Folate and human reproduction[1–3]

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ABSTRACT
The influence of folate nutritional status on various pregnancy outcomes has long been recognized. Studies conducted in the 1950s and 1960s led to the recognition of prenatal folic acid supplementation as a means to prevent pregnancy-induced megaloblastic anemia. In the 1990s, the utility of periconceptional folic acid supplementation and folic acid food fortification emerged when they were proven to prevent the occurrence of neural tube defects. These distinctively different uses of folic acid may well be ranked among the most significant public health measures for the prevention of pregnancy-related disorders. Folate is now viewed not only as a nutrient needed to prevent megaloblastic anemia in pregnancy but also as a vitamin essential for reproductive health. This review focuses on the relation between various outcomes of human reproduction (ie, pregnancy, lactation, and male reproduction) and folate nutrition and metabolism, homocysteine metabolism, and polymorphisms of genes that encode folate-related enzymes or proteins, and we identify issues for future research. *Am J Clin Nutr* 2006;83:993–1016.

KEY WORDS Folate, folic acid, pregnancy, complications, fetal growth, malformations, lactation, male reproduction

INTRODUCTION

The main objective of the present article was to review the evidence for the role of folate nutrition in human reproductive health. The term folate represents all forms of this B vitamin, including the many derivatives found in biological systems; folic acid (pteroylmonoglutamic acid) is the synthetic form found in dietary supplements and fortified foods. The effect of folate status on pregnancy outcomes has long been recognized (1). Since Wills (2) successfully treated megaloblastic anemia in pregnancy with a yeast extract (Marmite; Marmite Food Company Ltd, London, United Kingdom) in 1931, researchers have studied the prevalence and treatment of pregnancy-related folate deficiency and megaloblastic anemia (1). Studies conducted in the 1950s and 1960s led to the recognition that supplementing with folic acid reduced the prevalence of folate deficiency in pregnancy, and prenatal folic acid supplementation in the second and third trimesters became a common public health measure. In 1970, the US Food and Nutrition Board (3) recommended folic acid supplementation (200–400 μg/d) for pregnant women, and this became a common practice in developed countries and substantially reduced pregnancy-induced severe folate deficiency, which can lead to megaloblastic anemia. Prenatal folic acid, along with iron, supplementation reduced the prevalence of 2 of the most common pregnancy-related deficiencies.

The second major achievement with the use of folic acid occurred in the 1990s. For years, researchers suspected an association between maternal folate status and fetal malformations, particularly neural tube defects (NTDs) (4, 5). However, this relation was not confirmed until the early 1990s, when periconceptional folic acid supplementation was found to reduce both the recurrence (6) and occurrence (7) of NTDs. This periconceptional folic acid supplementation no longer aims to treat or prevent pregnancy-induced severe folate deficiency, but to correct abnormal folate metabolism or a subtle folate inadequacy that is possibly present in a certain segment of the population. These discoveries led to mandated folic acid food fortification in several countries (8–11). These distinctively different uses of folic acid—prenatal folic acid supplementation, periconceptional folic acid supplementation, and folic acid fortification of staple foods—may well be ranked among the most significant public health measures for the prevention of pregnancy-related disorders.

In the present review, we focus on the relation between human reproductive outcome and folate nutrition and metabolism, homocysteine metabolism, and polymorphisms of folate-related genes. We conducted a Medline literature search for the terms "folate, folic acid, pregnancy, and lactation." Over 2500 articles

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[2] Supported in part by the Intergovernmental Personnel Act (TT) from the National Institutes of Health.

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Received July 25, 2005.
Accepted for publication November 8, 2005.

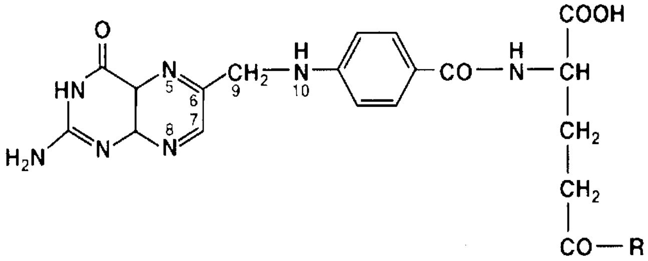

FIGURE 1. Structure of folic acid. Natural folates are generally reduced to either tetrahydrofolate with hydrogen at the 5,6,7, and 8 positions or dihydrofolate with hydrogen at the 7 and 8 positions, have a one-carbon unit (methyl, methylene, methenyl, formyl, or formimino) at the 5 or 10 positions or bridging the 5 and 10 positions, and exist as polyglutamyates with a glutamyl chain (R).

were identified after limiting the search to English language articles and studies conducted in humans, and our final update was in May 2005. However, despite our attempts at completeness, important publications may have been excluded from the review.

FOLATE STRUCTURE AND FUNCTION

Folic acid consists of a pteridine ring, *p*-aminobenzoic acid, and glutamic acid (**Figure 1**). Naturally occurring folates are generally reduced to tetrahydrofolate with hydrogen at the 5, 6, 7, and 8 positions or to dihydrofolate with hydrogen at the 7 and 8 positions, and they have a one-carbon unit (methyl, methylene, methenyl, formyl, or formimino) at the N-5 or N-10 positions, or both. Most folates exist as polyglutamyl folates with a γ-linked glutamic acid chain (12).

Folates function in various one-carbon transfer reactions, including purine and thymidylate biosynthesis, amino acid metabolism, and formate oxidation (12). Purine and thymidylate biosynthesis is a fundamental requisite event underlying DNA and RNA synthesis. Thus, it is unmistakably clear that these folate-dependent reactions are essential for fetal growth and development and for maternal and paternal well-being. The amino acids methionine, serine, glycine, and histidine are metabolized via folate-dependent reactions (**Figure 2**). Recent human reproduction studies have focused on reactions catalyzed by methionine synthase (Figure 2, reaction 1) and 5,10-methylenetetrahydrofolate reductase (MTHFR; reaction 2). These reactions are involved in homocysteine metabolism. Plasma total homocysteine (tHcy) is regulated by folate status (13), and hyperhomocysteinemia (ie, mildly elevated tHcy) is linked to occlusive vascular disease (14). Impaired placental perfusion due to hyperhomocysteinemia is implicated in having a negative effect on pregnancy outcome. Methionine formed from homocysteine is converted to *S*-adenosylmethionine, which is a methyl donor for numerous reactions including DNA methylation (reaction 12).

FOLATE METABOLISM IN PREGNANCY

Chanarin (1) summarized many studies on folate nutrition and metabolism in pregnancy that were performed in the 1950s and 1960s. The general conclusion drawn from these studies was that pregnancy was associated with an increased folate demand and in some cases led to overt folate deficiency. The increase in folate requirement during pregnancy is due to the growth of the fetus and uteroplacental organs. However, dietary folate intake does not always meet the increased folate needs in pregnancy. Pregnant women exhibit rapid plasma clearance of intravenously administered folic acid (1). Increased folate catabolism (15–18) and urinary folate excretion (19, 20) may also contribute to increased folate needs in pregnancy, but the findings are controversial.

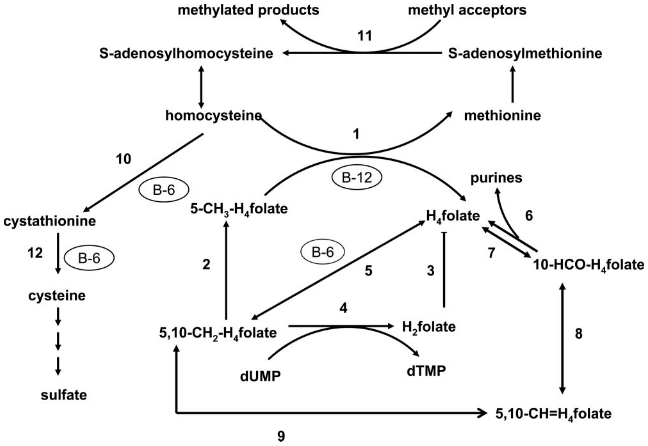

FIGURE 2. Folate and homocysteine metabolism. H_4folate, tetrahydrofolate; 5-CH_3-H_4folate, 5-methyltetrahydrofolate; 5,10-CH_2-H_4folate, 5,10-methylenetetrahydrofolate; H_2folate, dihydrofolate; 10-HCO-H_4folate, 10-formyltetrahydrofolate; 5,10-CH=H_4folate, 5,10-methenylterahydrofolate; B-12, methylcobalamin; B-6, pyridoxal phosphate; dUMP, deoxyuridylic acid; dTMP, thymidylate. Numbers represent enzymes. 1: methionine synthase; 2: 5,10-CH_2-H_4folate reductase; 3: dihydrofolate reductase; 4: thymidylate synthase; 5: serine hydroxymethyltransferase; 6: glycinamide and aminoimidazolecarboxamide ribotide transformylases; 7: 10-HCO-H_4folate synthetase and 10-HCO-H_4folate dehydrogenase; 8: 5,10-CH=H_4folate cyclohydrolase; 9: 5,10-CH_2-H_4folate dehydrogenase; 10: cystathionine β-synthase; and 11, cystathionase; 12, various methyltransferases.

