

Choline-related supplements improve abnormal plasma methionine-homocysteine metabolites and glutathione status in children with cystic fibrosis¹⁻³

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ABSTRACT

Background: Liver triacylglycerol accumulation and oxidative stress are common in cystic fibrosis (CF) and also occur in choline deficiency. Previously, we showed an association between elevated plasma homocysteine, reduced ratios of *S*-adenosylmethionine to *S*-adenosylhomocysteine (SAM:SAH) and of phosphatidylcholine to phosphatidylethanolamine, and phospholipid malabsorption in children with CF.

Objective: The objective was to address a possible relation between altered methionine-homocysteine metabolism and choline metabolism in children with CF.

Design: Children with CF were assigned without bias to supplementation with 2 g lecithin/d ($n = 13$), 2 g choline/d ($n = 12$), or 3 g betaine/d ($n = 10$) for 14 d. Plasma concentrations of methionine, adenosine, cysteine, cysteinyl-glycine, glutathione, glutathione disulfide (GSSG), and fatty acids; SAM:SAH; and red blood cell phospholipids were measured within each group of children with CF before and after supplementation. Plasma from healthy children without CF ($n = 15$) was analyzed to obtain reference data.

Results: Children with CF had higher plasma homocysteine, SAH, and adenosine and lower methionine, SAM:SAH, and glutathione:GSSG than did children without CF. Supplementation with lecithin, choline, or betaine resulted in a significant increase in plasma methionine, SAM, SAM:SAH, and glutathione:GSSG and a decrease in SAH ($n = 35$). Supplementation with choline or betaine was associated with a significant decrease in plasma SAH and an increase in SAM:SAH, methionine, and glutathione:GSSG. Supplementation with lecithin or choline also increased plasma methionine and SAM.

Conclusion: We showed that dietary supplementation with choline-related compounds improves the low SAM:SAH and glutathione redox balance in children with CF. *Am J Clin Nutr* 2007;85:702–8.

KEY WORDS Choline, betaine, phospholipids, ratio of glutathione to glutathione disulfide, GSH:GSSG, cystic fibrosis

INTRODUCTION

The cystic fibrosis (CF) gene maps on chromosome 7 and encodes the CF transmembrane conductance regulator (CFTR), a protein that spans the plasma membrane surface of epithelial cells and some intracellular membranes (1–4). When activated by cyclic AMP and protein kinase, CFTR opens to form an ATP-gated channel to allow chloride to enter the cell (3, 4). The most common mutation, which occurs in $\approx 70\%$ of patients with

CF, is a 3–base pair deletion encoding a phenylalanine at position 508 ($\Delta F508$) of the CFTR. Impaired exocrine pancreatic function with reduced secretion of pancreatic enzymes and sodium bicarbonate results in malabsorption of nutrients in 85–90% of patients with CF (5). In the remaining patients, enzyme secretion is present, although sodium bicarbonate and fluid secretion are impaired. Clinical management of patients with CF with pancreatic enzyme insufficiency involves pancreatic enzyme replacements, which greatly improves but does not completely correct the fat malabsorption (6–9). CF is also accompanied by several clinical complications, including hepatic steatosis for which neither the cause nor the connection to defective CFTR is clear (10–12).

CF-associated liver disease includes fatty infiltration of hepatocytes and focal biliary fibrosis or cirrhosis, which are believed to be multifactorial and to involve biochemical changes (11, 13). Oxidant damage and impaired glutathione metabolism were also extensively described in CF and may play a role in the pathophysiology of the disease (13–18). Hepatic triacylglycerol accumulation is a well-known feature of choline deficiency and is believed to be explained by failure of adequate phosphatidylcholine synthesis to support secretion of triacylglycerols from the liver in VLDL (19, 20). In addition, reduced glutathione, the most important intracellular antioxidant in animal cells (21), is reduced in the liver of choline-deficient animals (22). Choline deficiency also results in decreased betaine, which is an important source of methyl groups for remethylation of homocysteine (23). Phosphatidylcholine synthesis occurs through 2 pathways: the cytidine diphosphocholine pathway, in which preformed choline is converted to phosphatidylcholine by cytidine diphosphocholine, and by sequential transfer of methyl groups from methionine by *S*-adenosylmethionine (SAM) to phosphatidylethanolamine in the reaction catalyzed by

