Recommended dietary intakes (RDI) of folate in humans[1-3]

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ABSTRACT Extensive evidence is presented that 3 μg folate/kg (6.8 nmol/kg) body weight daily will not only maintain adequate folate nutriture but also a substantial reserve body pool in normal persons. Recommendations appropriate to this extensive evidence are presented. *Am J Clin Nutr* 1987;45:661-70.

KEY WORDS Folate requirement, folate, folic acid, folate body pool, folate absorption

Introduction

Folate and folacin are alternate generic descriptors for compounds that, at various oxidation states and with different numbers of glutamate residues, have nutritional properties and chemical structures similar to those of folicacid: N-[4-[[(2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl]amino-benzoyl]-L-glutamic acid, also known as pteroylglutamic acid (PGA). The amount of the vitamin is commonly measured by its ability to support the growth of folate-dependent organisms in an otherwise complete, chemically defined culture medium. *Lactobacillus casei* is generally accepted as the standard assay organism because it responds to the greatest number of different folate derivatives, including those with up to three L-glutamic acid residues. Its responses to derivatives with four or more such residues are less complete. The amount of folate is also measurable by radioisotope dilution and binding methods (1).

Requirements for folate can be met by a variety of chemical forms as long as the essential subunit structure of PGA remains intact. If the parent molecule PGA—consisting of pteridine, *p*-aminobenzoic acid, and glutamic acid—is broken, then nutritional activity is lost. Folate is heat labile; a diet comprised exclusively of thoroughly cooked foods is likely to be low in folate (2).

All naturally occurring folates show a variable degree of instability due to endogenous pteroylpolyglutamyl hydrolases that remove glutamate residues but leave an active compound. Heat, oxidation, and ultraviolet light cleave the folate molecule, rendering it inactive. Thus, naturally occurring, labile folates are lost in storage and cooking. Reducing agents, such as ascorbate, preserve folate but can damage vitamin B-12.

Although all members of the folate family may possess biological properties of their parent molecule under some conditions, they vary widely in their nutritional effectiveness, stability, and availability. Some of the tetrahydrofolate forms of folate (eg, N^5-formyl PGA H_4, N^{10}-formyl PGA H_4, and, to a lesser extent, N^5-methyl PGA H_4) are relatively heat stable, whereas others (eg, unsubstituted PGA H_4) are destroyed rapidly by heat (3). Methylene PGA H_4 is destroyed by acid but methenyl PGA H_4 is quite stable at low pH.

The principal function of PGA-containing coenzymes is the transport of fragments containing a single carbon atom from one compound to another. Many of these steps are essential for the synthesis of nucleic acid and for normal metabolism of certain amino acids. Hence, deficiency of the vitamin leads to impaired cell division and to alterations of protein synthesis—effects most noticeable in rapidly growing tissues. The dU suppression test, a sensitive indicator of folate-deficient DNA synthesis (2, 4), becomes clearly abnormal when intracellular folate levels fall below 0.2 ng/10^6 cells (4.5 pmol/10^6 cells) (5, 6).

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Folate is present in a variety of foods, especially liver, leafy vegetables, fruit, pulses, and yeast (7–11). It has recently been reported that breakfast cereals (12) and tea (13) may make a significant folate contribution to western diets. As of 1985, the best available data on folate in the food supply, as measured by microbiologic assay using ascorbate protection, are those summarized in the US Department of Agriculture (USDA) Handbook (14), which included Canadian data, and the UK data of Paul and Southgate (15). However, extraction conditions are critical and incomplete recovery may plague some of the data in these tables (16, 17).

Approximately three-fourths of the folate in mixed US diets is present in the form of polyglutamates (18), which are incompletely measured in microbiological assays unless pretreated with exogenous enzymes known as conjugases (also known as pteroylpolyglutamyl hydrolases). However, these polyglutamates may lose their extra glutamates and then be absorbed and utilized in higher organisms as a result of the presence of endogenous enzymes in the digestive tract (19–22).