Blood folate concentrations in pregnancy

Circulating folate concentrations decline in pregnant women who are not supplemented with folic acid (1, 19, 21–28). Chanarin (1) reported an average decline in serum folate of ≈10 nmol/L (from 20 to 10 nmol/L) during the 40-wk gestation. This decline may represent a physiologic response to pregnancy, but the mechanism is unknown. The pattern of changes in erythrocyte folate varies, with a decline observed in early pregnancy followed by a slight increase in midpregnancy (1, 25, 26). Possible causes for the declines in blood folate include increased folate demand for the growth of the fetus and uteroplacental organs (1), dilution of folate due to blood volume expansion (27), increased folate catabolism (15–18), increased folate clearance and excretion (19, 20), decreased folate absorption (1), hormonal influence on folate metabolism as a physiologic response to pregnancy (1), and low folate intake (1). Although the techniques used in the studies that were conducted in the 1950s and 1960s may be different from those used in recent days, the fundamental conclusions derived from the results are generally reasonable. It is apparent that the first and last causes mentioned above lead to a decrease in folate stores, but it is less apparent how much of the observed decline is due to the other factors. For example, Bruinse et al (24) measured plasma volume by a dye dilution method and estimated the total circulating amount of folate during both pregnancy and lactation (**Figure 3**). They found that serum folate declined 42% between 16 and 34 wk of gestation, and this decline

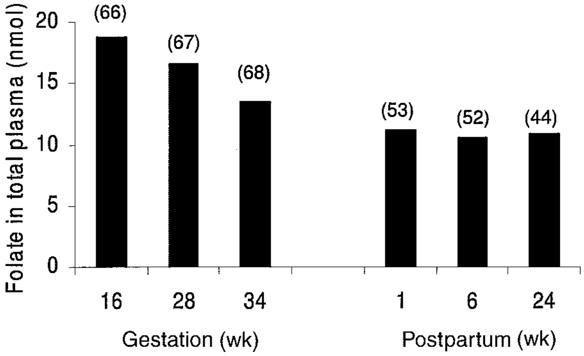

FIGURE 3. Total serum folate in circulation during pregnancy and lactation (24). Serum folate concentrations declined by 42% between 16 and 34 wk of gestation, and this decline was markedly greater than the 28% decline in total circulating folate in the same period. Serum folate was measured with a radiobinding assay. Numbers in parentheses represent the number of subjects. Data from this unique study suggest that the decline in serum folate could not be explained by hemodilution. The lack of recovery during lactation suggests that folate nutrition is a continuing burden.

was markedly greater than the decline in total circulating folate (28% in the same period), suggesting that the decline in serum folate cannot be explained by hemodilution.

In seemingly similar studies, folate catabolism was reported to increase or remain unchanged in pregnancy. One group reported that excretion of folate catabolites late in pregnancy was higher than in the nonpregnant state (15, 17). These catabolites are cleavage products of the C-9-N-10 bond of folate, including *p*-acetamidobenzoylglutamate (major urinary catabolite) and *p*-aminobenzolylglutamate, with the former involving *N*-acetylation of the latter. The folate-equivalent sum of the catabolites was 349 μg/d (0.79 μmol/d) in the third trimester, an amount double that of the nonpregnant state (0.31 μmol/d), indicating an accelerated folate breakdown. The amounts of catabolites excreted postpartum were similar to the level observed during the first trimester (15, 17). Increased catabolism may be consistent with placental expression of *N*-acetyltransferase type 1, which catalyzes the *N*-acetylation of *p*-aminobenzoylglutamate (29, 30). In contrast, another group did not find an increase in urinary catabolites in the second trimester in women who received a controlled diet (16). In the same study, with the use of stable-isotope-labeled folates, they reported no differences in urinary excretion of labeled folates or catabolites between the pregnant and nonpregnant women (18). The discrepancies between the findings of the 2 groups may be due to differences in the catabolite assay or in the gestational stages analyzed (17). Why folate catabolism increases late in pregnancy is unknown (15, 17). Additional studies are needed, particularly studies on how *N*-acetyltranferase type 1 (29, 30) and a ferritin-related folate-catabolizing enzyme that cleaves the C-9-N-10 bond of tetrahydrofolate possibly regulate intracellular folate concentrations (31).

Results on plasma folate clearance after folic acid administration in pregnancy are consistent. Chanarin et al (32) found that folate clearance after an injection of folic acid was higher in pregnant than nonpregnant women, accelerated as pregnancy progressed, and was greater in pregnant women with megaloblastic anemia than in those without. Landon and Hytten (19) estimated 24-h urinary folate serially during pregnancy and postpartum and reported that the mean urinary folate was 32 and 8 nmol/d, respectively. Fleming (20) also reported that mean folate clearance and urinary folate excretion was higher in pregnancy than in the nonpregnant state. Collectively, administered folic acid is more rapidly incorporated into cells and excreted in urine in pregnant than in nonpregnant women.

Whether a decrease in folate absorption contributes to an increased folate requirement in pregnancy is less certain. Chanarin et al (32) found that the peak serum folate concentration after an oral folic acid dose was significantly lower in pregnant than nonpregnant women, which suggested a decrease in folate absorption. However, Landon and Hytten (33) measured plasma folate after an oral folic acid dose in pregnant women, postpartum women, and adult men and found no difference between the 3 groups, which indicated that folate absorption is not altered in pregnancy. McLean et al (34) reported that oral loading with either folic acid or polyglutamyl folate (yeast) resulted in similar increases in serum folate in pregnant women, which suggested that malabsorption of polyglutamyl folate does not occur. The differences in the quantity of folate administered and the methods used to assess folate absorption may explain the discrepancies between these studies.

Several mechanisms, probably in combination, may explain the decline in blood folate in pregnancy. Whatever the reasons for the decline, it is essential that plasma folate be kept above a critical level ($>$7.0 nmol/L; 1) because plasma folate is the main determinant of transplacental folate delivery to the fetus. Adequate plasma folate is likely to be achieved if prenatal folic acid supplementation or folic acid fortification of foods is practiced. However, in countries without such measures, the risk for gestational folate deficiency remains a public health problem.

Placental folate transfer and metabolism

Although nutrient transfer via the placenta from the maternal plasma pool must be effective to satisfy the demand for fetal growth, information on placental folate transfer is scarce (35–38). Landon et al (35) measured the placental transport of an intravenous dose of [^{3}H]folic acid in women who were scheduled for pregnancy termination. Tritium uptake was greatest in the fetal liver, and an analysis indicated that a peak of reduced folates in the placenta was detected shortly after the dose was intravenously administered, which suggested that folic acid was rapidly metabolized before or at the time of placental transfer. Baker et al (36) found a strong positive association between maternal plasma, cord plasma, and placental folate concentrations, suggesting that transplacental folate delivery depends on maternal plasma folate concentrations.

In placental perfusion studies, Henderson et al (37) found that 5-methyltetrahydrofolate (the main form of folate found in plasma) is extensively and rapidly bound in the placenta but transferred to the fetus in reduced amounts at a slower pace, and that the transfer is bidirectional and saturable. The placental folate receptor (FR) favors the binding of 5-methyltetrahydrofolate and can transfer folate against a concentration gradient; hence, the fetal perfusate is about 3-fold that of the maternal perfusate, which indicates that folate is concentrated during placental transport. Bisseling et al (38) found that the transfer of 5-methyltetrahydrofolate from the maternal to the fetal perfusate was not saturable in a range well above typical physiologic concentrations.

TABLE 1
Total homocysteine concentrations in cord and maternal serum or plasma and in amniotic fluid[1]

Study	Cord serum or plasma	Maternal serum or plasma	Amniotic fluid
Steegers-Theunissen et al, Netherlands (72)	ND	8.7 (23)[2]	1.0 (23)
Malinow et al, United States (73)	4.5 ± 1.8 (35)[3,4] 3.5 ± 1.5 (35)[5]	5.4 ± 1.4 (35)	ND
Wenstrom et al, United States (82)	ND	ND	1.1 ± 0.6 (80)
Molloy et al, Ireland (54)	7.9 ± 2.9 (201)	8.3 ± 2.9 (201)[6]	ND
Guerra-Shinohara et al, Brazil (55)	6.6 ± 2.8 (69)	7.7 ± 3.1 (69)	ND

[1] ND, not determined.

[2] Median; *n* in parentheses (all such values).

[3] $\bar{x}$ ± SD; *n* in parentheses (all such values).

[4] Umbilical artery.

[5] Umbilical vein.

[6] Difference between cord and maternal plasma concentrations was significant, $P < 0.001$.

The placenta is rich in FRs and is one of the tissues (along with the choroid plexus and renal proximal tubules) that expresses the α-isoform of FR (FR-α) in abundance. FR-α is a membrane-bound glycosylphosphatidylinositol-linked glycoprotein and the primary form of FR in epithelial cells. The importance of FR-α to placental folate transfer is inferred from the fact that an FR-α knockout mouse is embryo-lethal, whereas the FR-β knockout is not (39). Placental folate transport may be mediated by FR-α via a 2-step process (40), which includes the binding of 5-methyltetrahydrofolate to placental FR-α to produce an intravillous concentration 3 times that of maternal plasma and transporting folate to the fetus against a concentration gradient. Maternal folate status should be kept adequate to maintain plasma folate above a certain concentration for placental transfer. High-affinity binding proteins in the maternal circulation, cord blood, and newborns are derived from membrane-associated precursors (41–43).

The activities of dihydrofolate reductase (Figure 2, reaction 3; 44), folylpoly γ-glutamate carboxypeptidase II (folate conjugase; 45), methionine synthase (46), MTHFR (47), and serine hydroxymethyltransferase (Figure 2, reaction 5; 48) were detected in human placenta. mRNA expression of mitochondrial C_1-tetrahydrofolate synthase [5,10-methylenetetrahydrofolate dehydrogenase (Figure 2, reaction 9); 5,10-methenyltetrahydrofolate cyclohydrolase (reaction 8); and 10-formyltetrahydrofolate synthetase (reaction 7)] was detected, although the activity was not measured (49). Daly et al (47) reported that placental MTHFR activities were related to C677T *MTHFR* variants, which suggests a possible association with NTD development. The biochemical and physiologic implications of placental folate metabolism and transport require additional studies, and the use of folates labeled with stable isotopes may make such human studies feasible.

Folate metabolism in the fetus

Many researchers have evaluated the relations between folate concentrations in maternal, cord, and neonatal blood at or shortly after delivery (50–55). They reported that blood folate is markedly elevated in fetuses and newborns, which indicates an effective placental folate transport against a concentration gradient. Despite a several-fold elevation of blood folate in cord or newborn blood over maternal blood, total fetal folate stores do not appear to be large, because fetal hepatic folate content is lower than that in adults. Fetal hepatic folate concentrations ranged from 1.5 to 4.0 μg/g (56–58), whereas adult hepatic folate concentrations were >5.0 μg/g (59, 60). These data suggest that fetal folate acquisition and utilization differ from those of adults. Amniotic fluid folate concentrations range between 3 and 33 nmol/L (61–63), but the metabolic significance of folate in amniotic fluid is unknown.

