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[¶,‖]

*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; [¶]Nutrition Research Institute, University of North Carolina, Chapel Hill, Kannapolis, North Carolina, USA; and [‖]Department 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, R^2 = 0.285). With the addition of the *PEMT* rs12325817 polymorphism, the association value was only marginally changed. Plasma betaine concentration, tHcy, and the *MTHFR* 677C>T 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, 1683–1688 (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 Adminsitration database (version 99.1).

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 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

TABLE 1. *Characteristics of 324 adolescents aged 15 yr*

Characteristic	Girls (n = 164)		Boys (n = 160)		P^a
	Mean	95% CI	Mean	95% CI	
Age (yr)	15.6	15.5–15.6	15.6	15.6–15.7	0.307
Weight (kg)	57.2	56.0–58.4	64.3	62.6–66.0	<0.001
Height (cm)	165.3	164.3–166.2	176.3	175.1–177.5	<0.001
Body mass index (kg/m^2)	20.9	20.6–21.3	20.6	20.2–21.0	0.243
Homocysteine (μM)	8.96	8.37–9.56	9.71	9.01–10.41	0.110
Betaine (μM)	27.9	26.8–29.2	32.3	31.1–33.5	<0.001
Betaine by Tanner groups (μM)					
Tanner 1–4	28.3	26.4–30.1	31.2	27.5–33.8	0.098
Tanner 5	26.6	24.8–28.4	32.3	30.8–33.7	<0.001
P for group differences[b]	0.201		0.491		
Choline (μM)	7.9	7.6–8.2	9.0	8.7 – 9.3	<0.001
Choline by Tanner groups (μM)					
Tanner 1–4	8.2	7.7–8.6	8.1	7.3–8.8	0.798
Tanner 5	7.6	7.1–8.0	9.1	8.7–9.5	<0.001
P for group differences[b]	0.052		0.020		
Folate intake (μg DFE/d)	224	207–239	285	268–302	<0.001
Folate intake by weight (μg/d/kg)	4.0	3.7–4.3	4.5	4.2–4.8	0.012
Folate intake by body mass index(μg/d/kg/m^2)	10.9	10.1–11.7	14.0	13.2–14.9	<0.001
Homocysteine by tertiles of folate intake (μM)					
Tertile 1: intake 42–173 in girls; 74–223 in boys	10.10	8.67–11.52	10.95	9.21–12.69	0.449
Tertile 2: intake 174–252 in girls; 228–335 in boys	8.78	7.88–9.68	9.72	8.67–10.78	0.174
Tertile 3: intake 254–593 in girls: 336–747 in boys	7.94	7.45–8.44	8.43	7.95–8.92	0.159
P for tertile differences[b]	0.014		0.016		
Sum of school grades	138.9	134.0–143.8	125.5	119.8–131.1	<0.001

Mean values and 95% confidence intervals (CI) are shown. For homocysteine, minimum and maximum homocysteine values per tertile of folate intake are also shown. [a]ANOVA was used to assess P values for sex differences. [b]ANOVA was used to assess P values for tertile differences within each gender group.

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 *PEMT* rs12325817 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* rs12325817 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 μM, the β values of approximately 2.4 to 2.5 school grade points per micromolar of choline that we found (Table 3) means that choline

TABLE 2. *Association of sum of school grades (dependent variable) with tHcy and selected covariates*

Model dependent variable	Covariate	β (covariates)	*P*	R^2 (adj model)
Model 1: school grades, 17 subjects	Sex	−24.63	<0.001	
	Smoking	−52.30	<0.001	
	Maternal education	30.39	<0.001	
	Homocysteine	−19.39	0.038	0.240
Model 2: school grades, 17 subjects	Sex	−22.64	<0.001	
	Smoking	−52.59	<0.001	
	Maternal education	33.58	<0.001	
	Homocysteine	−21.58	0.021	
	PEMT	−10.58	0.129	0.256
Model 3: school grades, 10 subjects	Sex	−12.46	0.001	
	Smoking	−29.72	<0.001	
	Maternal education	18.20	<0.001	
	Homocysteine	−12.64	0.048	0.186
Model 4: school grades, 10 subjects	Sex	−11.03	0.03	
	Smoking	−29.93	<0.001	
	Maternal education	20.24	<0.001	
	Homocysteine	−13.43	0.031	
	PEMT	−6.91	0.136	0.201

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/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).

could potentially affect the sum of school grades in 10 core subjects by 8 to 9% because the maximum point score is 200 (7 μM × 2.5 points/μM/200 = 8.8%).

Previous studies have documented positive effects of choline exposure during fetal life on neurocognitive functions (9–14). The present findings thus extend the scope of choline nutrition into adolescence, where brain plasticity is now recognized as an important factor in cognitive function (26, 27). In addition, short-term positive effects of choline on cognitive functions and learning have been demonstrated in trial studies using choline chloride supplements (28–31).

