

# Folic acid: influence on the outcome of pregnancy<sup>1-4</sup>

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**ABSTRACT** The periconceptional use of folic acid-containing supplements reduces the first occurrence, as well as the recurrence, of neural tube defects. Women of populations in which adverse pregnancy outcomes are prevalent often consume diets that contain a low density of vitamins and minerals, including folate. Folate intake may need to be sustained after complete closure of the neural tube to decrease the risk of other poor pregnancy outcomes. A central feature of embryonic and fetal development is widespread cell division; folate is central because of its role in nucleic acid synthesis. During gestation, marginal folate nutrition can impair cellular growth and replication in the fetus or placenta. Folate deficiency can occur because dietary folate intake is low or because the metabolic requirement for folate is increased by a particular genetic defect or defects. During pregnancy, low concentrations of dietary and circulating folate are associated with increased risks of preterm delivery, infant low birth weight, and fetal growth retardation. A metabolic effect of folate deficiency is an elevation of blood homocysteine. Likewise, the presence of maternal homocysteine concentrations have been associated both with increased habitual spontaneous abortion and pregnancy complications (eg, placental abruption and preeclampsia), which increase the risk of poor pregnancy outcome and of decreased birth weight and gestation duration. *Am J Clin Nutr* 2000;71(suppl):1295S–303S.

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## INTRODUCTION

An adverse gestational event, such as a conception culminating in spontaneous abortion or stillbirth, is an unwelcome, but not an unusual, pregnancy outcome. Poor outcomes can also occur in pregnancies that give rise to live-born infants and include preterm delivery (<37 wk gestation) and intrauterine growth restriction (<10th percentile), both of which encompass infant low birth weight (<2500 g).

About 30% of conceptions are spontaneously aborted; 10–15% of conceptions terminate after the pregnancy is recognized, whereas the remainder (20%) terminate before clinical recognition of pregnancy occurs (1). In certain women, spontaneous abortion is habitual; an estimated 4% of US women have 2 pregnancy losses and an estimated 3% of US women have  $\geq 3$  pregnancy losses in a lifetime (2).

Of live-born infants, 5–15% have low birth weights. Low birth weight can be caused both by preterm delivery and by slow in utero growth with delivery at term (3). Infants may also have both conditions because intrauterine growth restriction is particularly common in preterm births.

Preterm delivery contributes substantially to the incidence of infant low birth weight. In the developed world, most low-birth-weight infants are born preterm. In the United States, approximately two-thirds of infants weighing <2500 g are delivered preterm (<37 completed wk) (3). The remaining one-third of infants are born at term but are growth restricted in utero. In the developing world, most infants weighing <2500 g are growth restricted.

Preterm delivery is the leading underlying cause of infant mortality among infants with nonlethal congenital anomalies (4). Intrauterine growth restriction also carries a risk of perinatal mortality that is >6–10 times the risk for infants with normal growth (5). In addition to perinatal and infant mortality, several other morbidities associated with intrauterine growth restriction are more common in the short run (eg, respiratory distress syndrome, bronchopulmonary dysplasia, intraventricular hemorrhage, necrotizing enterocolitis, and hypoglycemia). In the long run, several developmental disorders associated with intrauterine growth restriction can emerge, including learning disabilities, childhood psychiatric disorders, mental retardation, and other disruptions of child growth and development (4, 5).

Adverse pregnancy outcomes are 2- to 3-fold more common among infants born to poor urban women from countries such as the United States (3). Other factors that can affect pregnancy outcome include inadequate gestational gain, inadequate prenatal care, smoking, drinking, and substance abuse (6, 7). A history of spontaneous abortion, preterm delivery, low birth weight, or intrauterine growth restriction in previous pregnancies can increase risk in the current pregnancy (7). A low intake of micronutrients

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and of vitamins such as folate also may increase risk of adverse pregnancy outcome (6, 8).

### FOLATE INTAKE

Humans are entirely dependent on dietary sources or dietary supplements for their folate supply. A significant proportion of women of reproductive age have low dietary folate intake and do not use folic acid-containing supplements or eat fortified cereals.

Subar et al (9) examined representative data from the second National Health and Nutrition Examination Survey (NHANES II) and found that the estimated mean folate intake of women surveyed ( $207 \pm 2.9 \mu\text{g/d}$ ) was approximately equivalent to the recommended dietary allowance (RDA) for the nonpregnant state ( $180 \mu\text{g/d}$ ). Approximately 90% of the women consumed  $<400 \mu\text{g}$  folate/d (the RDA for pregnancy) and only  $\approx 10\%$  of the women met the pregnancy RDA. More black (26%) than white (18%) women had very low folate intakes ( $\leq 100 \mu\text{g/d}$ ), potentially accounting for the consistently lower average folate intake reported for minority women ( $175\text{--}185 \mu\text{g/d}$ ). Despite this, there was little or no ethnic difference when daily intake of folate from the diet exceeded  $100 \mu\text{g/d}$ .