The ontogeny of folate-dependent enzymes in humans has not been extensively studied due to the obvious difficulty, with a few exceptions. Gaull et al (64) reported that the activities of methionine synthase in fetal tissues are higher than in adult tissues, whereas those of serine hydroxymethyltransferase were similar. Kalinsky et al (65) reported that the activities of hepatic MTHFR and methionine synthase in preterm infants were higher than those in full-term infants or young children, whereas the activities of hepatic formiminotransferase and 5,10-methylenetetrahydrofolate dehydrogenase (Figure 2, reaction 9) were just the opposite. These results suggest dynamic changes in folate-dependent reactions late in fetal life and in neonatal life. In studies conducted in animals, the data indicated that specific activities of some of the folate-dependent enzymes also changed during the perinatal period (66–68). Furthermore, Xiao et al (69) elucidated the effect of maternal folate status on the regulation of fetal FR in mice. However, it is unclear to what extent the findings from the animal studies can be extrapolated to human conditions.

Homocysteine metabolism in pregnancy

Homocysteine metabolism is regulated by the nutritional status of folate, vitamin B-12, and vitamin B-6; and folate status has the strongest influence on plasma tHcy concentration (13). Even though blood folate is generally low in pregnant women, plasma tHcy is low. Kang et al (70) first reported that plasma tHcy is significantly lower in pregnant than nonpregnant women. Subsequently, Andersson et al (71) reported that the decline in tHcy started in the first trimester with a nadir reached in the second trimester. Research interest in homocysteine metabolism intensified in the area of obstetrics in the 1990s (28, 54, 55, 72–77), because hyperhomocysteinemia could lead to altered placental circulation. The interest in this association was further strengthened by the finding that periconceptional folic acid supplementation prevented NTDs (78–83).

Possible mechanisms for the decline in plasma tHcy in pregnancy include increased methionine requirement for fetal growth (70, 71), hemodilution due to plasma volume expansion (73, 75), changes in endocrine functions (70, 71), increased renal homocysteine clearance (77), and decreased plasma albumin to which homocysteine is bound (75). Of these, endocrine changes are likely the major reason for the observed decline. As shown in **Table 1**, maternal plasma tHcy concentrations at delivery are slightly higher than those in cord plasma and are several-fold those in amniotic fluid (54, 55, 72, 73). Malinow et al (73) found large tHcy differences between umbilical vein and artery blood, indicating fetal homocysteine uptake and metabolism. These findings are consistent with elevated fetal methionine synthase activity (64). In the fetal liver, no cystathionase (Figure 2, reaction 11) activity was detected and cystathionine β-synthase (Figure 2, reaction 10) activity was only 20% of adult levels (84), which indicated that transmethylation is more active than transsulfuration in the fetus. Whether already low tHcy concentrations in pregnant women decline further after folic acid fortification remains to be seen.

FOLATE INTAKE AND REQUIREMENT IN PREGNANCY

Increased folate demand in pregnancy is generally not met by self-selected diets (1). Assessment of food folate intake is difficult because of the lack of accurate food tables (85). Food folate values were traditionally obtained by a *Lactobacillus rhamnosus* (formerly known as *L. casei*) assay after folate conjugase treatment for hydrolysis of polyglutamyl folate (86). The recently developed trienzyme extraction method (treatment with α-amylase, protease, and folate conjugase) has provided higher values for certain foods (85, 87). Although this method is becoming popular, only limited food folate data are available, and evaluation of folate intakes remains difficult (87). The concept of dietary folate equivalents (DFEs; 1 DFE = 1 μg food folate or 0.6 μg folic acid) for folate intake was introduced in 2000 (88). Folic acid added to or ingested with food is estimated to be ≈85% available, whereas natural food folate is only ≈50% available (89). Thus, folic acid is 1.7 times (85 divided by 50) more available than is food folate, and the amount of DFEs consumed equals the sum of the amount of food folate and 1.7 times the amount of folic acid ingested. The recommended folate intake during pregnancy is 600 DFEs/d (88).

These 2 factors—the new food folate assay and DFEs—make the interpretation of folate intake data challenging. Furthermore, only extremely limited information on the folate bioavailability of individual foods exists (90–92). This difficulty will remain until food composition tables incorporate reliable data and more information on food folate bioavailability is attained. Achieving these goals will take a lot of work, but knowledge of the composition and bioavailability of food folate is fundamental to understanding the role of folate in human nutrition.

Folate intake in pregnancy

Chanarin et al (93) measured folate content in individually prepared meals collected from pregnant women and found that mean folate intake was 676 μg/d, which significantly correlated with erythrocyte folate concentrations. However, this value is considered extremely high. Moscovitch and Cooper (94) measured the folate content of meals consumed by women who were in the second trimester of pregnancy and who prepared duplicate diets and found the mean folate intake was 242 μg/d. The large difference between the 2 groups may be due to differences in food selection and folate assay methods. Since these reports >30 y ago, there have been no reports on direct folate analyses of self-selected diets consumed by pregnant women. Instead, investigators have estimated dietary intakes by dietary recalls or food-frequency questionnaires and calculated the values for folate intake from food tables (95–101). In these reports, the mean folate intakes of pregnant women varied widely from 85 to 668 μg/d. These data were obtained without trienzyme extraction, and most were obtained before the initiation of folic acid fortification of foods. The results of the 2 studies that included fortified values in the calculation indicated that the mean folate intake of pregnant women was ≈600 DFEs/d (99, 101). Stark et al (101) reported that >50% of inner-city black pregnant women did not meet the recommended 600 DFEs/d.

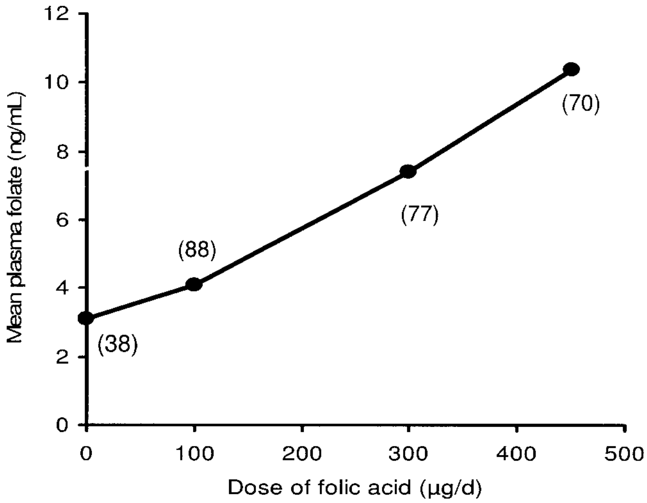

FIGURE 4. Dose-response effect of prenatal folic acid on serum folate concentrations 2–4 d after delivery (105). In addition to a mean dietary folate intake of ≈50 μg/d, subjects were given oral folic acid doses ranging from 0 to 450 μg/d from ≈3 mo of gestation to delivery. Numbers in parentheses represent the number of subjects. Serum folate at 2–4 d postpartum was measured with an *L. rhamnosus* assay. The data suggest that the minimum folic acid dose needed during pregnancy to keep postpartum serum folate concentrations >7.0 nmol/L was ≈300 μg/d (in addition to dietary intake).

Folate requirement for pregnant women

In 1970, the US Food and Nutrition Board (3) set the recommended folate intake for pregnant women at 400 μg/d; this was reduced to 270 μg/d in 1989 mainly because of data showing that this amount was typically ingested by healthy folate-replete adults (102). The third National Health and Nutrition Examination Survey dietary data (1989–1991) indicated that the mean folate intake of US women of childbearing age was ≈230 μg/d (103). The recommendation was increased to 600 DFEs/d in 1999, after the bioavailability of food folate and folic acid was considered (88). Caudill et al (104) monitored blood folate and urinary 5-methyltetrahydrofolate excretion in a metabolic study conducted in pregnant and nonpregnant women who consumed a diet containing only 120 μg folate/d with additional supplements of folic acid (330 or 730 μg/d). They concluded that 450 μg folate/d (≈600 DFEs/d) was sufficient to maintain adequate folate status in pregnant women. As reviewed above, most of the estimated dietary folate intakes were <400 μg/d.

Studies were conducted in the 1960s to determine the quantity of folic acid required, in addition to regular dietary intake, to maintain adequate folate status in pregnancy (1, 105–107). Willoughby and Jewell (105) measured the dose-response effect of prenatal folic acid (0–530 μg/d) on serum folate concentrations in the postpartum period and found that serum folate increased linearly with the amount of folic acid supplemented, which was given from ≈3 mo of gestation to delivery (**Figure 4**). To keep postpartum serum folate >7.0 nmol/L, they concluded that the minimum dose of folic acid needed during late pregnancy, in addition to a dietary folate intake of 50 μg/d, was close to 300 μg/d. Hansen and Rybo (106) conducted a similar study by monitoring blood folate concentrations in late pregnancy. Plasma folate increased linearly when folic acid was given at 200–500 μg/d. They suggested that an oral dose of 200 μg folic acid/d is close to the minimum requirement to maintain normal blood folate concentrations, although dietary folate intake was not reported in this study. Colman et al (108) conducted a pioneering study providing evidence that the folic acid fortification (300–1000 μg/d) of foods (maizemeal) improved folate status late in pregnancy. They found that erythrocyte folate responded linearly to the amount of folic acid added and suggested that maize containing 300 μg/d of fortified folic acid is effective in preventing folate depletion late in pregnancy.

Data from these studies suggest that 200–300 μg folic acid/d is needed in addition to dietary folate to maintain normal folate status and to prevent folate deficiency in pregnancy. In the past several years, it became feasible for pregnant women to achieve this intake in countries with folic acid fortification of foods, which aimed to provide an additional 100 μg folic acid/d. Pregnant women are still encouraged to consume foods high in folate, such as green leafy vegetables and fruits, in addition to folic acid–fortified foods.

FOLATE DEFICIENCY IN PREGNANCY

In addition to the blood folate assay, various biochemical (ie, formiminoglutamic acid analysis after a histidine load or deoxyuridine-suppression test) and hematologic (ie, neutrophil lobe count, mean corpuscular volume, or bone marrow test) tests were used to diagnose folate deficiency, assess the degree of folate deficiency, or measure responses to folic acid therapy in pregnancy (50, 93, 105, 109–113). In the 1990s, the plasma tHcy assay was added as a tool to assess folate adequacy. Of these tests, assays of folate and tHcy concentrations are the most extensively used; the other tests noted are used less because they lack sensitivity and specificity.