Metabolic-balance studies in which radioactive synthetic polyglutamates of PGA were given to humans (23) indicated that intestinal absorption of heptaglutamyl folate ranged from 50% to 75% (103) and of triglutamyl folate, 90% or more (as estimated from fecal losses). In other studies (24) that used a different quantity than the prior study, monoglutamates and polyglutamates were absorbed equally well. Differences in the relative absorption of folate measurable in different foods may relate to the presence of conjugase inhibitors, binders, or other unknown factors (25–27). Babu and Srikantia (28) confirmed that the mean absorption of folate in seven separate food items was close to 50% (range, 37–72%). In that study, the absorption of folate from brewers' yeast was only 10%. In a small sample (11 women), Iyenger and Babu (29) found percentage absorption of 2 mg of H^3PGA rose as red cell folate fell. This phenomenon of enhanced absorption as stores fall is similar to what occurs in iron absorption and needs further study.

Approximately 90% of folate monoglutamate and 50–90% of folate polyglutamate ingested separately from food is absorbed, but this percentage is decreased in the presence of many foods, irrespective of whether the folate was derived from or added to the food (30, 27). On the basis of food-composition and intestinal-absorption data, it is reasonable to assume that the bioavailability of folate in the typical US diet is about one-half to two-thirds that of separately ingested PGA. It is not clear whether polyglutamate absorbability decreases with increasing chain length. The absorbability of heptaglutamate has been reported to be 70–100% that of PGA (24, 31, 32, 105). About 100 μg (226 nmol) of free PGA taken orally each day will prevent the development of deficiency (33, 34).

There is relatively little conjugase activity in the contents of the intestinal lumen of humans and certain animals (31, 35–37). Rather, the hydrolysis of polyglutamates of PGA appears to be a function of mucosal cells (31, 35, 38, 104). Folacin enters portal plasma in the free, or monoglutamate, form following ingestion of the conjugated, or polyglutamyl, forms of the vitamin (35). Both the free and conjugated forms of folate can be utilized in meeting human nutritional requirements.

The fecal excretion of ~200 μg (453 nmol) of folate daily (39) is not a reliable indicator of folate intake or absorption because the feces also contain folate synthesized by bacteria in the colon. Normally nourished individuals excrete 5–40 μg (11–91 nmol) of free (ie, microbiologically measurable) folate in the urine daily (40). Moreover, the folate content of bile is approximately five times that of serum (41). Enterohepatic recirculation tends to conserve the body pool of folate (42, 43). Krumdieck et al (44) monitored complete stool and urine collections for radioactivity at sequential intervals after the ingestion of radioactive PGA by a healthy subject. After a period of equilibration, the disappearance curve indicated a biological half-life of 101 d. Fecal and urinary losses were approximately equal.

The size of the body's total folate pool has not been accurately determined but liver folate is a major part of the total (45). Of 560 assayed livers from autopsies in Canada (46), only two had a folate content of < 3 μg/g (< 6.8 nmol/g) of liver. Morphologic evidence of folate deficiency is not manifest until liver levels fall below 1 μg/g (2.3 nmol/g) (47). The mean levels of folate in the autopsied livers peaked at

8.8 μg/g (20 nmol/g) between the ages of 11 and 20 and then gradually decreased.

The male adult's total folate pool has been calculated to be 7.5 ± 2.5 mg (17 ± 5.7 μmol) (34, 48–50). Studies of normal human subjects (34, 48, 50) and patients with neoplastic disease (47) on low-folate diets containing < 5 μg (11.3 nmol) of folate daily indicate that the earliest morphologic manifestations of deficiency in the red cells develop in ~16 wk. During a 6-wk observation period of a normal woman in her mid-twenties on a no-folate diet (50), a daily oral supplement of 100 μg (227 nmol) of PGA maintained the serum-folate levels > 7 ng/mL (16 nmol/L), whereas with 50 μg (110 nmol) supplements in a second such woman, they dropped to a diagnostically indeterminate range of 4–6.9 ng/mL (9.1–16 nmol/L). At 25 μg (57 nmol), the serum folate levels in a third woman dropped to < 3 ng/mL (6.8 nmol/L), clearly indicating negative folate balance. Two alcoholic subjects with marginal folate reserves developed signs of depletion in ~8 wk (51) on a low-folate diet. Although some depleted subjects respond to 50-μg oral supplements of PGA daily (52, 53), others with disease complications do not (54, 55). Loss of folate from the liver on an intake of 2 μg (4.5 nmol) folate daily, as assessed by liver biopsies (47), varies from 35 to 47 μg (79 to 100 nmol) daily. Assuming that extrahepatic stores are approximately half those in liver, total daily folate loss in an adult eating essentially no folate would average ~60 μg (~140 nmol). In such adults, morphologic evidence of folate deficiency in bone marrow and peripheral blood does not appear until liver folate falls below 1 μg/g (2.3 nmol/g) (47).

