Plasma 1-carbon metabolites and academic achievement in 15-yr-old adolescents

Torbjörn K. Nilsson,*1 Anita Hurtig-Wennlöf,† Michael Sjöström,‡ Wolfgang Herrmann,§ Rima Obeid,§ Jennifer R. Owen,§ and Steven Zeisel*1,11

*Department of Medical Biosciences/Clinical Chemistry, Umeå University, Umeå, Sweden; †Faculty of Medicine and Health, School of Health and Medical Sciences, Örebro University, Örebro, Sweden; ‡Department of Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden; §Department of Clinical Chemistry and Laboratory Medicine, Saarland University Hospital, Homburg/Saar, Germany; 1Nutrition Research Institute, University of North Carolina, Chapel Hill, Kannapolis, North Carolina, USA; and 11Department of Nutrition, University of North Carolina Gillings School of Global Public Health, University of North Carolina, Chapel Hill, Chapel Hill, North Carolina, USA

ABSTRACT Academic achievement in adolescents is correlated with 1-carbon metabolism (1-CM), as folate intake is positively related and total plasma homocysteine (tHcy) negatively related to academic success. Because another 1-CM nutrient, choline is essential for fetal neurocognitive development, we hypothesized that choline and betaine could also be positively related to academic achievement in adolescents. In a sample of 15-yr-old children (n = 324), we measured plasma concentrations of homocysteine, choline, and betaine and genotyped them for 2 polymorphisms with effects on 1-CM, methylenetetrahydrofolate reductase (MTHFR) 677C>T, rs1801133, and phosphatidylethanolamine N-methyltransferase (PEMT), rs12325817 (G>C). The sum of school grades in 17 major subjects was used as an outcome measure for academic achievement. Lifestyle and family socioeconomic status (SES) data were obtained from questionnaires. Plasma choline was significantly and positively associated with academic achievement independent of SES factors (paternal education and income, maternal education and income, smoking, school) and of folate intake (P = 0.009, R² = 0.285). With the addition of the PEMTrs12325817 polymorphism, the association value was only marginally changed. Plasma betaine concentration, tHcy, and the MTHFR 677T>C polymorphism did not affect academic achievement in any tested model involving choline. Dietary intake of choline is marginal in many adolescents and may be a public health concern. — Nilsson, T. K., Hurtig-Wennlöf, A., Sjöström, M., Herrmann, W., Obeid, R., Owen, J. R., Zeisel, S. Plasma 1-carbon metabolites and academic achievement in 15-yr-old adolescents. FASEB J. 30, 000–000 (2016). www.fasebj.org

Key Words: betaine • choline • folate • school grades

Optimal early development of the human brain is important for future cognitive ability. An optimal nutritional status of the pregnant woman is essential for the child’s future cognitive status (1) as predicted by the Barker hypothesis (2). The B vitamin folate has been shown to be of importance for the developing nervous system both during organogenesis and fetal growth (3). More recently, neuroscientists have documented a continued structural development of the brain throughout adolescence, consisting of synaptic pruning and other fine-tuning mechanisms. Previously we hypothesized that folate intake by adolescents would affect brain development, and we tested this by assessing the impact of folate intake on academic achievement in a sample of 15-yr-old adolescents. A positive effect of folate intake on postbirth cognitive development is supported by other studies, as recently reviewed (4). We observed a significant positive impact of folate intake on school grades even after controlling for the established strong predictors of school achievement: sex, smoking, and mother’s education (5). We also reported a negative correlation between total plasma homocysteine (tHcy) concentrations and academic achievement, suggesting that altered 1-carbon metabolism (1-CM)—the interrelated metabolisms of folate, choline and methionine (6–8)—was involved. Both 5-methyl-tetrahydrofolate and betaine (derived from choline) can donate methyl groups needed to convert homocysteine to methionine. Choline is of special interest because maternal dietary intake of choline directly influences neuronal precursor proliferation, apoptosis, and differentiation in the hippocampus of rodents (9–11). There was a more than 2-fold difference in rates of hippocampal neurogenesis in fetal brain between fetuses from dams eating low-choline vs. high-choline diets (12), and hippocampal neurogenesis