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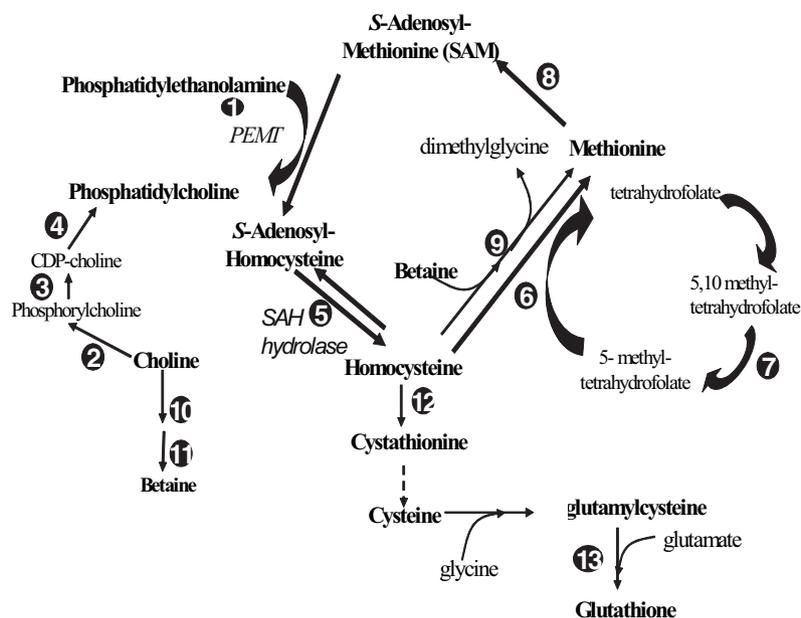


FIGURE 1. Schematic of phosphatidylcholine synthesis and the interrelation with methyl metabolism. Reactions catalyzed by 1) phosphatidylethanolamine-*N*-methyltransferase (PEMT); 2) choline kinase; 3) CTP:phosphocholine cytidyltransferase; 4) cytidine diphosphocholine (CDP)-choline; 5) *S*-adenosylhomocysteine (SAH) hydrolase; 6) 5-methyltetrahydrofolate homocysteine methyltransferase (MTHFR); 7) 5,10-methylene tetrahydrofolate reductase; 8) methionine adenosyltransferase; 9) betaine homocysteine methyltransferase; 10) choline oxidase; 11) betaine aldehyde dehydrogenase; 12) cystathionine β synthase; and 13) glutathione synthetase.

phosphatidylethanolamine-*N*-methyl transferase (PEMT) (19, 24), as shown in **Figure 1**. The other product of PEMT is *S*-adenosylhomocysteine (SAH), which is converted to homocysteine by SAH hydrolase. Homocysteine can be remethylated to methionine by methionine synthase in the pathway requiring 5-methyltetrahydrofolate (MTHF) or by betaine-homocysteine methyltransferase, with the use of methyl groups from betaine (25–27). Alternatively, homocysteine can enter the transsulfuration pathway that leads to cysteine, the precursor of glutathione (25, 27). Previously, we showed elevated plasma homocysteine, a low methionine concentration, a low ratio of SAM to SAH (SAM:SAH), and an inverse association between plasma homocysteine and phosphatidylcholine in children with CF (6, 28). More recently, we showed low plasma choline, as well as betaine and dimethylglycine in children with CF (29). In the present studies, we provide evidence of a functional relation between the choline-methyl pool and altered methionine-homocysteine metabolism and oxidant-antioxidant balance through the demonstration of an increase in plasma SAM:SAH and the ratio of glutathione to glutathione disulfide (glutathione:GSSG) after supplementation of children with CF with sources of choline.

SUBJECTS AND METHODS

Experimental design and subjects

This was a study of 3 separate supplements involving children with CF who were outpatients of the CF Clinic at the British Columbia Children’s Hospital (BCCH). The children were enrolled after description of the project to the child and his or her parents at a CF clinic appointment. The children were then assigned without bias to 1 of 3 choline-related supplements. Body