Block and Abrams (10) also examined data from NHANES II along with data from the Continuing Survey of Food Intakes by Individuals; they found that women who were near poverty or below poverty had intakes of folate and other nutrients (eg, iron, zinc, and vitamins A, C, and B-6) that were below the current RDA for nonpregnant women ( $3 \mu\text{g}$  folate/kg). One-third of low-income women ( $<131\%$  of poverty limit) and half of those women with higher incomes ( $>300\%$  of poverty limit) met the folate RDA for nonpregnant women. Folate intakes below the RDA were associated with infrequent consumption of folate-rich foods. Low-income women in particular ate few vegetables; about half of the women ate no vegetables at all, including potatoes, when surveyed over 4 nonsuccessive days.

The major food sources of folate include cooked dried beans, leafy green vegetables, and fortified cereals (9). Other foods of lower folate density are also important contributors of folate to the diet of US women because such foods are eaten frequently (eg, orange juice and white bread). Multivitamin tablets are an additional source of folate that is used by many people. Orange juice is the largest single source of folate consumed by the American public, contributing  $\approx 10\%$  of dietary folate, with white bread, dried beans, salad, and cold cereal contributing a cumulative one-third of folate from diet. One might presume that the use of vitamin and mineral supplements containing folic acid would offset the risk of low folate intake, particularly because the folic acid contained in supplements (eg, monoglutamate) has greater bioavailability than does the folate in food (eg, polyglutamate). However, supplements appear to be used the least by those individuals who need them the most.

In the United States, NHANES I data suggest limited supplement use by women of reproductive age; overall, about one-quarter of women (26% of whites, 15.5% of blacks) report regularly taking vitamin or mineral supplements (11). Suitor and Gardner (12) examined supplement use before and during pregnancy in low-income Massachusetts gravidas. About 16% of the women took vitamins before pregnancy, but this varied by ethnicity; white gravidas (23%) reported supplement use about twice as often as blacks (11%) or Hispanics (10%). During pregnancy, supplement use varied by ethnic group: 9% of whites, 20% of

blacks, and 13% of Hispanics used prenatal vitamins erratically (1–3 times/wk) or not at all. Reasons for noncompliance included maternal confidence that her diet was good, an unstable home life, or side effects attributed to the supplement by the women.

In Camden, NJ, 17% of low-income minority gravidas reported using supplements before they became pregnant (13). Preconceptional use of supplements was significantly more likely to have occurred among women with a history of adverse pregnancy outcomes, principally spontaneous abortion in past pregnancies, and was associated with increased spotting and bleeding during the current pregnancy (13). Thus, low-income women appeared to use supplements when they previously had, or currently anticipated, a problem with their pregnancies. On the basis of data from the Maternal and Infant Health Survey, other predictors of failure to use vitamin or mineral supplements before or during pregnancy include being black, unmarried, or  $<20$  y of age or having less than a high school education (14). In Camden, multiparity and late entry to prenatal care were additional factors associated with the nonuse of supplements (13).

Factors associated with improved folate status (measured as red cell folate) include use of fortified cereals in addition to the use of folic acid-containing supplements. This was shown by Cuskelly et al (15), who randomly assigned 62 women to 1 of 5 treatments, 3 of which provided an extra  $400 \mu\text{g}$  folate/d in the form of folic acid supplements, folic acid-fortified foods, and the incorporation of additional folate-rich foods in the diet. The remaining treatments included dietary advice and a control (ie, no intervention). The intervention treatments significantly raised the folate intake of the women. Even the group receiving dietary advice alone increased their intake by nearly  $100 \mu\text{g}$  folate/d. However, only women taking folic acid supplements or fortified foods had significantly increased red cell folate after 3 mo. Likewise, predictors of red cell folate among Minnesota women attempting pregnancy included use of folic acid-containing nutritional supplements and fortified cereals (16). Interestingly, there was a substantial ethnic difference in use of folic acid-fortified cereals, ranking 9th as a source of dietary folate among whites, but ranking 49th among black women (9).

Behavioral factors such as smoking cigarettes, using alcohol, or using oral contraceptives are also associated with poor folate status (17). Pregnant women living under circumstances in which preterm delivery and low birth weight are prevalent have diets with a low density of minerals and vitamins, including folate, and they also limit the use of folic acid-containing supplements (18). Low circulating concentrations of folate also have been reported in minority women (19). Thus, there exists an association of gestational problems with the population subgroup of low-income women who have diets low in folate before pregnancy.

### FOLATE METABOLISM

Folate is critically important for fetal development. Once absorbed, folate acts as a cofactor for many essential cellular reactions including the transfer of single-carbon units; it is required for cell division because of its role in DNA synthesis (20, 21). Folate is also a substrate for a variety of reactions that affect the metabolism of several amino acids, including the transmethylation and transsulfuration pathways.

