Homocysteine and Folate in Pregnancy

The importance of folate during pregnancy was addressed 40 years ago by Bryan Hibbard (1) in his study of folate status in 1484 low-income obstetric patients from Liverpool. He assessed folate status as urinary excretion of formiminoglutamic acid. Abnormal formiminoglutamic acid excretion was related not only to placental abruption and spontaneous abortion but also to adverse outcomes in previous pregnancies, including prematurity, congenital defects, and perinatal mortality. Shortly thereafter, Hibbard and Smithells (2) suggested that folate deficiency in pregnancy may be related to central nervous system malformations, and Smithells started a series of observational and intervention studies demonstrating that adequate folate status reduced the risk of neural tube defects (NTDs), observations that eventually in the early 1990s were confirmed in large, randomized intervention trials (3, 4).

It is now established that periconceptional folate supplementation reduces the occurrence and recurrence of NTDs (3, 4). The results obtained in many observational studies suggest that low folate intake or low circulating folate increases the risk of preterm delivery and low birth weight (5). However, a recent Dutch study on several B vitamins measured before and during pregnancy in healthy, well-nourished women demonstrated no association between the vitamin concentrations and birth weight or risk of early pregnancy loss (6). The results from randomized intervention trials with folic acid have been equivocal (5). Thus, the link between maternal folate status and birth weight is uncertain.

The conclusions of the observational studies on vitamins and adverse pregnancy outcomes have been questioned because of methodologic weaknesses. These include inaccurate assessment of vitamin intake, measurement errors attributable to variable plasma-volume expansion during pregnancy, and confounders such as drug use and stress, intake of other micronutrients, and energy intake and socioeconomic status (7). In particular, smoking is a potential confounder because it is related to poor vitamin status, high total homocysteine (tHcy) (8), and low birth weight (9). Analyses should therefore also be carried out among nonsmokers.

The concentration of tHcy in plasma is a responsive marker of impaired folate status. In 1991, Steegers-Theunissen et al. (10) suggested that maternal hyperhomocysteinemia was a risk factor for NTDs. Subsequent studies demonstrated increased tHcy in mothers of children with NTDs even in the absence of low circulating folate, suggesting a direct adverse effect of homocysteine on the developing fetus (4). Several studies also demonstrated that high tHcy is a risk factor of placenta-mediated diseases, such as preeclampsia, spontaneous abortion, placental abruption, and recurrent pregnancy loss (11–13).

Several malformations and obstetric complications associated with tHcy have been investigated in relation to the TT genotype of the methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism, which affects intracellular folate distribution and is associated with increased tHcy under conditions of impaired folate status (14). Because genotype, in contrast to vitamin or homocysteine status, is not changed during pregnancy or by pregnancy-related complications, associations with variant genotypes may give clues as to the mechanisms involved. NTDs show a consistent relationship with the MTHFR TT genotype (of both the mother and the baby), suggesting that the TT genotype may predispose to increased tHcy in women with NTD pregnancies and may also partly explain the protective effect of folate supplementation (4, 15).

However, the MTHFR 677C>T polymorphism shows a weak or inconsistent association with other pregnancy complications, including placenta-mediated disease, intrauterine growth retardation, and low birth weight (11, 16, 17), and the mechanisms involved are unclear. Homocysteine could be directly involved by causing vasculopathy leading to inadequate maternal–fetal circulation. This is in accordance with the observed relationship between high tHcy and defective chorionic villous vascularization in mothers with recurrent early pregnancy loss (18). Alternatively, increased tHcy may be only a marker of underlying conditions that are directly related to pregnancy complications, such a subclinical vascular disease, reduced glomerular filtration rate (19) (which is inversely associated with tHcy (20), and inadequate plasma-volume expansion (21, 22).