Some of the choline needed by young women is derived *via* phosphatidyl-ethanolamine-*N*-methyltransferase, *PEMT* (26). The *PEMT* gene, expressed in liver, enables endogenous biosynthesis of choline moiety (as part of phosphatidylcholine). This gene is induced by estrogen (32). However, 72% of women harbor a functional single nucleotide polymorphism in the *PEMT* gene (rs12325817 G>C), which makes them less able to induce this gene with estrogen (33), thereby abrogating much of endogenous production of choline. These women develop organ dysfunction on a low-choline diet (19). Homozygosity for the variant T allele in the *MTHFR* gene (rs1801133 C>T) increases the requirement for folate and may alter the metabolic use of choline (34). These well-established nutrigenetic connections notwithstanding, neither plasma betaine nor *PEMT* or *MTHFR* polymorphisms were significantly associated with academic achievement. This suggests that the positive association between plasma choline and academic achievement may reflect a specific effect of choline itself, which is likely to be related to its roles in neuronal development and integrity or neurotransmission (9–14, 17).

We attach particular significance to the fact that the previously documented positive association of folate intake with academic achievement (5) persisted in this study even after adding plasma choline and betaine concentrations and *PEMT* genotype to the explanatory model. Thus, 1-CM is heavily implicated in the metabolic underpinning of academic achievement in adolescents. This is further documented by our finding that tHcy, as a biomarker of methyl group sufficiency, was strongly negatively related to academic achievement (Table 2) in these adolescents. The fact that tHcy lost its significant association when folate and choline were included in the explanatory model does not detract from this conclusion but merely underscores that part of the variance in academic achievement is positively associated with nutritional sufficiency of 1-CM factors. To some extent, such an influence may be modulated by nutrigenetic interactions among key enzymes and the 1-CM nutrients as well as by sex because the *PEMT* gene activity is under estrogen control (33), although *PEMT* here did not quite reach statistical significance (Tables 2 and 3).

We studied a population that is not exposed to fortification of food with folate, which may be regarded as both a strength and a limitation of the study. The cross-sectional design, which precludes statements about causality, a lack of choline and betaine intake data in the available Swedish food databases and the single 24 h recall are limitations. Plasma choline concentrations are homeostatically regulated and drop about 30% when a person consumes a modestly low-choline diet but do not drop further, making this test unable to distinguish between moderately low and very low choline intake (35, 36). This could lead to an underestimation of choline effects when using plasma choline as a biomarker of choline intake. Thus, better food intake databases could shed further light on the associations demonstrated here.

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

TABLE 3. *Association of sum of school grades (dependent variable) with plasma choline and selected covariates*

Model dependent variable	Covariate	β (covariates)	*P*	R^2 (adj model)
Model 1: school grades, 17 subjects	Choline	3.52	0.011	
	Sex	−30.26	<0.001	
	Smoking	−52.56	<0.001	
	Maternal education	27.42	<0.001	
	Folate intake (tertiles)	11.70	0.001	0.274
Model 2: school grades, 17 subjects	Choline	3.62	0.009	
	Sex	−28.56	<0.001	
	Smoking	−52.93	<0.001	
	Maternal education	30.70	<0.001	
	Folate intake (tertiles)	10.76	0.002	
	PEMT	−10.72	0.117	0.285
Model 3: school grades, 10 subjects	Choline	2.44	0.008	
	Sex	−16.28	<0.001	
	Smoking	−29.94	<0.001	
	Maternal education	16.21	<0.001	
	Folate intake (tertiles)	7.69	0.001	0.225
Model 4: school grades, 10 subjects	Choline	2.48	0.007	
	Sex	−15.00	<0.001	
	Smoking	−30.19	<0.001	
	Maternal education	18.27	<0.001	
	Folate intake (tertiles)	7.12	0.002	
	PEMT	−7.05	0.120	0.235

As outcome variables, sum of school grades in either all 17 subjects on curriculum or in 10 core subjects was tested. A general linear model was 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.*, choline, points/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).