weight and height were measured, and routine blood work, including liver enzymes, hematology, serum zinc, selenium, and vitamins A and E, was completed as part of the clinic appointment, with additional blood collected for this study (day 0). The children were asked to return to the hospital after taking the assigned supplement for 14 d, at which time a second blood sample was collected. Routine hematology and clinical chemistry were done as part of the clinic visit on blood samples collected on study day 0 by the Hematopathology and Clinical Chemistry laboratories at the BCCH. CF genotype, sex, birth date, and medications and supplements were recorded from chart data. Forty children and their parents signed the informed consent, and 35 children completed the 14-d supplementation and provided blood samples before (day 0) and after (day 14) supplementation. Five children withdrew within 5 d of commencing the supplements or to return for blood sampling. Blood samples were collected from healthy children without CF ($n = 15$) to provide reference data for the laboratory measures; clinical chemistry and hematology tests were not done for the reference children. This study was approved by the University of British Columbia Clinical Screening Committee for Research and Other Studies Involving Human Subjects and the Children’s and Women’s Hospital Research Coordinating Committee. Approval for the use of betaine in this study was also obtained from Health Canada. All the parents and children provided written informed consent.

Supplements

The children with CF were assigned to receive phospholipid (lecithin, 2×1 g), choline (2×925 mg), or betaine (3×1 g) daily for 14 d. The lecithin used was soy lecithin providing 23% phosphatidylcholine and 20% phosphatidylethanolamine, the

TABLE 1Baseline characteristics of children with cystic fibrosis who took supplements for 14 d¹

	All children (n = 35)	Lecithin (n = 13)	Choline (n = 12)	Betaine (n = 10)
Age (mo)	128.4 ± 7.6 ²	123.2 ± 11.9	124.9 ± 13.7	139.9 ± 13.6
Sex				
Boy (n)	23	9	8	6
Girl (n)	12	4	4	4
Weight (z score)	-0.29 ± 0.14	-0.35 ± 0.28	-0.31 ± 0.21	-0.18 ± 0.30
Height (z score)	-0.30 ± 0.16	-0.16 ± 0.28	-0.39 ± 0.31	-0.34 ± 0.27
BMI (z score)	-0.31 ± 0.15	-0.32 ± 0.29	0.00 ± 0.20	-0.04 ± 0.33
Hemoglobin (g/L)	130 ± 1.80	129 ± 1.9	130 ± 3.7	132 ± 3.6
Vitamin A (μmol/L)	1.37 ± 0.05	1.36 ± 0.09	1.27 ± 0.05	1.51 ± 0.14
Vitamin E (μmol/L)	22.2 ± 1.21	24.0 ± 1.80	22.4 ± 2.22	19.6 ± 2.58
Zinc (μmol/L)	12.3 ± 0.30	12.4 ± 0.57	12.2 ± 0.62	12.4 ± 0.35
Selenium (μmol/L)	1.64 ± 0.03	1.59 ± 0.06	1.66 ± 0.06	1.68 ± 0.06
Alkaline phosphatase (U/L)	214 ± 11	209 ± 11	193 ± 20	236 ± 21
Aspartate transaminase (U/L)	35.6 ± 2.48	32.3 ± 1.61	40.6 ± 6.16	33.2 ± 2.51
Alanine transaminase (U/L)	27.7 ± 1.86	24.4 ± 3.46	30.6 ± 3.40	28.3 ± 2.70
γ Glutamyl transaminase (U/L)	19.6 ± 0.77	20.4 ± 1.52	18.8 ± 0.92	19.6 ± 1.68
Lactate dehydrogenase (U/L)	604 ± 22.4	609 ± 34.4	628 ± 38.6	566 ± 41.7
Triacylglycerol (mmol/L)	2.6 ± 0.27	2.7 ± 0.40	2.1 ± 0.51	3.0 ± 0.57
Cholesterol (mmol/L)	3.1 ± 0.11	3.2 ± 0.19	2.9 ± 0.19	3.1 ± 0.24

¹ Blood samples were collected on day 0, before supplementation began. No significant differences between groups were observed by ANOVA.² $\bar{x} \pm$ SEM (all such values).

choline was citrus-flavored choline chloride providing 925 mg choline/5 mL (Life Extension, Fort Lauderdale, FL), and betaine was from Sigma Aldridge Chemical Co (product no. B2629; Oakville, Canada). All the supplements were packaged by the BCCH pharmacy in coded bottles. Every bottle had a 2-wk diary printed on the label. The children were asked to take the supplements with meals and to tick on the diary each time the supplement was taken. The lowest observed adverse effect level, based on mild hypotension and fishy body odor, for choline is 7.5 g/d (30), whereas betaine has no reported adverse effects in clinical practice at doses of 6 g/d for >10 y (31). Thus, we used 2 g choline or lecithin/d or 3 g betaine/d and limited the study to 14 d. Each family was telephoned every 2–3 d throughout the supplementation period to monitor compliance and record any problems.