Interference with DNA synthesis gives rise to abnormal cell division. Rapidly dividing cells, such as those in the hematopoietic

system, are the most susceptible to irregularities in DNA production. Thus, one of the first clinical manifestations of folate deficiency is hypersegmentation of the neutrophils, followed later by the production of megaloblastic marrow cells, macrocytic red cells, and ultimately macrocytic anemia. Abnormalities in the division of epithelial cells and gonadal cells follow next in this progression (22).

A central feature of fetal development is widespread and sustained cell division. As a result of its role in nucleic acid synthesis, the need for folate increases during times of rapid tissue growth. During pregnancy, folate-dependent processes include an increase in red cell mass, enlargement of the uterus, and growth of the placenta and fetus.

Serum folate is a sensitive indicator of the folate available to replicating cells with high turnover rates, whereas red cell folate reflects folate status over preceding weeks. A metabolic effect of folate deficiency is an elevation of homocysteine (23). Hyperhomocysteinemia can occur as a result of folate deficiency when dietary folate intake is low. In other cases, genetic factors and the interaction between genes and the environment increase the metabolic requirement for folate.

Moderate to severe elevations in plasma homocysteine resulting from genetic defects in the production of the enzymes cystathionine  $\beta$ -synthase, 5,10-methylenetetrahydrofolate reductase, or methionine synthase [*O*-acetylhomoserine (thiol)-lyase] are rare in the population (23). A specific folate gene coding for the heat labile methylenetetrahydrofolate reductase (MTHFR) enzyme has been found to be common and also to have a harmful effect during pregnancy. The C-to-T substitution at nucleotide 677 (C677T) in the MTHFR gene has an allele frequency of  $>0.3$ ;  $\approx 9$ –10% of individuals are homozygous (24). Some studies suggest that in the presence of reduced folate intake, homozygotes have elevated blood homocysteine (25, 26). This mutation is believed to contribute to fetal nervous system malformation and spina bifida cystica (27, 28), and may affect other aspects of fetal development as well.

Molloy et al (29) suggested that common genetic polymorphisms, such as the thermolabile MTHFR mutation, may be responsible for the altered folate states that are widespread throughout the population. In their study, red cell folate was significantly lower among pregnant and nonpregnant women who were homozygous for C677T than in control gravidas without this mutation. Plasma folate was lower only when homozygotes were pregnant and their requirement for folate increased.

It has been hypothesized that maternal or fetal heterozygosity or homozygosity for MTHFR and other possible genetic polymorphisms affecting folate metabolism may influence common outcomes such as birth weight and gestation duration (30). If, for example, a gravida homozygous for the C677T mutation had a periconceptual folate intake sufficient to avoid a neural tube defect in her fetus, we might ponder the question of whether extra folate intake would need to be sustained throughout pregnancy. Would this minimize the risk of spontaneous abortion and, passing that benchmark, allow sufficient nucleic acid synthesis for cellular replication in the fetus and placenta? If the mother carried a fetus who was also homozygous for the C677T mutation, would maternal folate intake need to be even higher to support fetal growth and gestation? Mutation heterozygotes are more prevalent (42%) than homozygotes (9–10%) and might be affected as well. Mutation heterozygotes might also sustain an

increased risk of infant low birth weight or more modest birth weight reductions depending, in part, on how much extra folate was required to re-methylate methionine.

An estimated one-fourth of neural tube defects (NTDs) are attributable to the thermolabile C677T mutation, a fraction far below the 70% reduction in NTDs associated with folic acid supplementation (31). The protective effects of folate must therefore involve other environmental factors or gene-environment interactions as well. Several other functional polymorphic alleles of folate genes are common in the population. Their potential effects on pregnancy outcome are unknown. For example, a dihydrofolate reductase (DHFR) mutation of small effect would be perfectly poised to disrupt the flow of folate into body metabolism from the major environmental source—the diet. About half of dietary folate is in a reduced form and 100% of the folic acid contained in multivitamin and mineral supplements is unreduced. Unreduced folate requires the action of DHFR before it can be used in cellular reactions. The DHFR gene, synthesizing the DHFR enzyme, acts at the threshold where environmental folate enters the human metabolism.

Thus, during gestation, marginal folate nutrition alone or in combination with polymorphic alleles of folate genes can impair cellular growth and replication in the fetus or placenta. This could, in turn, increase the risk of spontaneous abortion, preterm delivery, or intrauterine growth restriction (6). A more abundant supply of folate to mother and fetus could, in turn, support growth and gestation leading to improved infant birth weight and increased gestation duration.

## FOLATE AND PREGNANCY OUTCOME

One of the first researchers to address the importance of folate during pregnancy was Brian Hibbard (32), who suggested that the detection of megaloblastic anemia in mid or late pregnancy implied an antecedent defect in folate metabolism. He hypothesized the existence of absolute and relative folate deficiencies during pregnancy. Absolute folate deficiency stemmed from too little folate in the diet—a supply and demand problem. In contrast, a relative folate deficiency was said to arise from a metabolic defect in the utilization of dietary folate that was manifested early in gestation under the stress of pregnancy. Hibbard went on to suggest that most fetal misfortunes occurred in women with early disturbances in folate metabolism (32, 33). Apart from NTDs, in which the nutrient-gene interaction to which Hibbard alluded has been identified, other effects of folate (ie, intake and metabolism) during pregnancy have been documented somewhat but not yet sorted out.