Abbreviations: 1-C, 1 carbon; 1-CM, 1 carbon metabolism; EYHS, European Youth Heart Study; MTHFR, methylenetetrahydrofolate reductase; PEMT, phosphatidylethanolamine N-methyltransferase; SES, socioeconomic status; tHcy, total plasma homocysteine

1 Correspondence: Department of Medical Biosciences/Clinical Chemistry, Umeå University, S-90185 Umeå, Sweden. E-mail: torbjorn.nilsson@umu.se
doi: 10.1096/fj.15-281097
rates remained elevated for more than 200 d in pups from high-choline-diet mothers compared to controls (13). Hippocampal function in the offspring of high-choline-diet dams was significantly enhanced compared to controls, as assessed by maze performance (14) and long-term potentiation (15). These effects lasted for the lifetime of the offspring, and age-related declines in memory that occurred in control mice were not observed in the mice born of high-choline-diet mothers (16). In a cohort study in people, higher maternal intake of choline during pregnancy was positively associated with memory performance in children at 7 yr of age (17).

Against this background, we hypothesized that, in addition to folate, choline and/or betaine might be predictors of academic achievement. We therefore studied academic achievement in 324 children aged 15 yr and measured biomarkers of choline in blood, 2 high-prevalence single nucleotide polymorphisms affecting their 1-CM (MTHFR, PEMT) (18, 19), and the major socioeconomic factors known to influence school achievement (5).

MATERIALS AND METHODS

Subjects and laboratory measurements

Blood samples were obtained from 324 adolescents aged 15 yr (164 girls and 160 boys) belonging to the Swedish part of the European Youth Heart Study (EYHS). EYHS is a cross-sectional school-based study of risk factors for future cardiovascular disease among children 9 to 10 yr old and adolescents 15 to 16 yr old. In Sweden, written school grades are given only during the last few years of compulsory school, so only adolescents were included in the present study. Mean age was 15.6 yr. Sampling procedures, participation rates, and representativeness, have been described previously (20, 21). The study was approved by the research ethics committees of Örebro County Council and Huddinge University Hospital. The subjects provided specific written informed consent.

A questionnaire was used to assess health-related lifestyle activities, including smoking habits (regular smoker/nonsmoker). The educational levels and incomes of the parents were obtained through a parent questionnaire and were used to classify background socioeconomic status (SES) of the pupils. Education at 2 levels [low/high (below university level or university level)] and income in 2 levels (above/below median) was used. Maternal educational status as an SES indicator has a high response rate and relatively unbiased responses, compared to questions regarding income and is extensively used in Swedish social sciences as the major marker of SES, but we evaluated education and income of both parents as SES indicators. We also assessed the possibility of a school effect on grades.

Blood samples were collected in the morning after an overnight fast and were centrifuged within 30 min. Homocysteine in citrated plasma was assayed by fluorescence polarization immunoassay on an IMx unit (Abbott Laboratories, Chicago, IL, USA). The coefficient of variation for the homocysteine assay was below 7.5%. The concentrations of choline and betaine were measured in EDTA–plasma samples at the University Hospital of Saarland by using ultra performance liquid chromatography–tandem mass spectrometry according to a previously published method (22). The between-day coefficient of variation of the choline/betaine assay was below 7%. No fresh samples were available for serum folate analyses, and estimated intake of folate was instead obtained from dietary assessment.

Total blood DNA was extracted and purified from 200 μl of whole blood using the QIAamp DNA Blood Mini Kit by the spin procedure, according to the manufacturer’s instructions (Qiagen, Germantown, MD, USA). Genotyping of the 677C>T polymorphism (rs1801133) in the human methylenetetrahydrofolate reductase (MTHFR) gene was performed using pyrosequencing (Biotage, Uppsala, Sweden) as previously described (23). Genotyping of human phosphatidylethanolamine N-methyltransferase (PEMT) rs12325817 G>C was performed by real-time PCR as previously described (24).

