Folate Fortification and Supplementation Do Not Provide Vascular Health Benefits in Type 1 Diabetes

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Objective To evaluate the lowest effective dose-response of folic acid on endothelial function in children with type 1 diabetes.

Study design A randomized, double-blind, crossover, placebo-controlled trial was conducted in 20 children with type 1 diabetes (age range 10-18 years) after mandatory folate fortification in Australia. Each child received orally 4 interventions (1 per month)—3 folic acid doses (0.5, 2, and 5 mg) and 1 placebo dose—in random order. The primary outcome was 2-hour postintervention change in endothelial function measured with flow-mediated dilatation (FMD). Thirty-five children with type 1 diabetes from our folic acid interventional trial before folate fortification were used for comparison.

Results All children completed the study. There were no differences in baseline FMD or folate status between the visits. Folic acid supplementation increased serum folate ($P = .0001$) and red cell folate ($P < .0001$), but none of the doses improved FMD ($P = .96$). Baseline serum folate and red cell folate levels and FMD and glyceryl trinitrate–mediated dilatation were significantly higher in these children compared with children from our trial before mandatory folate fortification ($P = .0001$, .0001, .014, and .04, respectively).

Conclusions Folate status and vascular function have improved in children with type 1 diabetes since the introduction of mandatory folate fortification, but the beneficial endothelial effects of additional folic acid are no longer present. *(J Pediatr 2013;163:255-60)*.

Vascular endothelial dysfunction is an early and fundamental event in the development of atherosclerosis.[1] Vascular endothelial and smooth muscle function can be evaluated by ultrasound assessment of brachial artery response to an increase in flow (flow-mediated dilatation [FMD]) and to glyceryl trinitrate (GTN). Abnormal FMD correlates with abnormal coronary angiography[2] and predicts coronary artery disease in adults.[3]

Children with type 1 diabetes have endothelial dysfunction that precedes the vascular complications of the disease.[4] Optimization of diabetes control is the initial strategy to improve endothelial function. However, despite the availability of better insulin delivery methods, children and adolescents with type 1 diabetes have higher than recommended glycosylated hemoglobin (HbA1c) levels for the prevention of complications.[5] Additional strategies with a potential to improve vascular function from childhood are therefore required.

In studies conducted before mandatory folate fortification in Australia, we have shown that endothelial dysfunction in children with type 1 diabetes is normalized by short-term high doses of folic acid supplementation (5 mg/day) without side effects.[6,7] Vascular response to folic acid is rapid (within hours), persists only with continuing daily supplementation, and is independent of homocyst(e)ine levels,[6,7] indicating a direct effect on the endothelium. Endothelial responses to lower doses of folic acid supplementation and/or mandatory folate fortification have not been evaluated in children, but a meta-analysis of randomized folic acid trials in adults shows a dose-response effect.[8]

Emerging data call into question the safety of long-term high doses of folic acid supplementation. Adults with established cardiovascular disease who received folic acid (0.8 mg/d) and vitamin B12 (0.4 mg/d) over 3 years had an increased

BMI	Body mass index
CV	Coefficient of variation
FMD	Flow-mediated dilatation
GTN	Glyceryl trinitrate
HbA1c	Glycosylated hemoglobin
HsCRP	High-sensitivity C-reactive protein
PGF2α	8-Epi-prostaglandin F2α
RCF	Red cell folate
tHcy	Total plasma homocysteine
WCH	Women's and Children's Hospital

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cancer incidence and greater all-cause mortality relative to the placebo group at a 3-year post-trial follow-up, despite no significant side effects during supplementation.[9] In addition, adults with type 1 or type 2 diabetes with advanced nephropathy who received folic acid (2.5 mg/d), vitamin B6 (25 mg/d), and vitamin B12 (1 mg/d) had a more rapid decline in glomerular filtration rate compared with those who received placebo.[10] Therefore, we aimed to identify the minimum folic acid dose required to improve endothelial function in children with type 1 diabetes.

Methods

Twenty children with type 1 diabetes (aged 10-18 years) without retinopathy or microalbuminuria were recruited from the diabetes clinics at the Women's and Children's Hospital (WCH), Adelaide, Australia (March 2010 to February 2011), after the introduction of mandatory folate fortification in Australia. Exclusion criteria were diabetes duration of <1 year, celiac or thyroid disease, vitamin B12 deficiency, treatment with statins or angiotensin-converting enzyme inhibitors, vitamin supplementation, or smoking history.