Hibbard (32) studied the folate status of 1484 low-income obstetric patients from Liverpool, United Kingdom, by use of an indirect method, the excretion of formiminoglutamic acid (FIGLU). In folate-deficient individuals, the conversion of histidine to glutamic acid is inhibited and the excretion of FIGLU into the urine is increased, particularly after histidine loading. A bone marrow biopsy was also obtained from a subsample of gravidas. Approximately 10% of gravidas had abnormal FIGLU excretion and 5% had megaloblastic marrow.

Abnormal FIGLU excretion was more frequent in young gravidas ( $\leq 20$  y) and in women with multiple pregnancies (eg, twins or triplets); this measure also increased with gravidity. Among grand multigravidas ( $> 5$  pregnancies), abnormal FIGLU excretion increased

3-fold. During the index pregnancy, placental abruption was 4-fold more likely and spontaneous abortion was 5-fold more likely, but the number of congenital defects was not significantly greater in gravidas with abnormal FIGLU excretion than expected rates. Adverse outcomes in prior pregnancies were significantly higher in women with abnormal FIGLU during the current pregnancy; these adverse outcomes included infant low birth weight, antepartum hemorrhage, fetal congenital defects, and perinatal mortality. Hibbard concluded that in most instances, folate deficiency during pregnancy resulted from an absolute deficiency—a diet inadequate to meet the needs of pregnancy. In other instances, a relative folate deficiency existed whereby a poor diet exacerbated an underlying defect in folate metabolism. His observations suggested that a relative folate deficiency was particularly closely associated with 2 outcomes: placental abruption and recurrent spontaneous abortion (33).

### SPONTANEOUS ABORTION AND FETAL DEMISE

The frequency of NTDs among spontaneously aborted fetuses is 10-fold higher than is the rate of NTDs at birth (34). Furthermore, there is a strong positive relation ( $r = 0.96$ ) between rates of NTDs in pregnancies coming to term and rates of NTDs among pregnancies terminating in spontaneous abortion (34). A rat model of folate deficiency suggests that severe gestational folate deficiency increases the risk of fetal demise whereas moderate folate deficiency does not, instead decreasing DNA synthesis and reducing litter size and weight (35).

Homocysteine, a sulfur amino acid and a byproduct of methionine metabolism, reflects an inadequate folate intake or abnormal folate metabolism. Homocysteine concentrations are significantly higher among women who have given birth to offspring with NTDs (36, 37). Likewise, about one-half of the pregnancies occurring in women with hereditary homocystinuria terminate in fetal demise (38). Thus, although circulating concentrations of folate bear little relation to risk of spontaneous abortion (39–42), high concentrations of homocysteine may be a marker for increased risk to the developing fetus.

Wouters et al (43) studied homocysteine at baseline and after oral methionine loading. There was no difference in serum or red cell folate concentrations, but there were statistically significant and clinically important differences in homocysteine concentrations (fasting and postload) for habitually aborting women. More than 20% of habitually aborting case subjects were hyperhomocysteinemic compared with an estimated 7% of control subjects. Other reports (**Table 1**) have suggested that women with a history of habitual spontaneous abortion, but not necessarily a single spontaneous abortion, have higher concentrations of homocysteine (44–45).

Thus, one might wonder about the efficacy of folate supplementation for reducing the risk of spontaneous abortion. In Hook and Czeizel's trial (46), periconceptional supplementation with folic acid-containing vitamins significantly increased the rate of diagnosed pregnancies without increasing that of achieved births. Rather, a slight but statistically significant increase (16%) in the risk of spontaneous abortion was found among women supplemented with vitamins containing folic acid (46). Hook and Czeizel hypothesized that folic acid-containing vitamins decreased the risk of NTDs by the spontaneous abortion of affected fetuses. Alternatively, it is possible that supplementation with folic acid-containing vitamins permitted pregnancies that would have otherwise been eliminated before they were clinically

recognized to persist until they became detectable. Hook and Czeizel's hypothesis has not been tested empirically—spontaneous abortions were not examined for NTD or any other anomaly. Consequently, the role of folate and folic acid supplementation in spontaneous abortion remains an open question.

### PREGNANCY COMPLICATIONS

More than 3 decades ago, Hibbard (32) described an increased risk of placental abruption in gravidas with abnormal FIGLU excretion and attributed it to a defect in folate metabolism. Other researchers showed that a genetic polymorphism affecting folate metabolism—the heat-labile C677T mutation—was associated with a >2-fold increase in the risk of preeclampsia among Japanese gravidas who were homozygous for the mutation (47).