Human requirement and basis for RDI

Adults

The minimal daily requirement for folate is ~50 μg (~1 μg/kg body weight) for adults based on observations that daily parenteral administration of this amount successfully treats uncomplicated folate-deficiency anemia (53, 56); however, Hoogstraten et al (57) and Marshall and Jandl (55) have reported that more complicated cases may fail to respond to such treatments. Approximately 85% (range, 50–94%) of a 10–200 μg (2.3–453 nmol) oral dose of free PGA is absorbed (58–61). Daily dietary folate intake correlates significantly with red cell folate (62).

Table 1 is a sequential list of events in the development of folate deficiency in persons consuming an experimental diet containing < 5 μg (11 nmol) of folate daily (1, 2, 4, 34, 48, 63).

In Canada, the mean national daily folate intake for ages 12–65 yr is 205 μg/d (465 nmol/d) for men and 149 μg/d (338 nmol/d) for women (210, 221, and 183 μg/d (476, 501, and 415 nmol/d) for males aged 12–19, 20–39, and 40–64, respectively; 153, 146, and 148 μg/d (347, 331, and 335 nmol/d) for females aged 12–19, 20–39, and 40–64, respectively), or ~3 μg/kg (~6.8 nmol/kg) body weight (64). This diet permits maintenance of normal and similar liver-folate levels (46, 65) in both sexes (*see* **Table 2**). Forty adult males living in a metabolic ward on a strictly controlled diet containing 200 ± 68 μg/d (66) maintained normal serum and red cell folates; at the end of 6 mo, they had normal serum folates (5.8 ± 1.4 ng/mL or 13 ± 3.2 nmol/L) and normal

TABLE 1
Folate deficiency in persons consuming < 5 μg (11 nmol) folate daily

Sequential changes	Time of appearance (weeks after initiation of diet)
Low serum folate (< 3 ng/mL or 6.8 nmol/L); slight increase in size of average bone marrow normoblast	3
Hypersegmentation in neutrophils in bone marrow (lobe average > 3.5); dU suppression test abnormal in bone marrow	5
Hypersegmentation in neutrophils; bone marrow shows increased and abnormal mitoses and basophilic intermediate megaloblasts; dU suppression test abnormal in peripheral blood lymphocytes	7
Bone marrow shows some large metamyelocytes and a number of polychromatophilic intermediate megaloblasts	10
High urine formiminoglutamate (FIGLU)	13
Orthochromatic intermediate megaloblasts in bone marrow	14
Low red blood cell folate	17
Macroovalocytosis; many large metamyelocytes in bone marrow	18
Overtly megaloblastic marrow	19
Anemia	20

TABLE 2
Liver-folacin levels in relation to age and sex*

Characteristic of subject	n	Folate levels	
		Range	Mean ± SD
		μg/g liver†	
Age (yr)			
At birth‡	7	3.3–8.5	5.9 ± 1.7
0–1	18	3.8–11.3	7.4 ± 2.0
1–10	13	4.3–10.5	7.4 ± 2.1
11–20	19	6.0–14.0	8.8 ± 2.2
21–30	32	3.6–14.8	8.0 ± 2.8
31–40	32	3.6–11.5	7.7 ± 1.7
41–50	61	4.1–14.6	7.1 ± 2.0
51–60	86	3.2–12.6	7.3 ± 1.9
61–70	128	3.2–15.6	7.7 ± 2.2
71–80	117	2.9–14.7	6.9 ± 2.1
80+	47	2.7–12.7	7.0 ± 2.0
Sex			
Male	370	2.7–15.9§	7.4 ± 2.2§
Female	190	3.2–14.0§	7.3 ± 2.9§

* Based on data in Happner and Lampi (46).
† To convert to SI (nmol/g) multiply by 2.266.
‡ Stillborn.
§ Average at all ages.

red cell folates (229 ± 44 ng/mL or 519 ± 100 nmol/L).