Before prenatal folic acid supplementation effectively reduced the prevalence of folate deficiency in developed countries, many cases of folate deficiency or megaloblastic anemia in pregnancy were reported (50, 114, 115). However, folate deficiency was prevalent worldwide in the 1970s. For example, >30% of women with pregnancy-related anemia in Venezuela were folate deficient (116), and a prevalence of folate deficiency of >10% was reported in pregnant women in Australia and the United States (117, 118). The presence of folate deficiency with or without megaloblastic anemia is still a public health problem for pregnant women in developing countries (119–121). A short interpregnancy interval associated with inadequate folate status was found to lead to unfavorable pregnancy outcome (96, 122, 123).

FOLATE AND PREGNANCY COMPLICATIONS

Various pregnancy complications have been associated with folate deficiency, but findings are equivocal. Discrepancies have resulted because many studies, out of necessity, were performed with a limited number of patients that yielded weak statistical power to provide firm conclusions, and because criteria for the evaluation of folate status varied between the studies. We review the relation of folate deficiency to each complication independently and discuss the findings on homocysteine metabolism or polymorphisms of genes encoding folate-related proteins.

Placental abruption

In the 1960s and 1970s, many studies evaluated the association of folate deficiency with placental abruption, a premature detachment of the placenta (124–133). Only 4 studies, which involved >600 cases, found folate deficiency to be associated with an increased risk of placental abruption (124, 125, 128, 129); the remaining studies, which involved ≈300 cases, found no association (126, 127, 130–133). These findings indicate that the association is possible, but not certain, and a mechanism for the possible association is unknown.

Because of the possible vasculotoxicity attributed to hyperhomocysteinemia (14), interest in studying the relation between tHcy and placental abruption was renewed in the 1990s. Most of the studies indicated an association of hyperhomocysteinemia with an increased risk for placental abruption (134–139). However, plasma tHcy analysis in these studies was made after the onset of symptoms; thus, the causal effect of tHcy cannot be established. Steegers-Theunissen et al (138) reported that an association between elevated tHcy and placental abruption was no longer significant after adjustment for the time between actual postpartum tHcy analysis and delivery.

The prevalence of placental abruption is reported to be associated with polymorphisms of folate-related genes. The abbreviations of these genes are shown in **Table 2**. A few research groups showed associations of placental abruption with maternal variants of the *MTHFR* gene (C677T, A1298C, or both; 140, 141), whereas others reported no such association (142, 143). Parle-McDermott et al (143) reported that the 1958*AA* variant of the gene encoding 10-formyltetrahydrofolate synthetase (Figure 2, reaction 7), a part of the C_1-tetrahydrofolate synthase, was an independent risk factor for placental abruption. Associations between placental abruption and altered folate or homocysteine metabolism appear to be weak. Possible associations between placental abruption and altered folate or homocysteine metabolism or polymorphisms of folate-related genes require additional study with attention to environmental factors, such as maternal folate status, that may exert an influence on these relations.

Preeclampsia

In the 1970s, 2 groups reported the lack of association of folate deficiency with preeclampsia (hypertension and proteinuria) or pregnancy-induced hypertension (144, 145). In the 1990s, research interest intensified on the premise that placental vasculopathy secondary to hyperhomocysteinemia may be the underlying cause of preeclampsia (77, 137–139, 141, 146–163). Of these studies, all but 5 indicated that plasma tHcy in women with preeclampsia was significantly higher than in women without. In 4 of the 5 studies that found no association, tHcy was measured before 27 wk of gestation (149, 152, 157, 160); in the fifth study,

TABLE 2

Polymorphisms of genes encoding folate-related enzymes or proteins that may be related to pregnancy complications or fetal malformations

Gene	Site of mutation
Methylenetetrahydrofolate reductase (*MTHFR*)	C677T
	A1298C
	C776G
	T1317C
C_1-tetrahydrofolate synthase (*MTHFD1:* 5,10-CH_2-H_4folate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase, and 10-formyltetrahydrofolate synthetase)	G1958A
Methionine synthase (*MTR*)	A2756G
Methionine synthase reductase (*MTRR*)	A66G
Folate receptor α (*FR-α*)	G762A
	T613C and A610C
Folate receptor β (*FR-β*)	A103G
	A660C
	A419C
Reduced-folate carrier (*RFC*)	A80G
Glutamate carboxypeptidase II (*GCII*)	C1561T
Dihydrofolate reductase (*DHFR*)	19–base pair deletion in intron 1

it was measured long after delivery (151). These findings may suggest that plasma tHcy is not elevated before clinical signs of preeclampsia appear, but that it increases considerably once signs develop. However, Cotter et al (153, 156) found that elevated tHcy at ≈15 wk of gestation was associated with an increased risk of preeclampsia. The reason for the difference between the study by Cotter et al (153, 156) and the other studies (149, 151, 152, 157, 160) is unknown. For data analyses, it is essential to consider when plasma tHcy was measured during gestation (138). Elevated tHcy may only be a surrogate of some metabolic event that responds to preeclampsia. A recent meta-analysis of 25 studies concluded that the evidence of hyperhomocysteinemia as the causative factor for preeclampsia was not compelling (164).

Of >30 studies reviewed, 11 included values for both plasma tHcy and folate (146–149, 153, 154, 156, 158, 159, 161–163) (**Table 3**). Most indicated that plasma folate concentrations were similar between women with and without preeclampsia. One showed decreased plasma folate in women with preeclampsia (148), whereas 3 indicated increased plasma folate (146, 158,

TABLE 3

Comparison of plasma total homocysteine (tHcy) and plasma or serum folate concentrations in women with preeclampsia and control subjects

Study	Time of blood draw	Plasma tHcy		Plasma or serum folate[1]	
		Preeclampsia subjects	Control subjects	Preeclampsia subjects	Control subjects
		μmol/L		*nmol/L*	
Rajkovic et al, United States (146)	Labor and delivery	8.7 ± 3.0 (20)[2]	5.0 ± 1.1 (20)[3]	23 ± 18 (20)	19 ± 11 (20)
Powers et al, United States (147)	Labor and delivery	9.7 ± 5.2 (20)	7.2 ± 2.3 (32)[3]	37 ± 15 (20)	37 ± 17 (32)
Laivuori et al, Finland (148)	29–39 wk gestation and delivery	6.7 ± 1.9 (22)	3.8 ± 0.8 (16)[3]	11 ± 7 (22)	14 ± 6 (16)[3]
		9.1 ± 2.2 (14)	8.2 ± 2.0 (11)[3]	11 ± 8 (14)	8 ± 3 (11)[3]
Hogg et al, United States (149)	26 wk gestation	5.2 ± 1.3 (16)	4.6 ± 1.4 (409)	30 ± 19 (16)	34 ± 20 (409)
	37 wk gestation	6.6 ± 2.1 (16)	5.3 ± 1.7 (409)[3]	26 ± 22 (16)	33 ± 21 (409)
Cotter et al, Ireland (153)	15–16 wk gestation (severe cases)	9.8 ± 3.3 (56)	8.4 ± 1.9 (112)[3]	6 ± 6 (56)	6 ± 5 (112)
Sanchez et al, Peru (154)	Third trimester	10.0 ± 6.7 (125)	8.4 ± 1.3 (179)[3]	12 ± 5 (125)	13 ± 7 (179)
Cotter et al, Ireland (156)	15 wk gestation (nonsevere cases)	8.4 ± 2.4 (71)	7.1 ± 1.5 (142)[3]	6 ± 4 (71)	6 ± 4 (142)
López-Quesada et al, Spain (158)	Third trimester	8.2 (32)[4]	6.3 (64)[3]	24 (32)	15 (64)[3]
Powers et al, United States (159)	Delivery	10.6 ± 7.3 (27)	7.2 ± 2.6 (30)	48 ± 14 (27)	35 ± 15 (30)
Patrick et al, United States (161)	> 31 wk gestation				
White women		7.5 ± 0.6 (34)	5.5 ± 0.3 (51)[3]	42 ± 3 (34)	42 ± 3 (51)
Black women		8.7 ± 1.4 (26)	7.6 ± 0.6 (52)[3]	33 ± 2 (26)	32 ± 3 (52)
Vanderjagt et al, Nigeria (162)	> 31 wk gestation	10.1 ± 3.7 (43)	8.4 ± 3.9 (130)[3]	16 ± 11 (43)	20 ± 10 (130)
Vadachkoria et al, United States (163)	Labor	9.0 (100)	6.7 (100)[3]	5 (100)	5 (100)

[1] All folate values originally reported in ng/ml were converted to nmol/L.

[2] $\bar{x}$ ± SD; *n* in parentheses (all such values).

[3] Significantly different from women with preeclampsia, $P < 0.05$.

[4] $\bar{x}$; *n* in parentheses (all such values).

159). The reason for this discrepancy is unknown. Folic acid supplementation in pregnancy decreases plasma tHcy (165), but whether such a reduction decreases the risk of preeclampsia is unknown. In a comparison of the rate of preeclampsia before (1990–1997) and after (1998–2000) folic acid fortification of food in Canada, Ray et al (166) reported no effect of increased folate intake on the risk of preeclampsia. Evidence appears to indicate that poor folate status is not responsible for the risk of preeclampsia; thus, improvement in folate status by folic acid supplementation or fortification may not be effective in preventing preeclampsia.

In 1997, the maternal 677*TT* variant of *MTHFR*, one of the thrombophilic genes, was reported to be associated with preeclampsia (167, 168). Since then, many groups have evaluated this association (151, 155, 160, 169–176); the 677*TT* variant is associated with elevated tHcy when folate status is poor (177). Only a few groups found an increased risk of preeclampsia in women with the 677*TT* variant compared with those with the wild type variant (151, 170, 173); thus, the 677*TT* variant alone may not be a risk factor. Kosmas et al (178) conducted a meta-analysis of 32 studies published before 2003 and suggested that early studies tended to indicate stronger associations than did later studies. Analysis of fetal and neonatal *MTHFR* polymorphisms indicated no association with preeclampsia (142, 172, 174). In addition, the maternal 1298*CC* and 1317*CC* variants of *MTHFR* were not significantly associated with the risk of preeclampsia (171, 175). Only 2 of these studies included plasma folate assays, and neither found an association of folate status with preeclampsia (169, 175). The pathogenesis of preeclampsia is clearly complex, and available data do not permit *MTHFR* polymorphisms to be included or excluded as causative factors; future studies should control for environmental and nutritional factors.