Additionally, hyperhomocysteinemia, a marker for folate deficiency or metabolic abnormality, has been associated with serious complications such as pregnancy-induced hypertension, preeclampsia, and placental abruption (48–52) (**Table 1**). All are risk factors for intrauterine growth restriction and preterm delivery, in particular, preterm delivery that is medically indicated.

Currently, many published studies of homocysteine status and pregnancy are uncontrolled or fail to adjust for potential confounders. For example, Leeda et al (53) tested 207 patients with a history of preeclampsia or fetal growth restriction for hyperhomocysteinemia. Those patients testing positive (17%) were supplemented with folic acid and vitamin B-6. Fourteen women subsequently experienced another pregnancy. On average, infant birth weight increased by nearly 1800 g and gestation duration increased by 7.2 wk when first and subsequent pregnancies were compared. Nonetheless, this study had no control group of untreated women. Consequently, it is uncertain whether these results are an effect of supplementation with folic acid and vitamin B-6 or whether they reflect confounding by parity—ie, that preeclampsia is less frequent and infant birth weight increases after a first pregnancy.

### BIRTH WEIGHT AND GESTATION

The role of folate in DNA synthesis and cell replication suggests that folate can influence fetal growth and gestation duration. Folate deficiency also interferes with growth of the conceptus, maternal erythropoiesis, growth of the uterus and mammary gland, and growth of the placenta (6).

The influence of dietary and circulating folate on preterm delivery and infant low birth weight was studied in 832 women from the Camden Study (8). Low intakes of folate from diet and supplements were associated with maternal characteristics reflecting poor nutritional status, including low energy intake, low rate of gestational weight gain, and a high frequency of iron deficiency anemia at entry to prenatal care. After the period of gestation at entry and the time during pregnancy when the samples were drawn were controlled for, there was a significant relation between dietary folate intake and serum folate at week 28 ( $r = 0.17$ ). Low folate intake (<240 ng folate/d) was associated with a >3-fold increase in risk of infant low birth weight and of preterm delivery, after maternal age, parity, ethnicity, smoking, gestational weight gain, and intake of energy and other nutrients (zinc, fiber, and vitamin B-12) were controlled for. Circulating folate at week 28 was also associated with risk; the adjusted odds ratio for low birth weight increased by 1.5% and the odds ratio for preterm delivery increased by 1.6% per unit (nmol/L) for