Dietary assessment

Dietary intake was assessed by an interviewer-mediated open 24 h recall. A qualitative food record completed the day before the interview served as a checklist once the 24 h recall was obtained (25). A food atlas was used to estimate portion sizes. The procedure has been validated against a weighed 7 d registration method as well as against doubly labeled water and found valid and sufficiently reliable with regard to energy intake for the age groups in the EYHS (data not shown). Dietary data were entered into nutrient analysis software (StorMats 4.02; Rudans Lättdata, Västerås, Sweden) and analyzed with regard to folate intake using the Swedish National Food Administration database (version 90.1).

Academic achievement

Information on written school grades was available for all subjects and was used here as a measure of academic achievement. The grades are in 4 levels for each subject (0, 10, 15, or 20 points): thus, a pupil can receive a maximum of 340 credit points in 17 school subjects. The 10 core subjects are Swedish, English, biology, chemistry, physics, mathematics, social science, history, geography, and religion. The final grades obtained by the subjects after completion of 9 yr of compulsory schooling were used in this study. Each subject was taught by a separate teacher, except for some popular combinations, such as when a teacher teaches 2 subjects, such as Swedish plus English or Swedish plus history. The sum of grades was thus a measure that represented the combined judgments of at least 10 different teachers for each pupil. It was normally distributed and is used here as a continuous outcome variable.

Statistical analysis

Statistical calculations were performed by SPSS 22.0 for Windows (IBM, Armonk, NY, USA). All variables were checked for normality of distribution before analysis. Qualitative data were used as categories in the analysis. Folate intake was grouped in tertiles using gender-specific cutoffs. The cutoffs obtained are shown in Table 1. Plasma concentrations of betaine and choline were analyzed as continuous variables.

To study the relationship between sum of school grades and selected predictor variables, we performed multivariate general linear model analyses. As an outcome variable, the sum of school grades was used for all 17 subjects and for the 10 core subjects. The independent variables were simultaneously entered as covariates and was used as a continuous outcome variable. As an outcome variable, the sum of school grades was used for all 17 subjects and for the 10 core subjects. The independent variables were simultaneously entered as covariates in the analyses. The MTHFR and PEMT genotypes were analyzed as recessive traits (i.e., 2 levels). No imputations were made for missing values in the questionnaire-based variables, and the general linear models are therefore based on 285 subjects. For all tests, P = 0.05 was used as the significance level.

RESULTS

Baseline characteristics

As shown in Table 1, plasma betaine was higher among boys, an effect that was solely due to lower betaine levels.
among the postpubertal girls (Tanner score 5). Likewise, choline was higher in boys; the effect was due to lower choline among the postpubertal girls as well as higher choline in postpubertal boys. Folate intake was higher in boys, even after adjustment for weight and body mass index; tHcy concentrations in both sexes were significantly lower with higher folate intake; when accounting for tertiles of folate intake, there was no sex difference in tHcy concentrations. Prevalences of the genotypes of the MTHFR rs1235817 G>C polymorphism were GG 31.1%, GC 48.7%, and CC 20.2% and were in Hardy-Weinberg equilibrium ($\chi^2 = 0.07, P = 0.78$), as was the MTHFR 677C>T polymorphism (CC 49.2%; CT 42%; TT 8.8%, $\chi^2 = 0.004, P = 0.95$).

### Determinants of academic achievement

The sum of school grades for either 17 main subjects or 10 core subjects was used as a measure of academic achievement, and its dependence on tHcy and plasma choline was tested. The previously established strong socioeconomic predictors of school grades (5) were entered as covariates (i.e., sex, smoking, and maternal education).