Spontaneous abortion and stillbirth

The causes of spontaneous abortion (loss before 20 wk of gestation) or stillbirths (baby born dead after 20 wk of gestation) are considered to be multifactorial and are often unclear.

Spontaneous abortion

In the 1960s, Martin et al (179) reported that serum folate was low in women who had a history of spontaneous abortion and that folic acid supplementation prevented recurrent abortion, whereas Chanarin et al (107) reported that women had similar erythrocyte folate concentrations regardless of their history of miscarriage. Researchers later reported no association between folate status and spontaneous abortion, but the statistical power was not sufficient due to small sample sizes (180–183). In a large Swedish cohort with and without a history of spontaneous abortion, George et al (184) reported that women with lower plasma folate (<4.9 nmol/L) had a greater risk for miscarriage than did those with higher plasma folate, particularly when fetal chromosomal anomalies were present. Gindler et al (185) evaluated the effect of folic acid supplementation on the risk of NTDs in China and reported that the supplementation did not alter the risk of miscarriage (186). Similarly, Czeizel et al (187) reported no effect of folic acid supplementation on the rates of spontaneous abortion or stillbirth.

After Steegers-Theunissen et al (134) provided the first evidence of an association between hyperhomocysteinemia and miscarriage in 1992, many researchers performed similar evaluations (188–190). These studies and a meta-analysis indicated that elevated plasma tHcy may be related to an increased risk of spontaneous abortion (191).

On the basis of the hypothesis that abnormal procoagulant activity has a potential role in the etiology of recurrent abortion due to impaired placental function, researchers examined whether the risk was associated with maternal polymorphisms of *MTHFR* (C677T, A1298C, or C776G) along with various coagulation factor genes (189, 192–195). Except for 2 reports (189, 194), these studies suggested that variants of *MTHFR* alone do not increase the risk of spontaneous abortion. A meta-analysis of data from all published studies should be performed to confirm or refute the association. Isotalo et al (196) found that the fetal 677*CT*/1298*CC* or 677*TT*/1298*CC* variants increased the risk of spontaneous abortion. Zetterberg et al (197) also reported an increased risk of spontaneous abortion for the combination of fetal 677*TT*/*TC* and 776*GG*/*CG* *MTHFR* variants, although Volcik et al (198) reported that the 677*CT*/1298*CC* variants did not affect fetal viability. These inconsistencies warrant additional studies, and the risk of miscarriage associated with maternal and fetal polymorphisms may have important implications for genetic counseling.

Stillbirth

Giles (50) and Ainley (115) reported that the stillbirth rate was higher in women with megaloblastic anemia than in those without, whereas Varadi et al (199) found no such association. In a large Norwegian female population with a history of stillbirth, Vollset et al (137) reported that women in the higher quartile for plasma tHcy had a significantly higher risk of stillbirth. However, the analysis of tHcy in this study was made ≥25 y after the index pregnancy. Whether it is reasonable to associate plasma tHcy with an incident that took place years before is uncertain (200). Only a few studies tested whether the risk of stillbirth was associated with *MTHFR* polymorphisms (141, 201, 202), and the findings are equivocal. Additional studies are needed to clarify whether such an association exists.

Other pregnancy complications

Other possible associations of abnormal folate nutrition and metabolism with pregnancy complications include relations between low blood folate, elevated tHcy, or variants of folate-related genes and threatened abortion (22), vaginal bleeding (22, 128, 131, 203), placental infarction (135, 151), or premature rupture of the membrane (204, 205). Conclusions about these associations cannot be reached because few cases have been examined and additional investigation is needed.

FOLATE AND FETAL GROWTH

Folate status and fetal growth

Birth weight is probably the most important pregnancy outcome, because fetal growth restriction (FGR; birth weight <10th percentile of a given population) is highly related to high mortality and morbidity (206). Many researchers examined the relations between birth weight and the rates of FGR, low-birth weight (<2500 g), or very-low-birth weight (<1500 g) and maternal folate status (207–211), folate intake or folic acid supplementation (93, 95, 212, 213), or megaloblastic anemia in pregnancy (111). Conclusions as to whether maternal folate nutrition

TABLE 4
Trials to evaluate the effect of prenatal folic acid supplementation on birth weight

Study	Folic acid dose	Subjects	Start of supplementation	Difference in birth weight[1]
	mg/d	*n*	*wk of gestation*	*g*
Baumslag et al, South Africa (223)	5.0	128	28	330
Giles et al, Australia (224)	5.0	620	≈10–30	None
Iyengar, India (225)	0.3	49	20–24	300[1]
Fletcher et al, United Kingdom (226)	5.0	643	14	None
Fleming et al, Australia (227)	0.5	89	20	None
Iyengar et al, India (228)	0.2–0.5	189	24–26	200[1]
Rolschau et al, Denmark (229)	5.0	36	21–25	407[1]
Blot et al, France (230)	0.35	109	≈28	158[1]
Tchernia et al, France (231)	0.35	108	≈24	157
Agarwal et al, India (232)	0.5	260	16–24	290
Czeizel et al, Hungary (187)	0.8	4672	Before conception (to 12 wk only)	None
Rolschau et al, Denmark (233)	1.0 or 2.5	3805	Before conception	≅40

[1] A significant ($P < 0.05$) difference between the supplemented and nonsupplemented groups was reported.

and metabolism affect fetal growth could not be made because of the lack of consistency between the studies and the insufficient statistical power due to small sample sizes. It is essential to understand that potential deficiencies in nutrients other than folate acting as confounding variables make it difficult to draw a solid conclusion, and this issue applies to interpreting data on the association between folate status and other pregnancy outcomes.

In 1992, Burke et al (214) first noted the possible relation between elevated tHcy and FGR. In a large Norwegian cohort, Vollset et al (137) later reported that the risk of FGR infants was significantly increased in women who were in the higher quartiles of tHcy than those in the lower quartiles, and others reported similar findings (139, 215). However, in other studies, elevated tHcy did not increase the risk of having an FGR infant (138, 149, 216–218). The relation of FGR risk with maternal or fetal *MTHFR* polymorphisms is also controversial (140–143, 170, 172, 219–222). Kupferminc et al (170) reported an increased risk of FGR in women who had the 677*TT* variant. In a large Norwegian cohort, Nurk et al (141) found that associations between the risk of FGR, low-birth weight, or very-low-birth weight and the C677T or A1298C variants were marginally significant. In contrast, Gebhardt et al (140) reported that C677T, A1298C, or both, variants were not related to FGR, and similar findings were reported by others (172, 219–221). Wisotzkey et al (222) reported that fetal growth was not related to fetal *MTHFR* polymorphisms. It appears that no firm consensus can be drawn about whether maternal folate nutrition and metabolism influences fetal growth.

Folic acid supplementation and fetal growth

Twelve studies (**Table 4**) evaluated the effect of prenatal folic acid supplementation on birth weight (187, 223–233). In 7 of the 12 studies, supplementation increased birth weight (223, 225, 226, 228, 229, 230–232). In contrast, no such effect was found in the remaining studies, probably due to sufficient maternal folate status early in pregnancy and the time of supplementation. Possible reasons for the discrepancy include race, maternal size, initial folate status, socioeconomic status, and dietary habits, including the intake of folate and other nutrients. For example, an impressive birth weight increase (300 g) was seen in Bantu women, whose diet consisted mainly of maizemeal with infrequent vegetable consumption, whereas no effect was seen in white women, whose diet habitually contained vegetables and fruit (223). The overall findings of these studies indicate that adequate folate status promotes fetal growth. This is supported by the recent report of an analysis of >5 million birth records in California that showed small but significant reductions in the rates of low-birth weight and very-low-birth weight infants and preterm delivery after folic acid fortification (234).

Folate status and preterm delivery

Preterm delivery (delivery before 37 wk of gestation), a leading cause of perinatal morbidity and mortality, was examined for its possible relation to maternal folate nutrition and metabolism (50, 98, 100, 137, 139, 141, 235–238). Biological plausibility for this association centers on the theory that elevated tHcy due to poor folate status along with the presence of the C677T *MTHFR* variant leads to decidual vasculopathy, which can result in preterm delivery (235). However, as with other complications, it is difficult to conclude whether the risk of preterm delivery is related to an altered folate status. The relation between the C677T variant and the risk of preterm delivery has been tested (141, 236, 237); but a significant association was found in only one study conducted in Mexico (236). Recently, Johnson et al (238) reported that a maternal 19-base pair deletion polymorphism in intron I of the dihydrofolate reductase gene is a risk factor for preterm delivery.

FOLATE AND FETAL DEVELOPMENT

Maternal folate status and child neurodevelopment

Mental retardation is one of the clinical features of inborn errors of folate metabolism, although the mechanisms by which an altered folate metabolism causes retardation are unknown (239). Studies of the consequences of inadequate prenatal folate status on the neurodevelopment of infants and children are scarce, although prenatal folate deficiency is known to be detrimental to neurodevelopment in animals (240–243). Two studies that evaluated this connection yielded conflicting data (244, 245). This may be due to differences in the degree of maternal folate deficiency, the age of children at assessment, and the sensitivity and specificity of the assessment tools used.

Folate and Down syndrome

Cystathionine β-synthase activity is high in patients with Down syndrome (trisomy 21) because the gene encoding for cystathionine β-synthase resides on chromosome 21 (246), and this leads to increased transsulfuration and reduced plasma tHcy (247). The distribution of the C677T variant was reported to be higher in mothers of children with Down syndrome than in mothers of non-Down syndrome children, which suggests that this variant is a risk factor for Down syndrome (248, 249). The *T* allele of the C677T variant was transmitted at a higher rate to children with Down syndrome than to children without Down syndrome (250), whereas no such increase was reported in the variant in mothers of children with Down syndrome (251–254). O'Leary et al (255) reported that the frequency of the A66G variant of the methionine synthase reductase gene was higher in mothers of children with Down syndrome than in mothers of children without Down syndrome. Fillon-Emery et al (256) analyzed polymorphisms of genes including *MTHFR* (C677T and A1298C), methionine synthase (A2756G), methionine synthase reductase (A66G), and reduced-folate carrier (*RFC1*; A80G) in adults with Down syndrome and found that only the distribution of the variant in the *RFC* gene was different from that of control subjects.