**TABLE 1**Summary of studies of maternal folate, folic acid, and homocysteine<sup>†</sup>

Author	Study design	Results
Scholl et al (8)	Prospective, observational study of folate from diet and supplements, and serum folate ( <i>n</i> = 832)	Women with low folate intake ( $\leq 240$ $\mu\text{g}/\text{d}$ ) had >3 times greater preterm delivery and infant low birth weight than women with folate intake >240 $\mu\text{g}/\text{d}$ ( $P < 0.05$ ). Risk of preterm delivery without premature rupture of membranes increased 3 times ( $P < 0.05$ ). Odds of preterm delivery increased 1.5% per unit decrease in serum folate ( $P < 0.05$ ).
Pietzrik et al (39)	Case-control study of serum folate in women with first-trimester spontaneous abortion ( <i>n</i> = 37) or habitual spontaneous abortions ( <i>n</i> = 46) compared with parous controls ( <i>n</i> = 116)	Low concentrations of serum folate found in habitually aborting gravidas than in controls ( $-3.1$ $\text{ng}/\text{mL}$ ; $P < 0.001$ ) and in first-trimester aborters than in controls ( $-2.3$ $\text{ng}/\text{mL}$ ; $P < 0.05$ ). Timing of comparison (pregnancy, postpartum) not reported.
Neiger et al (40)	Uncontrolled study of serum folate and spontaneous abortion in women with first-trimester vaginal bleeding ( <i>n</i> = 151)	No significant difference in spontaneous abortion for women with low (<4 $\text{ng}/\text{mL}$ ) or high serum folate.
Neela et al (41)	Case-control study of circulating folate in women with spontaneous abortion ( <i>n</i> = 115) and still-pregnant matched control subjects ( <i>n</i> = 115)	No significant difference in folate (RBC or whole blood) or other nutrients between cases and controls. Difference in timing of blood draw (pregnancy for controls, postpartum for cases) a confounder.
Sutterlin et al (42)	Case-control study of serum folate in 29 nonpregnant women with habitual spontaneous abortion (>3) and 29 control subjects	No significant difference in serum folate between cases and controls.
Wouters et al (43)	Case-control study of homocysteine (fasting and postload) in nonpregnant women with $\geq 2$ consecutive fetal losses ( <i>n</i> = 102) and in control subjects (parous women, no fetal loss) ( <i>n</i> = 41)	No significant difference in vitamin B-12, serum folate, or RBC folate. Cases had higher ( $P < 0.05$ ) fasting homocysteine (3 $\mu\text{mol}/\text{L}$ ), postload homocysteine (11 $\mu\text{mol}/\text{L}$ ), and the ratio of PLP to B6 (5 $\text{nmol}/\text{L}$ ) than controls.
Stegers-Theunissen et al (44)	Case-control study of homocysteine (fasting and postload) in nonpregnant women ( <i>n</i> = 24) with habitual spontaneous abortion ( $\geq 2$ ) or placental abruption and control subjects ( <i>n</i> = 15), day 21 of ovulatory cycle	25% of cases were hyperhomocysteinemic after the methionine load; fasting homocysteine, serum folate, and vitamin B-12 did not differ significantly between groups.
Quere et al (45)	Case series of 100 nulliparous women with habitual spontaneous abortion ( $\geq 3$ consecutive episodes)	12% of cases were hyperhomocysteinemic.
Hook and Czeizel (46)	Randomized controlled trial of folic acid-containing multivitamins (0.8 $\text{mg}/\text{d}$ ) ( <i>n</i> = 2787) or trace minerals ( <i>n</i> = 2653) in periconceptional period	Folic acid-supplemented group had more ( $P < 0.05$ ) diagnosed pregnancies (70.5% folic acid compared with 67.1% trace mineral) and spontaneous abortions (13.0% folic acid compared with 11.2% trace mineral).
Sohda et al (47)	Case-control study of C677T mutation in preeclamptic gravidas ( <i>n</i> = 68) and controls (98 pregnant and 260 normotensive population controls)	Preeclamptic gravidas were 2.5 times more likely than controls to be C677T homozygotes (OR = 2.5; 95% CI: 1.3, 4.8). C677T allele increased by 60% (OR = 1.6; 95% CI: 1.1, 2.3) among cases.
deVries et al (48)	Uncontrolled study of postload homocysteine in nonhypertensive women ( <i>n</i> = 62) with history of placental abruption, fetal demise, or intrauterine growth restriction during pregnancy	Hyperhomocysteinemia prevalence was 24% in cases compared with 2–3% expected for Dutch population.
Goddijn-Wessel et al (49)	Case-control study of nonpregnant women with history of placental infarction or abruption ( <i>n</i> = 84) and control subjects ( <i>n</i> = 46)	Fasting (2 $\mu\text{mol}/\text{L}$ ) and postload (8 $\mu\text{mol}/\text{L}$ ) homocysteine higher in cases than in controls ( $P < 0.05$ ). Serum folate ( $-2$ $\text{nmol}/\text{L}$ ), vitamin B-12 ( $-40$ $\text{pmol}/\text{L}$ ), and the ratio of PLP to B6 ( $-11$ $\text{nmol}/\text{L}$ ), lower in cases than in controls ( $P < 0.05$ ). RBC folate not significantly different.
Dekker et al (50)	Case series of women with early-onset preeclampsia ( <i>n</i> = 101) given a methionine load at 10 wk postpartum	Prevalence of postload hyperhomocysteinemia was 17.1%.
Rajkovic et al (51)	Case-control study of fasting homocysteine in preeclamptic gravidas ( <i>n</i> = 20) and controls ( <i>n</i> = 20) at delivery	Cases had higher plasma homocysteine (3.7 $\mu\text{mol}/\text{L}$ ) and delivered earlier than controls ( $35 \pm 4$ compared with $40 \pm 1$ wk) ( $P < 0.05$ for each).
Leeda et al (53)	Case series in which cases (history of preeclampsia or fetal growth restriction) were evaluated for hyperhomocysteinemia (methionine load) at 10 wk postpartum ( <i>n</i> = 207)	17.7% of preeclamptic women ( <i>n</i> = 181) and 19.2% of women with a history of fetal growth retardation ( <i>n</i> = 26) tested positive for hyperhomocysteinemia. In a subsequent pregnancy ( <i>n</i> = 14) with supplementation (folic acid, vitamin B6, and aspirin), birth weight increased from $1088 \pm 570$ to $2867 \pm 648$ g and gestation length increased from $29.5 \pm 3.7$ to $36.7 \pm 2.2$ wk. The proportion with preeclampsia decreased from 78.6% to 50% (no statistical testing).

(Continued)

TABLE 1 (Continued)