Blood collection

Venous blood samples were drawn from each child with CF at the outpatient laboratory of the BCCH immediately before supplementation and concurrent with collection of blood as part of their outpatient clinic appointment, then again after taking the supplements for 14 d (6, 28). A single blood sample was collected from each of the reference children. Two 7-mL blood samples, one with EDTA as an anticoagulant and one for serum, were taken. The samples were immediately centrifuged (2000 × g, 15 min, 4 °C), divided into aliquots for individual tests, and frozen at -70 °C within 20 min of blood collection (6, 28).

Analytic methods

Plasma and red blood cell (RBC) lipids were extracted, then the polar and nonpolar lipids were separated by HPLC, quantified with the use of an evaporative light-scattering detector, and recovered with the use of a fraction collector (28, 32). The fatty acid components in the plasma phosphatidylcholine and RBC

phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine were separated and quantified with the use of gas-liquid chromatography (33). Plasma thiols were measured with the use of reversed phase ion-pairing HPLC coupled to a coulometric electrochemical detector to allow simultaneous quantification, without derivatization of methionine, SAH, SAM, homocysteine, cystathionine, cysteine, cysteinyl-glycine, and glutathione as described previously (34, 35). Plasma and RBC folate and plasma vitamin B-12 were quantified by radioimmunoassay, and triacylglycerols and cholesterol were determined with the use of enzymatic methods (28).

Statistical analysis

All statistical analyses were performed with the use of SPSS for WINDOWS (version 10.0; SPSS, Chicago, IL). Data are presented as means ± SEMs. We used one-factor analysis of variance to compare the plasma thiols, glutathione, and GSSG and the plasma and RBC lipids in the children with CF with the group of reference children. This study was designed with 3 concurrent groups to address whether supplementation with any choline-containing compound is likely to alter thiol metabolism or redox status in children with CF. It was not designed to compare differences in efficacy between the 3 supplements. Paired *t* tests were used to analyze the effect of supplementation on plasma thiols, oxidant balance, and lipids within each of the supplemented groups of children with CF.

RESULTS

The characteristics of the children with CF who completed the 14-d supplementation are shown in **Table 1**. Of the 35 children who completed the study, 26 were homozygous and 7 were heterozygous for the ΔF508 mutation; 1 child was homozygous for the G85E mutation, and 1 child was G542X/G5511D. No



TABLE 2

Plasma thiols in children with cystic fibrosis (CF) and in a group of reference children without CF¹

	Reference children (n = 15)	Children with CF (n = 35)	95% confidence limit ²
Methionine (μmol/L)	25.9 ± 1.43 ³	20.7 ± 0.96 ⁴	-8.7, -2.1
Hcy (μmol/L)	6.06 ± 0.35	7.94 ± 0.45 ⁴	10.6, 2.6
SAM (nmol/L)	91.0 ± 6.23	83.1 ± 3.93	-17.7, 11.7
SAH (nmol/L)	15.8 ± 1.16	26.8 ± 1.63 ⁴	6.9, 15.0
SAM:SAH	6.22 ± 0.06	3.45 ± 0.27 ⁴	-3.9, -1.1
Adenosine (μmol/L)	0.13 ± 0.02	0.46 ± 0.04 ⁴	0.27, 0.47
Cysteine (μmol/L)	209 ± 9.25	237 ± 10.7	3.7, 49.4
Cyst-glyc (μmol/L)	45.0 ± 1.77	43.3 ± 2.30	-9.1, 0.64
Free GSH (μmol/L)	2.07 ± 0.17	1.45 ± 0.13 ⁴	-1.3, -0.40
Free GSSG (μmol/L)	0.16 ± 0.02	0.42 ± 0.04 ⁴	0.2, 0.64
GSH:GSSG	16.7 ± 2.78	4.60 ± 0.50 ⁴	-18.0, -6.0

¹ Hcy, homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Cyst-glyc, cysteinyl-glycine; GSH, glutathione; GSSG, glutathione disulfide. Blood samples for children with CF were collected on day 0 of the study before commencing any supplementation.

² Difference between the children with CF and the reference group of children.

³ $\bar{x} \pm SEM$ (all such values).