Red cell folate reflects liver folate fairly closely (45, 67, 68); by a coincidence of nature, the red cell life-span is 4 mo and the liver-folate stores will last for 4 mo (67). Not more than 8% of Canadian men and 10% of Canadian women have low folate stores as judged by red cell concentrations < 140 ng folate/mL (317 nmol/L) (69). The NHANES II data (70) for American men is also 8%, and for American women 13%. Therefore, for ~90% of the adult population, Canadian and American diets provide not only adequate folate for daily metabolic needs but also adequate folate to sustain a substantial folate storage (> 140 ng folate/mL red cells) against periods of dietary deprivation.

Tamura and Stokstad (personal communication cited in ref 71) calculated the US dietary folate intake as 227 μg (514 nmol) per capita daily. This estimate is likely to be high because they did not account for food wastage or nutrients lost in home food preparation (71). It is important to remember that heat destroys folate. Earlier, higher estimates of average folate intake in various countries were based on older methodology; because they were apparently in error, they need reevaluation (9, 62, 72).

Recognition that diets contain about half as much folate as previously believed and still result in liver stores > 3 μg/g (6.8 nmol/g) provides the basis for lowering the folate RDA (**Table 3**) below that stated in 1980. Because folate absorbability from an average North American diet is adequate to sustain normal liver stores and the diet contains an average of 3 μg folate/kg body weight (46, 64, 65), the recommended dietary intake (RDI) for folate is set at 3 μg/kg (6.8 nmol/g) body weight (as measured in a microbiological assay) for normal nonpregnant, nonlactating adults and adolescents—that is, 240 μg (544 nmol) (rounded) for a 79-kg reference man and 190 μg (431 nmol) for a 62-kg reference woman. This allowance appears to provide an adequate margin of safety for adequate storage in the liver against periods of negative folate balance and is well above the ~50 μg/d (~110 nmol/d) minimal adult requirement (~1 μg/kg or ~2.3 nmol/kg body weight) delineated above.

Pregnancy and lactation

The added burden of pregnancy increases the risk and incidence of folate deficiency among populations with low or marginal intakes of the vitamin (73–75). The usual problems of establishing folate requirements are further complicated by necessary safeguards against the use of radioactive tracers and other experimental procedures in pregnant subjects. PGA supplements ranging from 100 to 1000 μg/d (227–2266 nmol/d) have been recommended by different investigators in addition to the folate present in a mixed diet of good quality (30, 76–78). The report of Baumslag et al (79) was confirmed by data (80) indicating that an oral PGA supplement of 500 μg/d (1130 nmol/d) was associated with a 50% reduction in the incidence of small-for-date births among 134 pregnant women in India. In women consuming a usual diet in the United Kingdom, a daily oral supplement of 100 μg of PGA prevented any fall in the mean red cell folate during pregnancy (76). The dietary folate content in that country was subsequently reported to be ~190 μg/d (~431 nmol/d) (9, 62, 81). All manifestations of folate deficiency in women who start pregnancy with moderate folate stores could probably be prevented by diets containing the equivalent of 299 μg PGA/d (678 nmol/d) (67). In women