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. FJ

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.); Örebro Läns Landsting, Nyckelfonden, Örebro, 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

1. Anjos, T., Altmäe, S., Emmett, P., Tiemeier, H., Closa-Monasterolo, R., Luque, V., Wiseman, S., Pérez-García, M., Lattka, E., Demmelmair, H., Egan, B., Straub, N., Szajewska, H., Evans, J., Horton, C., Paus, T., Isaacs, E., van Klinken, J. W., Koletzko, B., and Campoy, C.; NUTRIMENTHE Research Group. (2013) Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. *Eur. J. Nutr.* **52**, 1825–1842
2. Calkins, K., and Devaskar, S. U. (2011) Fetal origins of adult disease. *Curr. Probl. Pediatr. Adolesc. Health Care* **41**, 158–176
3. Guéant, J. L., Namour, F., Guéant-Rodriguez, R. M., and Daval, J. L. (2013) Folate and fetal programming: a play in epigenomics? *Trends Endocrinol. Metab.* **24**, 279–289
4. Breimer, L. H., and Nilsson, T. K. (2012) Has folate a role in the developing nervous system after birth and not just during embryogenesis and gestation? *Scand. J. Clin. Lab. Invest.* **72**, 185–191
5. Nilsson, T. K., Yngve, A., Böttiger, A. K., Hurtig-Wennlöf, A., and Sjöström, M. (2011) High folate intake is related to better academic achievement in Swedish adolescents. *Pediatrics* **128**, e358–e365
6. Ueland, P. M., Refsum, H., Stabler, S. P., Malinow, M. R., Andersson, A., and Allen, R. H. (1993) Total homocysteine in plasma or serum: methods and clinical applications. *Clin. Chem.* **39**, 1764–1779
7. Zeisel, S. H. (2006) Choline: critical role during fetal development and dietary requirements in adults. *Annu. Rev. Nutr.* **26**, 229–250
8. Stover, P. J. (2011) Polymorphisms in 1-carbon metabolism, epigenetics and folate-related pathologies. *J. Nutrigenet. Nutrigenomics* **4**, 293–305
9. Albright, C. D., Siwek, D. F., Craciunescu, C. N., Mar, M. H., Kowall, N. W., Williams, C. L., and Zeisel, S. H. (2003) Choline availability during embryonic development alters the localization of calretinin in developing and aging mouse hippocampus. *Nutr. Neurosci.* **6**, 129–134
10. Albright, C. D., Mar, M. H., Craciunescu, C. N., Song, J., and Zeisel, S. H. (2005) Maternal dietary choline availability alters the balance of netrin-1 and DCC neuronal migration proteins in fetal mouse brain hippocampus. *Brain Res. Dev. Brain Res.* **159**, 149–154
11. Niculescu, M. D., Craciunescu, C. N., and Zeisel, S. H. (2006) Dietary choline deficiency alters global and gene-specific DNA methylation in the developing hippocampus of mouse fetal brains. *FASEB J.* **20**, 43–49
12. Craciunescu, C. N., Albright, C. D., Mar, M. H., Song, J., and Zeisel, S. H. (2003) Choline availability during embryonic development alters progenitor cell mitosis in developing mouse hippocampus. *J. Nutr.* **133**, 3614–3618
13. Glenn, M. J., Gibson, E. M., Kirby, E. D., Mellott, T. J., Blusztajn, J. K., and Williams, C. L. (2007) Prenatal choline availability modulates hippocampal neurogenesis and neurogenic responses to enriching experiences in adult female rats. *Eur. J. Neurosci.* **25**, 2473–2482
14. Meck, W. H., Smith, R. A., and Williams, C. L. (1988) Pre- and postnatal choline supplementation produces long-term facilitation of spatial memory. *Dev. Psychobiol.* **21**, 339–353
15. Pyapali, G. K., Turner, D. A., Williams, C. L., Meck, W. H., and Swartzwelder, H. S. (1998) Prenatal dietary choline supplementation decreases the threshold for induction of long-term potentiation in young adult rats. *J. Neurophysiol.* **79**, 1790–1796