Author	Study design	Results
Tamura et al (54, 56), Goldenberg et al (55), Negggers et al (57)	Prospective observational study of dietary and serum folate in women with risk factors for intrauterine growth restriction ( $n = 1200$ ); most analyses involved a subsample ( $n = 289$ ) for whom dietary and serum folate were assayed	Folate from diet and supplements correlated with serum folate ( $r = 0.25$ ) at weeks 18 and 30. Higher serum folate at week 30 predicted higher infant birth weight (2.1 g birth weight per unit serum folate; $P < 0.05$ ) and decreased intrauterine growth restriction ( $P < 0.05$ ). Infant birth weight (47.6 g) for mothers whose dietary folate intake was above the 90th percentile was significantly higher ( $P < 0.05$ ) than that for mothers whose dietary folate intake was below the 10th percentile.
Frelut et al (58)	Case-control study of red cell folate in gravidas with ( $n = 8$ ) and without ( $n = 13$ ) fetal growth retardation diagnosed at $27 \pm 3$ wk gestation	Positive bivariate correlation (week 32) between maternal RBC folate and infant birth weight ( $r = 0.48$ , $P < 0.02$ ).
Ek (59)	Observational data at delivery ( $n = 139$ )	Positive correlation between maternal RBC folate at delivery and birth weight ( $r = 0.18$ , $P < 0.05$ ) and birth length ( $r = 0.22$ , $P < 0.01$ ).
Malinow et al (60)	Observational study of plasma homocysteine and serum folate in healthy nulliparas at delivery (37–42 wk) ( $n = 35$ )	High maternal homocysteine correlated negatively with low infant birth weight ( $r = -0.36$ , $P < 0.05$ ) and short gestation duration ( $r = -0.42$ , $P < 0.05$ ). High maternal serum folate correlated positively with increased birth weight ( $r = 0.47$ , $P < 0.01$ ) and gestation duration ( $r = 0.23$ , $P < 0.05$ ). Homocysteine and serum folate were negatively correlated ( $r = -0.54$ , $P < 0.001$ ).
Burke et al (61)	Case-control study of fasting homocysteine (at delivery) in cases ( $n = 37$ ) with growth-restricted infants ( $n = 37$ ) and controls ( $n = 35$ )	No significant difference in fasting homocysteine between cases and controls.
Whiteside et al (62)	Prospective, observational data from gravidas who entered care at $\leq 12$ wk gestation ( $n = 60$ )	Shorter gestation duration ( $< 39$ wk) associated with lower serum folate at week 26 ( $-1.9$ ng/mL, $P < 0.05$ ).
Tchernia et al (63)	Observational study of serum folate in women using iron, or iron and vitamin C supplements ( $n = 100$ )	Serum folate lower by 1.1 $\mu\text{g/L}$ in women delivering at $\leq 39$ wk ( $P < 0.01$ ).
Tchernia et al (63)	Observational study of RBC folate in high-risk women ( $n = 100$ )	Gestation duration reduced by 0.8 wk in women with RBC folate $\leq 200$ $\mu\text{g/L}$ ( $P < 0.025$ ).
Tchernia et al (63)	Supplementation trial (open) of iron compared with iron and folate (350 $\mu\text{g/d}$ ) ( $n = 108$ )	Gestation duration increased by 0.8 wk among iron- and folate-supplemented women ( $P < 0.001$ ).
Rondo et al (64)	Case-control study of fetal growth retardation ( $n = 356$ )	More growth-restricted infants (25.7%) than control infants (19.9%; $P < 0.01$ ) had abnormally low RBC folate in cord blood. No significant difference in maternal RBC folate (at delivery).
Martin et al (65)	Prospective, observational study of serum folate at first antenatal visit ( $n = 808$ )	Bacteriuria ( $> 100000$ organisms/mL) associated with lower serum folate at week 28 ( $P < 0.05$ ). In the presence of low serum folate, bacteriuria was associated with a 2-fold increase in prematurity (14.7% compared with 7.0%) (no statistical testing).
Fleming et al (66)	Randomized controlled study of iron (60 mg/d) with or without a low (0.5 mg/d) or high (5 mg/d) dose of folic acid or low (0.5 mg/d) folic acid alone compared to placebo ( $n = 146$ )	No significant differences in placental weight, birth weight, or gestation duration among groups.
Fletcher et al (67)	Double-blind, randomized study of folic acid plus iron (5 mg/d) or of iron alone (200 mg/d) ( $n = 643$ )	No significant difference in gestation duration, birth weight, or placental weight between groups.
Giles et al (68)	Randomized study of folic acid (5 mg/d) or iron supplementation ( $n = 692$ ) with patients stratified by gestation at entry to care: $< 10$ wk, 10–20 wk, 20–30 wk, or $> 30$ wk	No significant difference in gestation duration or birth weight between groups.
Baumslag et al (69)	Randomized controlled study of iron alone (200 mg) or in combination with folic acid (5 mg/d) or with folic acid and vitamin B-12 (50 $\mu\text{g/d}$ )	No effect of the folic acid among white women studied. Among Bantu women, risk of bearing an infant $< 2.25$ kg ( $< 5$ lb) reduced 4-fold in the folate groups compared with the iron alone group (6.2% compared with 30%, $P < 0.001$ ).
Iyengar et al (70)	Nonrandomized trial of iron alone (60 mg/d) or in combination with folic acid (500 $\mu\text{g/d}$ ) ( $n = 189$ )	Folic acid group had significantly ( $P < 0.001$ ) higher infant birth weight (200 g) and placental weight (61g).
Rolschau et al (71)	Paired trial (matched by age, parity, smoking, pregravid weight, and housing conditions) with allocation (method not stated) to iron (200 mg Fe) or multivitamins with and without folic acid (5 mg)	Among folic acid-supplemented subjects, infant birth weight was greater by $\geq 400$ g ( $P < 0.01$ ) and placental weight was greater by $\approx 50$ g (NS).
Blot et al (72)	Nonrandomized, double-blind study of iron and ascorbic acid compared with iron, ascorbic acid, and folic acid (350 $\mu\text{g/d}$ ) at 6 mo gestation ( $n = 200$ )	Gestation duration was longer by 0.8 wk with folic acid supplementation, birth weight greater by 158 g, birth length greater by 1.7 cm, and placental weight greater by 56 g ( $P < 0.05$ for each).