⁴ Significantly different from reference children, $P < 0.05$ (one-factor ANOVA).

significant difference was observed in age; z scores for height, weight, or body mass index; or the plasma measures of liver function, hemoglobin, vitamins A or E, zinc, selenium, triacylglycerols, or cholesterol between the 3 groups of children with CF who took the different supplements (Table 1). All of the children with CF had pancreatic insufficiency and were taking pancreatic enzyme replacements (500–2500 U lipase/kg per meal). None of the children were taking folate or vitamin B-6 antagonists, had chronic renal disease or malignancy, or were taking N-acetyl cysteine or any medications reported to increase homocysteine (36). Amino thiols, such as penicillamine, can reduce plasma homocysteine; however, no significant differences were observed in any of the plasma metabolites measured in this study between the children taking no antibiotics (n = 22) and children taking antibiotics (n = 13). No medication changes were made in any child during participation in this study.

The plasma concentrations of metabolites for methionine cycle and transsulfuration pathway in the children with CF before supplementation and in the reference children are shown in **Table 2**. The children with CF had higher plasma homocysteine, SAH, adenosine, and GSSG but lower methionine, glutathione, SAM:SAH, and glutathione:GSSG than did the reference children.

The plasma metabolite concentrations in the children with CF before and after 14 d of supplementation with lecithin, choline, or betaine are shown in **Tables 3, 4, and 5**, respectively. Supplementation with lecithin was associated with a significant increase in plasma methionine and SAM, but it had no statistically significant effect on the concentration of any other metabolite. Children with CF assigned to choline had significantly higher plasma concentrations of methionine and SAM, higher SAM:SAH and glutathione:GSSG, and lower plasma concentration of SAH after supplementation than before supplementation (Table 4). In the

TABLE 3

Effect of supplementation with lecithin for 14 d on plasma thiols in children with cystic fibrosis¹

	Lecithin (n = 13)	
	Day 0	Day 14
Methionine (μmol/L)	19.1 ± 1.23	25.8 ± 1.58 ²
Hcy (μmol/L)	8.56 ± 0.79	8.52 ± 0.64
SAM (nmol/L)	83.1 ± 4.77	95.0 ± 6.05 ²
SAH (nmol/L)	25.6 ± 2.96	21.2 ± 1.77
SAM:SAH	3.93 ± 0.55	4.90 ± 0.39
Adenosine (μmol/L)	0.42 ± 0.07	0.36 ± 0.06
Cysteine (μmol/L)	244 ± 9.60	246 ± 12.2
Cyst-glyc (μmol/L)	43.0 ± 2.6	44.0 ± 3.8
Free GSH (μmol/L)	1.62 ± 0.21	1.94 ± 0.26
Free GSSG (μmol/L)	0.46 ± 0.09	0.41 ± 0.10
GSH:GSSG	4.91 ± 0.81	7.63 ± 1.38

¹ All values are $\bar{x} \pm SEM$. Hcy, homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Cyst-glyc, cysteinyl-glycine; GSH, glutathione; GSSG, glutathione disulfide.

² Significantly different from day 0, $P < 0.05$ (paired t test).

group of children with CF assigned to betaine, the plasma concentration of methionine was higher, SAM:SAH and glutathione:GSSG were lower, and the concentrations of homocysteine, SAH, adenosine, and cysteine were higher on day 14 than on day 0 of the study (Table 5).

The analysis of the RBC phospholipids showed no significant differences in the concentration of individual phospholipids for phosphatidylcholine, sphingomyelin, lysophosphatidylcholine, or phosphatidylinositol between the children with CF and the group of reference children. However, RBC phosphatidylethanolamine was higher (1026 ± 34 and 86.3 ± 2.7 mg/L) and the ratio of phosphatidylcholine to phosphatidylethanolamine was lower (0.63 ± 0.02 and 0.84 ± 0.02; $P < 0.05$) in the children with CF (n = 35) than in a group of reference children (n = 15).