Because of a possible influence of folate inadequacy on genetic expression, the effect of folic acid fortification on the chromosomal anomalies was examined. No changes in the prevalence of chromosomal abnormalities were found after fortification (257, 258), and the risk for autosomal trisomy was not affected by maternal periconceptional multivitamin use (259). The possible association of folate-dependent enzyme gene polymorphisms with the increased risk of Down syndrome is attractive. Whether these positive data withstand additional scrutiny remains to be seen.

FOLATE AND FETAL MALFORMATIONS

Folate and NTDs

In 1976, Smithells et al (5) suggested that folate deficiency was a cause of NTDs because women with an NTD infant had low blood folate; later, Smithells et al (260) reported that periconceptional vitamin supplementation, which included folic acid, reduced the recurrence of NTD pregnancies. Others also reported the same effectiveness of periconceptional supplementation with folic acid alone or in combination with multivitamins (261, 262). Although the nonrandomized nature of these trials was criticized, the apparently clear and positive findings became a powerful driving force for the Medical Research Council to launch a large-scale, randomized trial to evaluate the effect of multivitamin supplementation with and without folic acid on the recurrence of NTDs (6). Several studies evaluated the association between folate status in early pregnancy and the risk of NTDs and provided conflicting data (263–266), indicating the difficulty of identifying NTD pregnancies by a single blood folate analysis early in pregnancy. Similarly, epidemiologic studies conducted in the 1980s provided mixed results (267–269), whereas additional studies conducted in the 1990s were consistent with the effectiveness of folate suppplementation (270–272).

Periconceptional folic acid supplementation and NTD prevention

In 1991, the Medical Research Council group (6) performed a randomized daily periconceptional folic acid (4.0 mg) supplementation trial to evaluate the effect on the recurrence of infants born with NTDs in women who had a history of infants born with NTDs (high-risk population) and found that the recurrence was only 5 in 593 women who received folic acid supplements and 21 in 602 women who did not. The mean risk of recurrence was 0.28 (95% CI: 0.12, 0.71) for the women who received folic acid, which showed the benefit of folic acid given before the critical period for neural-tube closure (≈4 wk of gestation). The outcome of the trial may be the most significant for disease prevention in the folate research area, and provided support for the hypothesis put forward by Smithells (5, 260). Periconceptional folic acid supplementation is a clear departure from the prenatal supplementation that was established earlier for the prevention of folate deficiency.

After 1991, research on the mechanisms by which folic acid prevents NTDs intensified. In the 10 y before the trial (1981–1990), there were 4 articles per year on "NTDs and folic acid;" the rate increased to 67 articles per year in the next 10 y (1992–2001). The topics included the relation between the risk of NTDs and altered folate or homocysteine metabolism and polymorphisms of folate-related genes. Interest in homocysteine and polymorphisms was strong, because these coincided with the recognition of possible vasotoxicity of elevated tHcy (14) and rapid advances in molecular genetics (273, 274).

In 1992, Kirke et al (275) reported that periconceptional folic acid supplementation (0.36 mg/d) reduced the recurrence of NTDs in a small group of Irish women who had an NTD infant, which provided supporting evidence for the protective effect of folic acid. In 1992, Czeizel and Dudás (7) reported on a large-scale trial of periconceptional folic acid (0.8 mg/d) in Hungarian women without a history of NTDs (first occurrence). None of the 2394 women who received folic acid supplements had an NTD infant, whereas 6 of the 2310 women who did not receive supplementation had an NTD infant. The prevention of first NTD occurrence by periconceptional folic acid supplementation was thus established. The importance of this finding cannot be overemphasized, because most NTDs are first occurrences.

Berry et al (186) conducted a study in 2 areas of China between 1993 and 1995. Although this was not a randomized trial, the NTD occurrence rate was compared between 130 142 women who elected to receive folic acid supplements (0.4 mg/d) starting at their premarital examination until the end of the first trimester and 117 689 women who elected not to receive folic acid supplementation. Overall, 102 fetuses or infants of women who received folic acid supplementation and 173 of those who did not receive folic acid supplementation had NTDs, a significant difference. In northern China, where the prevalence of NTDs was high, folic acid supplementation reduced the rate from 4.8 to 1.0 per 1000 births (80% reduction), and in the southern region, folic acid supplementation reduced the rate from 1.0 to 0.6 per 1000 births (40% reduction). Periconceptional supplementation of a relatively low dose of folic acid reduced the risk for NTDs in areas with high and low NTD prevalence.

Against seemingly solid scientific evidence of folic acid supplementation for the prevention of NTDs, Kalter (276) cautioned that trials tend to have unavoildable methodologic uncertainties, such as subject selection and recruitment, type of supplements, and unexplained reasons for high or low NTD risk in certain populations. However, with endorsements from scientific communities, governments moved to implement policies for periconceptional folic acid supplementation and folic acid fortification of foods.

Awareness of the importance of folate intake for NTD prevention

The above studies provided firm scientific evidence of the importance of folic acid supplementation for the prevention of NTDs. Although folic acid supplementation was encouraged by prenatal health care workers, the awareness and practice of supplementation by women of childbearing age was often unsatisfactory. In the past decade, the reported rates of knowledge of the importance of adequate folate intake were 17–77% in young women worldwide (277–281). Reports by the Centers for Disease Control and Prevention indicated that the rate improved from 48% to 77% in the past decade (278, 280). Ray et al (282) reviewed 34 studies on the use of periconceptional folic acid by young women and found that the rate varied from 0.9% to 50%. The connection between awareness and practice depended on the women's socioeconomic status, education, race, location of residence, and the presence of an NTD-affected child within the family. Efforts to educate young women on the importance of high folate intake before conception should be intensified.

Results of periconceptional folic acid supplementation

The transfer of a successful intervention to community programs is not always straightforward. Not surprisingly, the prevalence of NTDs either declines or remains unchanged in areas of the world that have programs promoting folic acid supplementation (283–286). Botto et al (286) analyzed >13 million birth records from 10 countries and found no detectable reduction in the NTD prevalence between 1988 and 1998. Busby et al (287) reported that the NTD prevalence declined by only 0.9% in European countries without governmental policies on folic acid supplementation but by 17% in countries with clear policies. However, the ability to detect a reduction in NTD prevalence secondary to folic acid supplementation or fortification is hampered by a folate-independent decline in NTD prevalence in many countries (288).

Possible mechanisms for NTD prevention by folic acid

Investigations into the mechanisms underlying the prevention of NTDs by folic acid have focused on folate absorption (289, 290), abnormal one-carbon metabolism (291), and homocysteine metabolism (78–83, 292–294). Heightened efforts were also made to relate NTD risk to variants of folate-related genes, including the C677T variant of *MTHFR* (79–83, 141, 294–313), the A1298T variant of *MTHFR* (300, 306, 309, 314, 315), methionine synthase (302, 303, 305, 312, 314–317), methionine synthase reductase (311, 314, 317), *FR-α* (303, 314, 318, 319), *FR-β* (303, 319, 320), *RFC* (310, 321–323), folate conjugase (310, 323), C_1-tetrahydrofolate synthase (mitochondrial C_1-H_4folate synthase; 324, 325), dihydrofolate reductase (326), and the combination of C677T and A1298T variants of *MTHFR* (300, 306, 309). Recently, Rothenberg et al (327) reported that serum samples from 9 of 12 women with a history of NTDs contained autoantibodies against FRs, suggesting an altered folate transfer.

Researchers compared the intestinal absorption of mono- and polyglutamyl folates between mothers of an NTD child and mothers of children without NTDs (289, 290). Absorption of polyglutamyl folate was similar between case and control mothers, suggesting that decreased folate absorption is not an etiologic factor.

Altered homocysteine metabolism had been proposed as a mechanistic link between NTD prevention and folic acid supplementation. A Dutch group (78, 292) was the first to report elevated amniotic fluid tHcy in women who were carrying an NTD fetus. Since then, there have been many reports that plasma or amniotic fluid tHcy is higher in NTD infants and their mothers than in non-NTD infants and their mothers (79–83, 292–295). Although it is reasonable to conclude that plasma tHcy is generally higher in NTD infants and their mothers than in non-NTD infants and their mothers, it remains to be clarified whether abnormal homocysteine metabolism is a causal factor of NTDs.

Among the polymorphisms of folate-related genes in relation to NTDs, the prime candidate is the C677T variant of *MTHFR*; however, results from studies on that relation are conflicting (79–83, 141, 294–313). We selected 8 studies that involved >180 NTD cases to evaluate the association between the C677T variant and the risk of NTDs. Four showed no association and 4 showed a risk ratio for NTDs between 1.6 and 2.0 for those with the 677*TT* variant. The conflicting data may be confounded by factors including race, time of the study, and gene-nutrient interactions. For example, Muñoz-Moran et al (328) found an increase in the *T* allele frequency in younger subjects in Spain and hypothesized that folic acid treatment early in pregnancy resulted in the increase in the birth of infants with a *T* allele. Johanning et al (329) showed a shift of the distribution of the *T* allele in NTD fetuses by determining frequencies of fetal *MTHFR* genotypes in NTD cases and controls between 1988 and 1998. Before increased folate intake was recommended in young women in 1994, the rate of the 677*CT* variant in the cases was 51%; this rate decreased to 25% after 1994, whereas there were no changes in allele distribution in the controls. Johanning et al (329) suggested that the increase in folate intake reversed abnormal folate metabolism and prevented NTDs in ≈50% of fetuses with the 677*CT* variant who would have had NTDs. In light of these findings, it is recommended that calculations of odds ratios based on the *MTHFR* polymorphism should be made with consideration for various factors that can affect allele frequencies.

Some studies indicate an association of A1298T variants of *MTHFR* with an increased NTD risk (300, 306, 309, 314, 315). Richter et al (306) reported that the 1298*AC*/677*CT* combination was found significantly more in NTD patients than in those without NTDs, whereas others observed no such association (300, 309). However, other polymorphisms of folate-related genes have not been associated with the risk of NTDs [eg, the A2756G variant of methionine synthase (302, 303, 305, 315–317) and variants of *FR-α* and *FR-β* (314, 318–320)]. In animal studies, Hansen et al (330) showed that antisense modulation of the *FR-α* sequence increased the rate of NTDs in cultured mouse embryos, suggesting that altered expression of the gene affects NTD development. However, the application of this finding to humans remains unclear.