TABLE 3
Recommended dietary intakes (RDI) for folate

Category	Age	Weight	RDI	RDI for reference individual*
		kg		*μg*
Infants	0–2.9 mo	4.5	16 μg† (36.26 nmol)	
	3–5.9 mo	6.6	24 μg† (54.38 nmol)	
	6–11.9 mo	8.8	32 μg† (72.51 nmol)	
Children	1–1.9 yr		3.3 μg/kg (7.5 nmol/kg)	35 (79 nmol)
	2–5.9 yr		3.3 μg/kg (7.5 nmol/kg)	50 (110)
	6–9.9 yr		3.3 μg/kg (7.5 nmol/kg)	80 (180)
Males	11–11.9 yr		3 μg/kg (6.8 nmol/kg)	110 (249)
	12–17.9 yr		3 μg/kg (6.8 nmol/kg)	170 (385)
	18–24.9 yr		3 μg/kg (6.8 nmol/kg)	220 (499)
	25–49.9 yr		3 μg/kg (6.8 nmol/kg)	240 (544)
	50–69.9 yr		3 μg/kg (6.8 nmol/kg)	230 (521)
	70+ yr		3 μg/kg (6.8 nmol/kg)	220 (499)
Females	11–14.9 yr		3 μg/kg (6.8 nmol/kg)	130 (295)
	15–17.9 yr		3 μg/kg (6.8 nmol/kg)	170 (385)
	18–24.9 yr		3 μg/kg (6.8 nmol/kg)	170 (385)
	25–49.9 yr		3 μg/kg (6.8 nmol/kg)	190 (431)
	50–69.9 yr		3 μg/kg (6.8 nmol/kg)	190 (431)
	70+ yr		3 μg/kg (6.8 nmol/kg)	190 (431)
Pregnancy	0–2.9 mo		500 μg/d (1130 nmol/d)	500 (1130)
	3–5.9 mo		500 μg/d (1130 nmol/d)	500 (1130)
	6–9 mo		500 μg/d (1130 nmol/d)	500 (1130)
Lactation	0–5.9 mo		3 μg/kg + 100 μg/d (6.8 nmol/kg + 227 nmol/d)	280 (635)
	6+ mo		3 μg/kg + 100 μg/d (6.8 nmol/kg + 227 nmol/d)	280 (635)

* Values in this column are for a reference individual. Actual figures expressed per kilogram of body weight are 3.3 μg/kg (7.5 nmol/kg) for ages 1–9.9 and 3 μg/kg (6.8 nmol/kg) for males and nonpregnant nonlactating females ages 10–70+. (Assumes reference lactating woman is 59 kg). SI (nmol) are given in parentheses.

† Human milk or 3.6 μg/kg (8.2 nmol/kg).

with poor folate stores who received essentially no other dietary folate, the progression of folate deficiency was as effectively prevented by administering 300 μg PGA/d (680 nmol/d) in a food that impaired availability by 44% (reducing effective dose to 168 μg PGA/d or 381 nmol/d) as it was by higher doses or more efficient vehicles (30).

Oral supplementation or food fortification appears desirable to maintain maternal stores (9, 30, 71, 73) and to keep pace with the increased folate turnover in rapidly growing tissue. On the basis of a 50% food-folate absorption, the RDI for folate is set at 500 μg/d (1130 nmol/d) during pregnancy (Table 3) with the recognition that this level cannot usually be met without oral supplementation. This RDI is higher than needed for most pregnant women; it is intended to meet the needs of those with poor folate stores, essentially no other dietary folate, and multiple or twin pregnancies.

The burden of lactation on maternal folate reserves was estimated to be 20 μg/d (45 nmol/d) (82), varying with the folate content and volume of milk. This estimate was based on daily production of 850 mL of milk with an average folate content; however, content may be as high as 50–60 μg folate/L (110–140 nmol/L) (83). Ek (84) reported that supplementation was unnecessary in women in the middle socioeconomic class in Sweden. On the basis of a daily production of 750 mL of milk and 50% absorption of food folate, the allowance for folate during lactation is set at 3 μg/kg (6.8 nmol/kg) body weight plus 100 μg/d (227 nmol/d) (Table 3).

Infants and children

Folacin deficiency is the most common cause of megaloblastic anemia in infants and children (85, 86). Although serum folate at birth is three times that of maternal folate, body stores at birth are small and are rapidly depleted by the requirements for growth, especially in small premature infants whose liver stores are ~159 μg (360 nmol) (87). By 2 wk of age, serum and red cell folate of newborns fall below adult values and remain low for several months (83, 86–88). In a premature infant, an appropriate maintenance dose is 50–100 μg/d (100–227 nmol/d), which is adequate to prevent the folate deficiency that commonly accompanies childhood hemolytic anemias (85). In full-term infants, liver stores are ~224 μg (508 nmol) (89). In a study of 20 infants aged 2–11 mo, Asfour et al (90) demonstrated the nutritional adequacy of diets providing 3.6 μg folate/kg (8.2 nmol) body weight daily for 6- to 9-mo periods.