The Children, Youth and Women's Health Service Human Research Ethics Committee approved the study. Written informed consent was obtained from parents and children. Thirty-five children with type 1 diabetes and the same selection criteria who participated in our previous acute interventional trial,[6] before mandatory folate fortification in Australia, were used for comparison.

A dose-response, randomized, double-blind, placebo-controlled, crossover trial (Australian New Zealand Clinical Trials Registry: 12610000176066) was conducted to compare the acute effects of folic acid and placebo on endothelial function measured by FMD, 2 hours after the intervention. Each participant received in a random order 4 interventions (1 per month): a single treatment of 1 of 3 folic acid doses (0.5, 2, and 5 mg) or placebo, as a 10-mL solution, on the study morning immediately after baseline fasting investigations. Solutions were prepared by the WCH Pharmacy using folic acid British Pharmacopeia powder (Professional Compounding Chemists, Matraville, New South Wales, Australia). Randomization was performed by the WCH Pharmacy using a computerized sequence generator[11] that created random balanced permutations of interventions. Folic acid doses and the 2-hour reassessment timeframe were chosen taking previous studies into account.[7,8] There was no systematic change to diabetes management throughout the study period. All participants had their usual insulin dose the night before the study and their usual morning insulin immediately after fasting baseline investigations.

We assessed FMD, blood pressure, folate status, high-sensitivity C-reactive protein (HsCRP), glucose, and 8-epi-prostaglandin F2α (PGF2α) before and 2 hours after each intervention. Other baseline assessments included GTN, anthropometry, HbA1c, lipid profile, and vitamin B12.

Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer. Weight with minimal clothing worn was measured on an electronic digital scale to the nearest 0.1 kg. Body mass index (BMI) z score was calculated using EpiInfo database version 3.2.2 and Centers for Disease Control and Prevention 2000 standardized reference charts (www.ncdc.gov/epiinfo). Blood pressure was measured with appropriate-size cuff on the left arm after 10 minutes of rest in supine position, and the mean of 3 consecutive measurements was recorded.

The ultrasound assessments of vascular endothelial and smooth muscle function (FMD and GTN, respectively) were performed after overnight fasting, as previously described.[12] Brachial artery diameter was measured in a longitudinal section using B-mode ultrasound with a 17-MHz linear array transducer (iU22; Philips, Bothel, Washington). Each study included 4 scans: (1) resting scan; subsequently, reactive hyperemia was induced by occluding arterial blood flow using a sphygmomanometer inflated to 250 mm Hg for 4 minutes; (2) FMD scan recorded 45-75 seconds after cuff deflation; (3) re-control scan 10-15 minutes later; and (4) last scan, GTN, taken 4 minutes after the sublingual administration of GTN (400 μg, Nitrolingual Pump Spray; Sanofi Aventis, Macquarie Park, Australia). For each scan, measurements were made over 4 consecutive cardiac cycles, incident with the R wave on the electrocardiogram, by blinded observers. Measurements were averaged and expressed as percentages of the resting scan. Our coefficient of variation (CV) was 3.9% for FMD and 4.0% for GTN.[12]

Serum folate and red cell folate (RCF) levels were measured using chemiluminescent microparticle folate binding protein immunoassay (Abbott Architect Analyzer, Abbott Diagnostics [Sydney, Australia]; normal range 6.5-45 nmol/L and 220-1300 nmol/L, respectively). Total plasma homocyst(e)ine (tHcy) was measured by competitive immunoassay using direct, chemiluminescent technology (ADVIA Centaur homocyst(e)ine assay, Siemens Healthcare Diagnostics [Bayswater, Australia]; normal range of 4-14 μmol/L]). Vitamin B12, HbA1c, glucose, HsCRP, and lipids were measured as previously described.[13] Oxidative stress was assessed by measuring urinary PGF2α using a competitive enzyme-linked immunoassay (CV 2.38%), and values obtained were corrected for urinary creatinine.

Statistical Analyses

Data were analyzed using Stata IC version 10.1 statistical software (StataCorp, College Station, Texas). Spearman's rank correlations were performed between baseline FMD and other variables. Comparison of baseline characteristics between the 4 visits was performed using Skilling-Mack tests or ANOVA, as appropriate. Analyses followed the "intention-to-treat" principle. ANOVA was used to determine variables that had significant effects on FMD and brachial artery diameter over time. Skilling-Mack tests were used to evaluate changes in FMD, artery diameter, serum folate, RCF, tHcy, glucose, HsCRP, and urinary PGF2α between the 4 visits, according to the intervention. Statistical significance was inferred with a value of $P < .05$.