A limited number of studies are available for certain genotypes; thus, the statistical power may be too low to provide firm conclusions. Some studies (299, 301, 307, 309, 313, 315, 320, 325) included >200 cases, and the conclusions derived from these are likely to survive future scrutiny; however, these large studies did not necessarily provide unequivocal conclusions. Nevertheless, additional studies for certain genotypes in a large sample size are awaited. Despite the intense effort, the mechanisms of the preventive effect of folic acid on NTDs remain unknown 15 y after the Medical Research Council study.

Sites of NTDs

Neural-tube closure occurs as a sequential fusion and is controlled by various genes (331). Seller (332) found no association between failed closure sites and periconceptional folic acid supplementation, which suggests that altered folate metabolism does not control the closure sites. However, a few groups reported that the defective closure sites are associated with fetal C677T variants or amniotic fluid tHcy (82, 304, 308, 311). These data indicated a possible interaction between neural-tube closure sites and folate metabolism; additional studies with a large sample size are required to confirm the relations.

Folate and malformations other than NTDs

Because of the success in preventing NTDs with folic acid supplementation, studies were performed to associate a possible influence of folate status with other malformations. We review orofacial clefts (OFCs) and congenital heart defects in relation to folate nutrition and metabolism.

Orofacial clefts

Although the critical period for fetal lip and palate formation is at 6–12 wk of gestation, researchers speculated that orofacial structure and neurocrest closure were linked (at ≈4 wk of gestation; 333). In 1982, Tolarova (334) reported that periconceptional folic acid supplementation (10 mg/d) in women who had a child with OFCs (high-risk population) prevented its recurrence. Many researchers examined the possible connection of altered folate metabolism with OFC risk, but the findings were equivocal. Czeizel et al (335) reported that a high dose of folic acid (10 mg/d), but not a lower dose (<1.0 mg/d), given early in pregnancy prevented OFCs. However, because of safety issues, careful consideration is needed before a primary prevention trial is planned, particularly in countries where folic acid fortification of foods is practiced. Kim (336) pointed out the potential cancer-promoting effect of a large dose of folic acid supplementation, and suggested careful monitoring of the long-term effect of folic acid fortification of foods in the general population. In some epidemiologic studies, maternal periconceptional folic acid use reduced OFC risk (337–339), but Hayes et al (340) reported no such protective effect. In a study conducted in the Philippines, Munger et al (341) reported a negative correlation between plasma and erythrocyte folate concentrations and OFC risk. In studies conducted in the Netherlands, one group noted that maternal elevated tHcy was a risk factor for OFCs (342), whereas another group found that neither tHcy nor folate were associated with OFC risk (343). Ray et al (344) reported no changes in the OFC risk before (1994–1998) and after (1998–2001) the folic acid fortification of foods in Canada.

Mills et al (345) reported that the frequency of the 677*TT* variant of *MTHFR* is high in patients with OFCs in Ireland. Other researchers did not detect associations between maternal C677T or A1298T variants of *MTHFR* and OFC risk, although risk was increased with low folate intakes (346–348). Shaw et al (349) found that persons with the heterozygous variant of the *RFC-1* gene had an increased risk of OFCs when their mothers did not receive vitamin supplements early in pregnancy, suggesting the presence of a gene-nutrient interaction in the development of OFCs. A similar interaction was suggested for variants of other genes that encoded for enzymes involved in folate catabolism (ie, acetyltransferase types 1 and 2) (350). Evidence is not sufficient for conclusions as to whether folate nutrition and metabolism is causally related to OFCs.

Congenital heart defects

Congenital heart defects are among the most common causes of infant mortality, and their causes are unknown. A histologic study conducted in mice indicated that folate-deficient dams had fetuses with delayed heart morphogenesis, suggesting the importance of folate in normal heart formation (351). Several groups evaluated the association between folate nutrition and metabolism and congenital heart defects in humans. In the NTD prevention trial (7), Czeizel (352) found a significant reduction of congenital heart defects in infants whose mothers received folic acid supplementation. Survey data from the United States in the 1990s showed that multivitamin use or increased folate intake reduced the risk of the malformations by 30–60% (353, 354).

Wenstrom et al (355) reported that the C677T variant and elevated amniotic fluid tHcy were associated with an increased cardiac defect risk. However, McBride et al (356) found neither maternal C677T nor A1298C variants of *MTHFR* to be associated with the increased risk of the defects (left ventricular outflow tract malformations). Hobbs et al (357) observed increased plasma tHcy or *S*-adenosylhomocysteine and decreased *S*-adenosylmethionine and methionine in mothers of a child with cardiac malformations, suggesting abnormal transmethylation. Studies to date have used small sample sizes and, thus, statistical power for defining any association between altered folate metabolism and congenital heart defects is weak. Many types of congenital heart defects exist, and additional studies are needed to clarify this issue.

Folic acid fortification of staple foods

To reduce the prevalence of NTDs, the US Food and Drug Administration mandated in 1998 that enriched cereal-grain products should be fortified with folic acid at 140 μg/100 g product (8). In the United States, grain industries were allowed to test folic acid fortification in early 1996; thus, folate intake in the general population started to increase in 1996. Although this population-wide approach is the most effective means to improve folate status, issues on safety, economic advantages, and minimum effective fortification were discussed before the initiation of the US program (358, 359). Fortification is also practiced in Canada, Costa Rica, and Chile (9–11), and the program started in the summer of 2004 in Brazil. Shortly after the US mandate, the folate content of grain products was more than predicted (360); however, Johnston and Tamura (361) recently reported that the amount of folate in commercial breads declined after 2001.

Effect of folic acid fortification of foods on NTD prevalence

Folic acid fortification of foods resulted in a markedly increased folate intake in the United States, Canada, Costa Rica, and Chile and in a dramatic improvement in folate status as

TABLE 5
Human milk folate concentrations obtained by using the trienzyme extraction method

Study and month of lactation	Folic acid supplementation	Samples	Milk folate concentration
	μg/d	*n*	*nmol/L*
Lim et al, United States (369)			
3	None	42	206 ± 9[1]
6	None	42	186 ± 9
Mackey and Picciano, United States (370)			
3	None	21	224 ± 11
6	None	21	186 ± 11
3	1000	21	186 ± 9
6	1000	21	181 ± 11
Villalpando et al, Mexico (371)			
< 1	None	68	109 ± 32

[1] $\bar{x}$ ± SD (all such values).

assessed by blood folate or tHcy (9–11, 362, 363). Fortification programs are, however, not embraced in Europe. Despite global declines in the NTD prevalence in the past decades (288), researchers were able to detect an additional decline in the NTD prevalence in the United States after folic acid fortification of foods (364, 365). Honein et al (365) reported that the prevalence declined by 19%, a value lower than the target of 50% (8). Such a disappointingly low decline may be due to the limited validity of birth defect data from birth certificates, exclusion of elective abortions, or spontaneous abortions of NTD fetuses (365) and possibly a high unintentional use of nonenriched products (361). In Canada, however, where the level of fortification is similar (150 μg/100 g product), NTDs declined by 32–50% (9, 366), suggesting that the amount of folic acid used to fortify foods is sufficient to reduce NTDs in pregnancies as originally intended. The difference in the apparent reduction rates of NTDs between the United States and Canada is considered to be due to the validity of the Canadian registry, where all information on elective abortion or spontaneous abortion of NTD fetuses is included. In Costa Rica and Chile, where the levels of fortification are 40–180 and 220 μg folate/100 g product, respectively, NTD reductions after fortification were reported to be 35% and 40%, respectively (10, 11).

Evans et al (367) reported a 32% decline in the fraction of women with an abnormally high serum α-fetoprotein from 1997 to 2000. This protein is a biomarker for NTDs, and the decline in an abnormal value indicates a reduction in the prevalence of NTD pregnancies. Although this 32% reduction is less than the predicted 50%, it is higher than the 19% reduction in NTDs reported by Honein et al (365), suggesting unintentional elimination of aborted NTDs. Careful monitoring of the degree of fortification; folate status of the general population, particularly in women of childbearing age; and the rate of prevalence of NTD pregnancies is warranted in countries with a fortification program. Such a surveillance system is not in place in the United States.

FOLATE METABOLISM DURING LACTATION

Human milk feeding is the preferred method for infants because it provides a balance of essential nutrients and bioactive components with both short- and long-term health benefits. Breastfeeding is endorsed by health and nutrition professionals for at least the first year of life. The nutritional requirement for producing milk adequate in essential nutrients, including folate, is high (368). We review milk folate content and its relation to folate status and the requirements of mothers and infants.

Human milk folate

The reliable estimation of human milk folate and the quantity delivered to breastfed infants was accomplished with the advent of improvement in the folate assay method. Before the 1980s, investigators underestimated human milk folate content because they often used assay organisms that did not respond to all milk folates, did not use a reducing agent (such as ascorbate) during storage and assays to protect labile folates, or did not include heating or folate conjugase to properly extract folates before assay. The application of a trienzyme extraction is the latest method advance that permits a more reliable estimation than previously possible (85, 87). Folate concentrations in human milk obtained by trienzyme extraction are summarized in **Table 5**; the mean values reported ranged from 109 to 224 nmol/L (369–371).

A significant fraction of human milk folates exists as polyglutamates with ≥4 glutamyl residues (372, 373). Microbiological assays and HPLC analyes indicate that most folates exist in the reduced form and that 20–40% is 5-methyltetrahydrofolate (372–374). Because most plasma folate is in the monoglutamyl 5-methyltetrahydrofolate form, the presence of other polyglutamyl folates in milk indicates that mammary epithelial cells can interconvert folates and synthesize polyglutamates. Human milk contains folate conjugase, although the activity is only 5% that of human plasma and is not sufficient to hydrolyze endogenous polyglutamates (373). As to mammary folate metabolism, the appearance of [^{14}C]folates in milk was monitored after a dose of [^{14}C]5-methyltetrahydrofolate (375). However, the study was performed in women with breast abscesses; thus, this may not represent folate metabolism in healthy lactating women.

