Effects of Stage of Reproduction, Nutrients, and Genes on Serum Total Homocysteine Concentrations in Reproductive Age Women (17-44 Years) in the United States from the Third National Health and Nutrition Examination Survey DNA Bank

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Background and Objectives: Significantly elevated or low micronutrient levels prior to and during pregnancy have been implicated in adverse reproductive outcomes, such as the association between decreased folate consumption and neural tube defects. Therefore, it is important to characterize the subpopulations of reproductive-age women with significantly elevated or low micronutrient or metabolite levels, since these women could be at a higher risk for adverse pregnancy outcomes.

Elevation of serum homocysteine, a metabolite in the folate pathway, is associated with the development of cardiovascular disease and stroke. Elevated serum homocysteine concentrations have also been associated with adverse pregnancy outcomes, such as prematurity, stillbirth, and low birth weight. Serum homocysteine concentrations can be affected by both environmental and genetic factors. Age, cigarette smoking, caffeine intake, and the physiologic and hormonal effects of pregnancy can modify homocysteine concentrations. Supplementation with B vitamins, such as folic acid, vitamin B6, and vitamin B12, has been shown to reduce homocysteine concentrations. Several common polymorphisms in genes encoding enzymes involved in folate and homocysteine metabolism have been shown to influence homocysteine concentrations and disease risks. None of these factors act in isolation, so their potential effects on disease risk, as well as adverse reproductive outcomes, need to be considered in an interdependent fashion with multiple potential levels of interaction.

The objectives of this study are (1) to describe the serum homocysteine concentrations among reproductive age women in relation to stage of reproduction (currently pregnant, pregnant in the previous two years, pregnant more than two years ago, and never pregnant), race/ethnicity, and age, and (2) to evaluate the influence of genetic variants on the serum homocysteine concentration in women within each of four reproductive stages and three major racial/ethnic groups.

Methods: Survey data from interviews of 2,012 women (aged 17-44 years) who participated in the Third National Health and Nutrition Examination Survey (NHANES III) DNA Bank (1991-1994) were evaluated to determine patterns of vitamin supplementation in different age groups, racial/ethnic groups, and among four stages of reproduction. Among the 1,769 women in the study group who had a serum homocysteine determination, mean homocysteine concentrations were compared for the different reproductive stages with the unpaired Student's t-test using pairwise contrasts. Women were subdivided into quartiles, based on homocysteine concentrations, and the χ^2 statistic was used to evaluate differences in the number of women in each quartile according to stage of reproduction, race/ethnicity, or age.

Genetic modeling in relation to logarithmically transformed homocysteine concentrations was performed for each of five genetic variants within three enzymes involved in folate and homocysteine metabolism (MTHFR 1298A-C, MTHFR 677C-T, MTHFR 116C-T, MTRR 66A-G, and CBS 844ins68). Pairwise differences between the three genotypes of each variant with regard to the outcome (mean log homocysteine) were compared by a Student's t-test. Subsequently, linear regression with the best genetic model (dominant, recessive, complete overdominant, or codominant) was used to estimate sample-weighted and adjusted geometric means and 95% confidence intervals for homocysteine concentrations. Some modeling included interaction terms between genotype and stage of reproduction or race/ethnicity. Models were adjusted for age, smoking status, caffeine intake, folate intake and vitamin B6 intake from food and dietary supplements, and serum vitamin B12 concentration. Models were further stratified by folate intake of ≤ 20th percentile (≤ 131 µg/day) and >20th percentile (>131 µg/day).

Results: Compared with women in the other stages of reproduction, currently pregnant women had a higher reported frequency of vitamin supplement use in the 24 hours prior to interview (53% vs. 17-18%) and during the previous month (61% vs. 34-38%). Mean homocysteine concentrations were significantly lower for currently pregnant women (mean ± SE: 5.2 ± 0.3 µmol/L) than for never pregnant women (7.7 ± 0.2 µmol/L, $p<0.0001$). Fewer Mexican Americans were in the higher homocysteine quartiles compared with non-Hispanic whites ($\chi^2=17.7$, df=3, $p=0.001$), whereas more women aged 36-44 years were in the higher homocysteine quartiles than women aged 17-24 years ($\chi^2=10.9$, df=3, $p=0.013$).

When analyses were performed for each of the five gene variants to determine the best genetic model, significant pairwise differences in mean homocysteine concentrations were seen only between genotypes for

MTHFR 677C-T, and the pattern of differences suggested a recessive model (CC/CT vs. TT). Since no significant differences were seen for MTHFR 1298A-C, MTHFR 116C-T, MTRR 66A-G, or CBS 844ins68, further analyses were restricted to the recessive model for MTHFR 677C-T.

A significant increase (p=0.0001) in serum homocysteine was observed among women homozygous for the MTHFR 677T polymorphism (9.48 µmol/L), compared to women with the CC/CT genotypes (7.17 µmol/L). With stratification by race/ethnicity, this increase in relation to genotype was observed in non-Hispanic whites (9.56 compared to 7.19 µmol/L) and in Mexican Americans (7.77 compared to 6.63 µmol/L). The model could not be applied to non-Hispanic blacks because of the low prevalence of the TT genotype (0.8%, compared to 11.9% and 19.6% for non-Hispanic whites and Mexican Americans, respectively). With stratification by stage of reproduction, this difference between genotypes was significant for women pregnant in the previous 2 years (9.76 compared to 7.19 µmol/L, p=0.0060) and for women pregnant more than 2 years ago (11.04 compared to 7.41 µmol/L, p<0.0001). The increased homocysteine level among women homozygous for the MTHFR 677T genotype was not observed in never pregnant (7.49 compared to 7.42 µmol/L) or in currently pregnant women (5.18 compared to 4.74 µmol/L).

The effect of the TT genotype on increasing the serum homocysteine concentration relative to the CC/CT genotypes was greater in women with a reduced daily intake of folate (≤ 131 µg) (17.8 compared to 8.04 µmol/L) than in women with an intake of >131 µg (8.18 compared to 6.98 µmol/L). This effect based on folate intake was mirrored in non-Hispanic whites, but in Mexican American women, the pattern of effect, although present, did not reach statistical significance (p=0.1051). Never pregnant women with the TT genotype had significantly elevated serum homocysteine concentrations (p<0.0001) only with a reduced intake of folate. However, women in the stage of reproduction categories of pregnant in the previous 2 years and pregnant more than 2 years ago showed significant elevations of homocysteine concentration for both folate intake strata. The model could not be applied to currently pregnant women because of the low prevalence of women with an intake of ≤ 131 µg/day of folate (5%, compared to 19-22% for the other reproductive stages).

Discussion/Conclusion: In reproductive-age women, homozygosity for the MTHFR 677T polymorphism, relative to the CC/CT genotypes, was associated with moderate increases in serum homocysteine concentrations. No effects were observed for the other four polymorphisms in the folate and homocysteine pathway enzymes that were evaluated. The effects of the MTHFR 677C-T polymorphism were significantly more pronounced for women with a reduced daily folate intake. The observation that the effect of the MTHFR 677C-T genotype varies with stage of reproduction bears further investigation.