Most studies on maternal tHcy and pregnancy complications have measured tHcy near the time of delivery (23, 24) or up to years after the index pregnancy (5, 13), whereas only a few studies have measured tHcy before or during pregnancy (25–27). This is a possible shortcoming because the time interval between exposure and event may attenuate the association, because the disease itself may affect the tHcy concentration, and because of marked changes in plasma tHcy during pregnancy (16).

Low plasma tHcy during an uncomplicated pregnancy was first demonstrated by Kang et al. (28) almost 20 years ago, and this has subsequently been confirmed by numerous investigators (16). Plasma tHcy concentrations are 30–60% lower in pregnant women than in nonpregnant women, and the lowest tHcy values are observed in the second trimester. In a recent longitudinal study of tHcy during pregnancy, Murphy et al. (29) demonstrated that the reduction cannot be accounted for by folic acid supplementation, plasma-volume expansion, or a decrease in serum albumin. They suggest that low tHcy represents a physiologic adaptation to pregnancy, mediated by endocrine changes. In line with this, it has been speculated whether homocysteine plays a role in regulating hemostasis during pregnancy (16) and myometrial contractility at labor (30).

In this issue of Clinical Chemistry, Murphy et al. (31)

present additional analyses of data from their longitudinal study on tHcy in plasma from 93 healthy women, collected 2–10 weeks before conception; at gestational weeks 8, 20, and 32; and immediately before delivery. The novel data presented here show an increase in tHcy from week 32 of gestation, and the tHcy concentration at delivery in mothers not supplemented with folic acid was essentially similar to that measured before conception. Furthermore, maternal tHcy concentrations correlated from preconception throughout pregnancy and at birth, which in turn correlated with tHcy concentrations in cord blood. The concentrations of both maternal and fetal tHcy were lowered by folic acid supplementation. Finally, maternal tHcy at preconception, at 8 weeks, and at birth was inversely related to birth weight. This association was upheld after adjustment for maternal smoking.

As emphasized by the authors (31), the correlation between preconceptional tHcy and tHcy during pregnancy points to the possibility that preconceptional tHcy may predict tHcy-associated pregnancy complications. Large prospective studies are needed to investigate this possibility. It also seems rational that preconceptional tHcy may identify mothers at increased risk of complications and who may benefit from folic acid supplementation, but this idea gains limited support from the equivocal results from the intervention trials with folic acid cited above (5). Finally, the observed lower birth weights in babies of mothers with the highest tHcy agrees with some (13, 32–34), but not all (24, 26, 35, 36), published studies and adds to an apparently confusing body of literature on the relationship between maternal tHcy and birth weight or intrauterine growth retardation.

The discrepant results may be related to study design, including the population investigated. The study of Murphy et al. (31) and two other studies reporting an inverse association (34), including a large population-based study of ~6000 mothers (13), investigated birth weight and tHcy in healthy unselected mothers. These studies had a cross-sectional design. Infante-Rivard et al. (24) compared tHcy in 483 mothers (cases) giving birth to babies with birth weights below the 10th percentile with that in 409 mothers with healthy babies (controls). The authors unexpectedly observed lower tHcy among mothers of low-birth-weight babies. A notable characteristic of this study is that it was carried out in a folate-fortified population, and the overall maternal tHcy was low. Furthermore, tHcy concentrations were measured after birth. Conceivably, nutritional or hemostatic factors that predict severe growth restriction may be different from those that are associated with moderate variability in birth weight.

In conclusion, impaired folate status, the associated high tHcy, and the *MTHFR* TT genotype are associated with NTDs. The prevention of ~50% of recurrent and first NTDs by folic acid supplementation probably represents one of the most important advances in preventive medicine of the 1990s. Low folate status and hyperhomocysteinemia have been linked to other malformations and pregnancy complications and adverse outcomes, but the

direction of causality and the importance are uncertain. Large intervention trials as well as prospective studies measuring tHcy and folate status before and during pregnancy are needed to establish the role of these and related factors as predictors or etiologic factors of adverse pregnancy outcomes and complications.

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