Milk folate is bound to folate-binding proteins (such as FR-α) that may be involved in regulating folate secretion (376). Antony et al (377) reported that the molecular weights of soluble and particulate folate-binding proteins were ≈40 kDa and 160 kDa, respectively. Two isoforms of FR-α have been identified in human milk, one with a molecular weight of 27 kDa that is a cleavage product of the other, which has a molecular weight of 100 kDa and contains a hydrophobic membrane anchor (378). A positive relation exists between human milk folate and folate-binding protein concentrations, and human milk folate binding capacity exceeds folate concentrations by ≈68 nmol/L. The excess folate-binding capacity may act to concentrate human milk folate for secretion against a concentration gradient (376). Milk folate is 5–10-fold that of maternal plasma. Additionally, the presence of protein-bound folates in milk may enhance folate bioavailability (379, 380). Additional studies are warranted to elucidate the functional role of milk folate-binding protein, in particular reference to the regulation of mammary folate secretion.

Milk folate concentrations are generally higher in hindmilk (at the end of feeding) than in foremilk (at the beginning of feeding) (374, 381), and diurnal variations in folate content (higher in the late afternoon than in the morning or early afternoon) exist (374, 381–383). Reported changes in milk folate concentrations with the progression of lactation are not consistent; some researchers found a gradual increase as lactation progresses (381, 384–387) and others found no change (388, 389). Possible reasons for the apparent differences are maternal folate status, sample collection procedures, and assay methodologies. The information on human milk folate concentrations after the folic acid fortification program should be updated.

Folate requirement in breastfed infants

Recommended folate intake in infancy is based on intakes achieved by breastfed infants with normal growth who were nursed by mothers who had adequate folate status. Under these circumstances, milk furnished between 46 and 98 μg folate/d (370, 379, 381, 390). The Recommended Dietary Intake for folate in infancy is 65 μg/d for 0–6-mo-olds and 80 μg/d for 6–12-mo-olds. Infants who were breastfed by mothers with sufficient folate status maintained plasma folate concentrations far above maternal concentrations (379, 381, 385, 390, 391), and the incidence of folate deficiency is extremely low in breastfed infants. Positive correlations exist between maternal and infant erythrocyte folate concentrations during lactation (381, 391) and between milk and infant plasma or erythrocyte folate concentrations (379, 390). Plasma folate concentrations in breastfed infants are generally 45–68 nmol/L in the first 6 mo of life and decline to 23–45 nmol/L at 12 mo when foods other than human milk contribute a sizable portion of total intake (379, 390, 391). Again, information on the folate status of breastfed infants after folic acid fortification is needed.

Folate status and requirement in lactating women

Researchers have examined maternal folate status during lactation (24–28, 379, 384, 385). Plasma folate concentrations during lactation generally decrease below those at delivery when women do not receive folic acid supplementation (19, 24, 25, 95). Bruinse et al (24) reported that total folate in the circulation declines as pregnancy progresses and remains low during lactation in nonsupplemented women (Figure 3). They also reported that serum folate concentrations were significantly lower in women who breastfed for ≥6 wk compared with those who did not breastfeed, which suggests that folate nutrition is an extra burden on lactating women (24). Smith et al (381) reported that erythrocyte folate concentrations declined from 6 to 12 wk of lactation in mothers without folic acid supplementation although serum folate concentrations remained unchanged. The findings may indicate that folate status can deteriorate during lactation if folic acid is not given. However, such conditions are likely to be rare in countries with folic acid fortification of food.

Milk folate secretion may be strictly regulated to keep an adequate folate supply for infants, as suggested by Metz et al (392). They monitored serum and milk folate concentrations and reticulocyte responses in 2 cases of lactating women with megaloblastic anemia in the course of folic acid therapy. These cases had markedly low serum and milk folate concentrations. Within 4 d of the therapy, milk folate concentrations increased appreciably; however, maternal serum folate and reticulocyte counts remained at baseline even after 10 d of therapy. These data indicate that folate was taken up by mammary epithelial cells preferentially over the hematopoietic system, suggesting a strong regulation of the mammary gland to maintain milk folate concentrations to meet the demand for infants, even by sacrificing maternal well-being.

Two studies on plasma tHcy concentrations during lactation exist. Andersson et al (71) found that plasma tHcy increased rapidly from 8 to 11 μmol/L within 6 d after delivery and that complete recovery of plasma tHcy to prepregnancy concentrations occurred within 35 wk postpartum. Mackey and Picciano (370) conducted a 3-mo double-blind trial to examine the effect of folic acid supplementation (1 mg/d) on folate status and milk folate concentrations in lactating women who consumed ≈380 μg dietary folate/d. They reported that plasma tHcy in their subjects were within normal range and slightly increased from 3 to 6 mo of lactation in both folic acid–supplemented and nonsupplemented women.

In the study by Mackey and Picciano (370), plasma and erythrocyte folate concentrations declined during the 3-mo period in nonsupplemented women. Based on the findings, they suggested that a dietary folate intake of ≈380 μg/d is not sufficient to maintain adequate folate stores in lactating women. Reported dietary folate intakes in lactating women were 205 μg/d (393) and 87–130 μg/d (394) in the United Kingdom and 169 μg/d in Navajo women (395). These data, although obtained without trienzyme extraction, indicate that overall folate intake may be lower than desirable in countries where folic acid fortification of foods is not practiced.

Willoughby and Jewell (396) reported that supplementation with 300 μg folic acid/d in addition to dietary folate intake is suitable to maintain blood folate concentrations of lactating women. In Gambia, Bates et al (25) suggested that folic acid supplementation (500 μg/d) is necessary to maintain adequate folate status in pregnancy and should be continued during lactation. They found that the erythrocyte folate concentration was sufficiently high (>560 nmol/L) in women who received supplementation of folic acid (500 μg/d) and iron (47 mg/d) until delivery. However, once supplementation was discontinued, erythrocyte folate concentrations showed a steep decline in the first 3 mo of lactation then plateaued at ≈453 nmol/L (25). The current US Recommended Dietary Intake is set at 500 DFEs/d. Information on folate status of lactating women and milk folate concentrations after the folic acid fortification mandate went in effect in several countries is not available.

FOLATE AND MALE REPRODUCTION

Relations between folate and male reproduction have been largely ignored and more research is needed (397). We summarize the available articles on this topic.

Effect of folate supplementation on male reproduction

We found 4 articles that related folic acid supplementation to fertility or seminal quality, and data are equivocal (398–401). Landau et al (398) reported that folic acid supplementation (10 mg/d) for 30 d did not change sperm quality in normo- and oligospermic men, although a 3-fold increase in seminal plasma folate concentrations was found. In contrast, Bentivoglio et al (399) showed successful treatment of infertility by 3-mo oral administration of 5-formyltetrahydrofolate (15 mg/d). Wong et al (400) reported that sperm counts increased after 26 wk of supplementation with both folic acid (5 mg/d) and zinc (66 mg/d), but not after supplementation with folic acid or zinc alone in fertile and subfertile men. The effect of supplementation was found only in subjects with the wild type variant (677*CC*) of the *MTHFR* gene (401), although the mechanism is unknown. Whether folate status influences semen quality cannot be established from available data and additional study is needed.

Folate in seminal plasma

Seminal fluid is a mixture of combined secretions of the male accessory sex glands (402). Thus, it is difficult to determine where folate in seminal plasma comes from and how much is secreted from each gland. For this reason, it is challenging to interpret data on the biochemical roles of folate in seminal plasma. Wallock et al (403) measured seminal and blood plasma folate concentrations in healthy subjects and found that total seminal plasma folate significantly correlated with blood plasma folate. Folate concentrations in seminal plasma (median: 18 nmol folate/L) were higher than in blood plasma (median: 10 nmol folate/L), and 76% of seminal plasma is 5-methyltetrahydrofolate, which was measured by differential microbiological assay (combined use of *L. rhamnosus* and *Enterococcus hirae*). The polyglutamyl chain of seminal plasma folates is <4. Folates other than 5-methyltetrahydrofolate in seminal plasma correlated significantly with sperm counts. These data suggest that seminal plasma folate reflects folate status, which may be important in male reproduction. The concentration (18 nmol folate/L) reported by Wallock et al (403) is lower than those (30–39 nmol folate/L) reported by Wong et al (400), who used a radiobinding assay. Seminal plasma contains about 26% folates other than 5-methyltetrahydrofolate, and these can provide erroneously high values in a radiobinding assay (404). The presence of high-affinity folate binding proteins with molecular weights of 100 and 25 kD were identified in semen and the prostate gland, respectively (405, 406), although the functions of these proteins are unknown. Additional research to identify the role of folate in male reproduction is warranted.

Polymorphisms of *MTHFR* and male reproduction

Bezold et al (407) reported the prevalence of the 677*CT* variant of *MTHFR* to be 19% in the infertile group and 10% in the control group. It is interesting to connect this observation with the findings by Ebisch et al (401), who reported that sperm counts of men with the wild-type *MTHFR* gene increased after folic acid and zinc supplementation, whereas those with the 677*TT* or 677*CT* variant did not respond to the supplementation. However, whether this finding represents a causal relation between infertility and the variant of this folate-related gene is unknown.

SUMMARY AND FUTURE STUDIES

Folate is now viewed not only simply as a nutrient needed to prevent megaloblastic anemia in pregnancy but also as a vitamin essential for reproductive health, disease prevention, and health maintenance. We reviewed articles that examined various reproductive outcomes in relation to folate nutrition and metabolism, including homocysteine metabolism and polymorphisms of folate-related genes. However, many studies involved small sample sizes and methodologic heterogenicity, making it difficult to draw firm conclusions. We identified the following issues that may be important for future research: *1*) the mechanistic relation of periconceptional folic acid supplementation for the prevention of NTDs; *2*) the investigation on whether folate status affects pregnancy complications, such as preeclampsia or miscarriage; *3*) the association of polymorphisms of folate-related genes with various pregnancy outcomes including birth defects; *4*) functional aspects of folate-binding proteins, particularly FR-α, for placental transfer and mammary secretion where folate must be transported against a concentration gradient (the use of folates labeled with stable isotopes may make such studies feasible); and *5*) the role of folate in male reproduction. These investigations should include sample sizes sufficiently large for statistical power and with uniform research methods. Finally, we recommend careful systematic monitoring of the consequences (benefits and possible adverse effects) of folic acid fortification of foods.

We thank Paul Coates for his encouragement during the preparation of this review and Susan Pilch for her help with our Medline search.

Both TT and MFP contributed significantly to the preparation of the manuscript and to the literature search. This work was completed while TT was on assignment as a Visiting Senior Scientist in the Office of Dietary Supplements at the National Institutes of Health. The authors did not have any conflicts of interest.

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