16. Glenn, M. J., Kirby, E. D., Gibson, E. M., Wong-Goodrich, S. J., Mellott, T. J., Blusztajn, J. K., and Williams, C. L. (2008) Age-related declines in exploratory behavior and markers of hippocampal plasticity are attenuated by prenatal choline supplementation in rats. *Brain Res.* **1237**, 110–123
17. Boeke, C. E., Gillman, M. W., Hughes, M. D., Rifas-Shiman, S. L., Villamor, E., and Oken, E. (2013) Choline intake during pregnancy and child cognition at age 7 years. *Am. J. Epidemiol.* **177**, 1338–1347
18. Kohlmeier, M., da Costa, K. A., Fischer, L. M., and Zeisel, S. H. (2005) Genetic variation of folate-mediated one-carbon transfer pathway predicts susceptibility to choline deficiency in humans. *Proc. Natl. Acad. Sci. USA* **102**, 16025–16030
19. Da Costa, K. A., Kozyreva, O. G., Song, J., Galanko, J. A., Fischer, L. M., and Zeisel, S. H. (2006) Common genetic polymorphisms affect the human requirement for the nutrient choline. *FASEB J.* **20**, 1336–1344
20. Wennlöf, A. H., Yngve, A., and Sjöström, M. (2003) Sampling procedure, participation rates and representativeness in the Swedish part of the European Youth Heart Study (EYHS). *Public Health Nutr.* **6**, 291–299
21. Wennlöf, A. H., Yngve, A., Nilsson, T. K., and Sjöström, M. (2005) Serum lipids, glucose and insulin levels in healthy schoolchildren aged 9 and 15 years from Central Sweden: reference values in relation to biological, social and lifestyle factors. *Scand. J. Clin. Lab. Invest.* **65**, 65–76
22. Kirsch, S. H., Herrmann, W., Rabagny, Y., and Obeid, R. (2010) Quantification of acetylcholine, choline, betaine, and dimethylglycine in human plasma and urine using stable-isotope dilution ultra performance liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **878**, 3338–3344
23. Börjel, A. K., Yngve, A., Sjöström, M., and Nilsson, T. K. (2006) Novel mutations in the 5′-UTR of the *FOLR1* gene. *Clin. Chem. Lab. Med.* **44**, 161–167
24. Corbin, K. D., Abdelmalek, M. F., Spencer, M. D., da Costa, K. A., Galanko, J. A., Sha, W., Suzuki, A., Guy, C. D., Cardona, D. M., Torquati, A., Diehl, A. M., and Zeisel, S. H. (2013) Genetic signatures in choline and 1-carbon metabolism are associated with the severity of hepatic steatosis. *FASEB J.* **27**, 1674–1689
25. Villa, I., Yngve, A., Poortvliet, E., Grjibovski, A., Liiv, K., Sjöström, M., and Harro, M. (2007) Dietary intake among under-, normal- and overweight 9- and 15-year-old Estonian and Swedish schoolchildren. *Public Health Nutr.* **10**, 311–322
26. Konrad, K., Firk, C., and Uhlhaas, P. J. (2013) Brain development during adolescence: neuroscientific insights into this developmental period. *Dtsch. Arztebl. Int.* **110**, 425–431
27. Spear, L. P. (2013) Adolescent neurodevelopment. *J. Adolesc. Health* **52**(2, Suppl 2)S7–S13
28. Sitaram, N., Weingartner, H., Caine, E. D., and Gillin, J. C. (1978) Choline: selective enhancement of serial learning and encoding of low imagery words in man. *Life Sci.* **22**, 1555–1560
29. Sitaram, N., Weingartner, H., and Gillin, J. C. (1978) Human serial learning: enhancement with arecholine and choline impairment with scopolamine. *Science* **201**, 274–276
30. Davis, K. L., Mohs, R. C., Tinklenberg, J. R., Hollister, L. E., Pfefferbaum, A., and Kopell, B. S. (1980) Cholinomimetics and memory. The effect of choline chloride. *Arch. Neurol.* **37**, 49–52
31. Mohs, R. C., and Davis, K. L. (1980) Choline chloride effects on memory: correlation with the effects of physostigmine. *Psychiatry Res.* **2**, 149–156
32. Resseguie, M., Song, J., Niculescu, M. D., da Costa, K. A., Randall, T. A., and Zeisel, S. H. (2007) Phosphatidylethanolamine N-methyltransferase (*PEMT*) gene expression is induced by estrogen in human and mouse primary hepatocytes. *FASEB J.* **21**, 2622–2632
33. Resseguie, M. E., da Costa, K. A., Galanko, J. A., Patel, M., Davis, I. J., and Zeisel, S. H. (2011) Aberrant estrogen regulation of PEMT results in choline deficiency–associated liver dysfunction. *J. Biol. Chem.* **286**, 1649–1658
34. Yan, J., Wang, W., Gregory III, J. F., Malysheva, O., Brenna, J. T., Stabler, S. P., Allen, R. H., and Caudill, M. A. (2011) *MTHFR* C677T genotype influences the isotopic enrichment of one-carbon metabolites in folate-compromised men consuming d9-choline. *Am. J. Clin. Nutr.* **93**, 348–355
35. Fischer, L. M., daCosta, K. A., Kwock, L., Stewart, P. W., Lu, T. S., Stabler, S. P., Allen, R. H., and Zeisel, S. H. (2007) Sex and menopausal status influence human dietary requirements for the nutrient choline. *Am. J. Clin. Nutr.* **85**, 1275–1285
36. Savendahl, L., Mar, M. H., Underwood, L. E., and Zeisel, S. H. (1997) Prolonged fasting in humans results in diminished plasma choline concentrations but does not cause liver dysfunction. *Am. J. Clin. Nutr.* **66**, 622–625
37. Vennemann, F. B. C., Ioannidou, S., Valsta, L. M., Dumas, C., Ocké, M. C., Mensink, G. B. M., Lindtner, O., Virtanen, S. M., Tlustos, C., D'Addezio, L., Mattison, I., Dubuisson, C., Siksna, I., and Héraud, F. (2015) Dietary intake and food sources of choline in European populations. *Br. J. Nutr.* **114**, 2046–2055

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