Human and cow's milk both contain 50–60 μg free folate/L (110–140 nmol/L), although the concentration of folate in human colostrum and early milk is much lower (83). Hoppner et al (91) reported that fresh cow's milk contains 5 μg folate/100 g (50 μg/L). The needs of infants are adequately met by human or cow's milk, but not by goat's milk, which has a much lower folate content (83, 85).

Heat sterilization of infant formulas may destroy portions of the folate content, depending on the quantity of reducing agent added to the formula to protect its folate content against heat destruction. Milk from humans, cows, and goats contains a factor that is essentially unaffected by pasteurization and that facilitates folate uptake by gut cells (92, 93). Presumably, this factor facilitates both absorption of dietary folate and reabsorption of bile folate. Boiling, or the preparation of evaporated milk, destroys an average of 50% of the folate in cow's milk (88) so that infants receiving boiled formulas prepared from pasteurized, sterilized, or powdered cow's milk should be given additional folate to ensure an adequate intake (94). If the diet consists of goat's milk, folic acid supplementation should be given.

Megaloblastic anemia due to dietary folate deficiency is rare in children who drink vegetable or fruit juice or eat one fresh, uncooked fruit or vegetable each day (85). However, this disease is common among children whose entire diet consists of thoroughly cooked foods, especially finely particulate foods cooked for a long period (eg, diets consisting primarily of beans and rice). The cooking of beans not only destroys part of their folate content but also causes them to release a substance that may reduce folate absorbability (95, 96). Up to age 2 yr, 3.5 μg (7.9 nmol) dietary folate/kg body weight appears to be adequate (61).

On the basis of the above considerations (82–91), the RDI for folate is set at 3.6 $\mu g \cdot kg^{-1} \cdot d^{-1}$ (8.2 $nmol \cdot kg^{-1} \cdot d^{-1}$) for healthy offspring from birth to age 1.9 yr. This value

should provide an adequate margin of safety and is compatible with the folate content of human milk.

In Canadians, whose average diet contains 3 μg folate/kg (6.8 nmol/kg) body weight, folate stores in the liver appear to be satisfactory in children as well as in adults (46). The folate RDI for healthy children 2–9.9 yr of age is interpolated from the allowances for infants and adolescents to be 3.3 μg/kg (7.5 μmol/kg) body weight (Table 3).

The elderly

The elderly are considered in the same category as other adults with respect to folate needs (97). On diets estimated to contain 135 μg folate/d, all of 21 elderly men and women living at home sustained red cell folate > 100 ng/mL (> 227 nmol/L) and were hematologically normal; nine had red cell folate < 150 ng/mL (< 340 nmol/L) (98).

Other data sources

Rodriguez (24) published an extensive review of human folate requirements that covers 818 references. For further discussion, the reader is referred to the published proceedings of a workshop held by the Food and Nutrition Board in 1975 (9), a technical report prepared for the Food and Drug Administration by Anderson and Talbot (71), and a book by Chanarin (45) that contains 4258 references.

Toxicity

Folic acid and the anticonvulsant drug phenytoin inhibit uptake of each other at the gut cell wall and possibly at the brain cell wall (45, 72). Very large doses of folic acid (100 or more times the RDA) may precipitate convulsions in persons whose epilepsy is in continuous control by phenytoin (72). As little as 1 mg of intravenous PGA produced an abnormal EEG in an adult with phenobarbitol-controlled epilepsy (99). In experimental animals, very large doses of folic acid given parenterally may precipitate in the kidneys, producing kidney damage and hypertrophy (72). Although fragile chromosomes are associated with folate deficiency, it does not necessarily follow that excesses of folate protect against malignancy. In fact, large excesses may promote the growth of certain tumors (100).

In January 1987 it was reported that "pregnancy supplements" containing 100 mg (1.79 mmol) iron and 350 μg (0.79 μmol) folate significantly reduce zinc absorption, as do iron alone and folate alone, and may promote maternal zinc depletion, resulting in intrauterine growth retardation (101). This adverse nutrient–nutrient interaction from widely used pregnancy supplements provides further support for the recommended reduction by the 1980–1985 RDA Committee of the size of supplemental dietary intakes. With respect to iron, the Committee in its draft report recommended only a 10 mg (180 μmol) iron supplement in pregnancy, but raised it to 30 mg (540 μmol) on the strong recommendation of the draft's reviewers (102). ■

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