Table 2 shows that plasma tHcy concentrations were significantly and negatively correlated with school grades independent from the established SES covariates and irrespective of whether all 17 or just the 10 core subjects were used as the outcome variable. tHcy is a global biomarker of 1-CM, which points to a relation between 1-carbon (1-C) nutrients and/or polymorphisms in 1-CM genes being associated with school grades. To further delineate this, we tested whether 2 major 1-CM nutrients could substitute for tHcy in the models.

Table 3 shows that plasma choline was significantly and positively associated with academic achievement independent of the SES variables and independent of folate intake. The addition of the PEMT rs1235817 polymorphism (models 2 and 4) only marginally changed the $R^2$ of the model and did not attenuate the choline effect. The addition of the following variables were found not to affect academic achievement in any tested model involving choline: plasma betaine concentration, tHcy, MTHFR 677C>T, paternal education, paternal income, maternal income, and school.

### DISCUSSION

A main finding of this study was that plasma choline concentrations are positively correlated with academic achievement in 15-yr-old adolescents (Table 3)—an effect independent of previously documented predictors of academic achievement such as sex, smoking, maternal education, and folate intake (5). Because plasma choline concentrations vary between 5 and 12 $\mu$M, the $\beta$ values of approximately 2.4 to 2.5 school grade points per $\mu$M of choline that we found (Table 3) means that choline could...
As outcome variables, sum of school grades in either all 17 subjects on curriculum or in 10 core subjects was tested. General linear model used. **PEMT** polymorphism rs12325817 (G>C) was entered as factor on 2 levels (recessive trait; GG+GC vs. CC, models 2 and 4). Units of β values are school grade points per unit of the variable (i.e., homocysteine, points per μM; sex, points for male vs. female; smoking, points for yes vs. no; maternal education, points for university graduate vs. nongraduate; **PEMT**, points for CC vs. GG+GC).

<table>
<thead>
<tr>
<th>Model dependent variable</th>
<th>Covariate</th>
<th>β (covariates)</th>
<th>P</th>
<th>R² (adj model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1: school grades, 17 subjects</td>
<td>Sex</td>
<td>−24.63</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>−52.30</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>−30.39</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>−19.39</td>
<td>0.038</td>
<td>0.240</td>
</tr>
<tr>
<td>Model 2: school grades, 17 subjects</td>
<td>Sex</td>
<td>−22.64</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>−52.59</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>−33.58</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>−21.58</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>PEMT</strong></td>
<td>−10.58</td>
<td>0.129</td>
<td>0.256</td>
</tr>
<tr>
<td>Model 3: school grades, 10 subjects</td>
<td>Sex</td>
<td>−12.46</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>−29.72</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>18.20</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>−12.64</td>
<td>0.048</td>
<td>0.186</td>
</tr>
<tr>
<td></td>
<td><strong>PEMT</strong></td>
<td>−11.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Model 4: school grades, 10 subjects</td>
<td>Sex</td>
<td>−29.93</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal education</td>
<td>20.24</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>−13.43</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>PEMT</strong></td>
<td>−6.91</td>
<td>0.136</td>
<td>0.201</td>
</tr>
</tbody>
</table>

We conclude that the 1-C nutrients folate and choline are positively related to academic achievement in 15-yr-old adolescents. The underlying mechanisms are...
unclear but could involve enhanced adult neurogenesis in the hippocampus or increased release of acetylcholine from neurons of the hippocampus. Dietary intake of choline is now known to be marginal in many European adolescents and is therefore a public health concern (37). Identification of novel factors that can improve academic achievement in children is of great interest to parents, neuroscientists, and society in general. From a public health perspective, our findings suggest that it would be highly important to perform randomized controlled trials evaluating whether diets rich in foods that contain choline and folate improve academic achievement in schoolchildren and adolescents.  

This study was supported by the U.S. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (DK56350, to S.Z.); Orebro Länns Landsting, Nyckeludden, Orebro, and Umeå University (to T.N.); the Stockholm County Council, and the Swedish Heart and Lung Foundation (to M.S.). The authors declare no conflicts of interest.

REFERENCES


Received for publication September 4, 2015. Accepted for publication December 14, 2015.