(Continued)

TABLE 1 (Continued)

Author	Study design	Results
Czeizel et al (73–75)	Randomized controlled trial of periconceptional supplementation with folic acid–containing multivitamins	The folic acid group had more multiple pregnancies (3.8% compared with 2.7%, $P < 0.05$ ) and girls (50.1% compared with 48.1%) than did the trace mineral group but the difference was not significant ( $P = 0.18$ ). In analysis of all births, there was a significant excess of low-birth-weight infants among folic acid–supplemented subjects (5.8% compared with the trace mineral group (4.2%) ( $P < 0.05$ ). Among singleton pregnancies, rate of low birth weight was 4.3% (folic acid group) and 3.5% (trace mineral group) ( $P = 0.17$ ).

<sup>1</sup> PLP, pyridoxal 5' phosphate; OR, odds ratio; RBC, red blood cells; B6, vitamin B6. To convert serum folate in ng/mL (or µg/L) to nmol/L, multiply by 2.266.

each unit decrease in serum folate at week 28. Thus, lower concentrations of serum folate at week 28 were also associated with a greater risk of preterm delivery and low birth weight.

Many observational studies of folate during pregnancy suggest a potential benefit of good folate status—an improvement in birth weight and gestation (54–65) (Table 1). Unlike observational studies, randomized trials of folic acid supplementation have shown less uniform benefit (66–75) (Table 1). Although the results of many randomized trials were positive, they imply that routine supplementation may not benefit all pregnant women. Some who are potentially at risk—from common genetic polymorphisms that alter folate metabolism or from environmental factors associated with folate—may benefit the most through an improved diet.


For example, Baumslag et al (69) administered iron alone (200 mg) or in combination with folic acid (5 mg/d) to South African women. There was no effect of the folate among the white South Africans who were studied. However, among Bantu women, who subsist primarily on maize porridge, mean birth weight was increased by nearly 0.45 kg (1 lb) and the risk of bearing an infant weighing <2.25 kg (<5 lb) was reduced 4-fold with folic acid supplementation.

Not all supplementation trials yield a benign result. Czeizel et al (73–75) reported the effect of periconceptional supplementation (>28 d before conception to second missed menstruation) with folic acid–containing multivitamins (0.8 mg folic acid) or trace minerals. After the randomized periconceptual period, ≈60% of women in each group received multivitamins with folic acid or received folic acid alone as part of routine prenatal care. Thus, only the effect of periconceptional folic acid supplementation was addressed. In addition to increasing the number of recognized conceptions, supplementation with folic acid–containing multivitamins significantly increased the rate of multiple births (74). The number of female births was marginally greater among the folic acid–supplemented women ( $P = 0.18$ ). In an analysis that included both singleton and multiple births, there was a small but statistically significant excess of low-birth-weight infants in the folic acid–supplemented group than in the trace mineral group (75).

The increased risk of fetal growth restriction and preterm delivery among multiple pregnancies is well known. When the analysis was confined to singletons, the excess of low-birth-weight infants persisted among the folic acid group but was no longer statistically significant (73, 75). Czeizel suggested that periconceptional folic acid supplementation improved fertility (higher rate of conceptions and multiple births) and shifted the sex ratio slightly, thus increasing the risk of low birth weight because females have lower birth weights than do males.

## IMPLICATIONS

In summary, both types of studies—observational and supplemental—suggest that poor dietary folate intake and low circulating concentrations of folate are associated with an increased risk of adverse birth outcomes. Supplementation studies likewise suggest that some women—most likely poor women—may benefit from receiving additional folic acid during, as well as before, pregnancy. Some negative effects have been reported in association with periconceptional supplementation with folic acid–containing vitamins, including potential increases in spontaneous abortion and infant low birth weight. These risks may be more apparent than they are real, occurring in association with increased fertility, survivorship of marginal conceptions, or a small shift in sex ratio.

Likewise, high concentrations of homocysteine have been associated with increased habitual spontaneous abortion and serious complications of pregnancy, including pregnancy-induced hypertension, preeclampsia, and placental abruption. Currently, there are no data from well-controlled studies to document the security of these observations or from clinical trials to determine whether supplementation with folic acid and B vitamins reduces the risk associated with maternal hyperhomocysteinemia. 

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