TABLE 4

Effect of supplementation with choline for 14 d on plasma thiols in children with cystic fibrosis¹

	Choline (n = 12)	
	Day 0	Day 14
Methionine (μmol/L)	21.6 ± 1.03	24.7 ± 1.32 ²
Hcy (μmol/L)	7.83 ± 0.32	7.52 ± 0.42
SAM (nmol/L)	81.3 ± 2.27	95.4 ± 5.16 ²
SAH (nmol/L)	29.4 ± 2.63	20.2 ± 1.84 ²
SAM:SAH	2.97 ± 0.23	5.16 ± 0.71 ²
Adenosine (μmol/L)	0.52 ± 0.08	0.43 ± 0.07
Cysteine (μmol/L)	238 ± 9.8	248 ± 10.9
Cyst-glyc (μmol/L)	39.2 ± 2.00	41.5 ± 1.72
Free GSH (μmol/L)	1.53 ± 0.17	1.80 ± 0.28
Free GSSG (μmol/L)	0.38 ± 0.06	0.36 ± 0.08
GSH:GSSG	5.02 ± 0.74	8.09 ± 1.01 ²

¹ All values are $\bar{x} \pm SEM$. Hcy, homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Cyst-glyc, cysteinyl-glycine; GSH, glutathione; GSSG, glutathione disulfide. The effect of supplementation was analyzed by using 2-factor ANOVA.

² Significantly different from day 0, $P < 0.05$ (paired t test).

TABLE 5Effect of supplementation with betaine for 14 d on plasma thiols in children with cystic fibrosis¹

	Betaine (<i>n</i> = 10)	
	Day 0	Day 14
Methionine (μmol/L)	21.6 ± 0.65	23.8 ± 2.71 ²
Hcy (μmol/L)	7.26 ± 0.38	6.18 ± 0.38 ²
SAM (nmol/L)	85.1 ± 3.72	87.4 ± 4.16
SAH (nmol/L)	26.1 ± 1.73	19.3 ± 2.92 ²
SAM:SAH	3.39 ± 0.25	5.32 ± 0.84 ²
Adenosine (μmol/L)	0.45 ± 0.02	0.29 ± 0.03 ²
Cysteine (μmol/L)	228 ± 9.01	185 ± 33.2 ²
Cyst-glyc (μmol/L)	44.6 ± 2.85	46.3 ± 5.78
Free GSH (μmol/L)	1.14 ± 0.19	1.35 ± 0.18
Free GSSG (μmol/L)	0.40 ± 0.06	0.25 ± 0.02
GSH:GSSG	3.68 ± 0.73	5.40 ± 0.63 ²

¹ All values are $\bar{x} \pm \text{SEM}$. Hcy, homocysteine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Cyst-glyc, cysteinyl-glycine; GSH, glutathione; GSSG, glutathione disulfide.

² Significantly different from day 0, $P < 0.05$ (paired *t* test).

We found no statistically significant differences in the concentrations of individual phospholipids in the RBC membranes of the children with CF after 14 d of supplementation with lecithin, choline, or betaine (data not shown). However, in the group of children given choline, the RBC membrane ratio of phosphatidylcholine to phosphatidylethanolamine was lower ($P < 0.05$) on day 0 (0.52 ± 0.02) than on day 14 (0.91 ± 0.04) of supplementation, which was explained by a higher phosphatidylcholine and lower phosphatidylethanolamine concentration on day 14 (824 ± 3 and 1043 ± 70 mg/mL, respectively) than on day 0 (752 ± 23 and 929 ± 58 mg/L, respectively) of the study. The plasma phospholipid fatty acid analyses showed that, compared with the group of reference children ($n = 15$), the children with CF ($n = 35$) had significantly lower concentrations of linoleic acid (22.1 ± 0.50 and 25.4 ± 0.80 g/100 g fatty acids) and docosahexaenoic acid (2.31 ± 0.11 and 2.88 ± 0.30 g/100 g fatty acids) and significantly higher concentrations of eicosatrienoic acid (4.5 ± 0.15 and 3.74 ± 0.12 g/100 g fatty acids) and eicosapentaenoic acid (0.89 ± 0.5 and 0.70 ± 0.07 g/100 g fatty acids) ($P < 0.05$); arachidonic acid concentrations were not significantly different between the children with CF and the reference group (9.65 ± 0.29 and 9.79 ± 0.49 g/100 g fatty acids, respectively). We found no significant differences in n-6 (linoleic acid, eicosatrienoic acid, arachidonic acid) or n-3 (docosahexaenoic acid, eicosapentaenoic acid) fatty acid concentrations between day 14 and day 0 in the group of children with CF who took lecithin, choline, or betaine (data not shown).

DISCUSSION

This study was the first to show that supplementation with choline-related compounds alters plasma methionine-homocysteine cycle metabolites, which leads to increased plasma methionine and SAM:SAH and to an improved glutathione antioxidant status in children with CF. This study was based on our recent work that showed increased plasma homocysteine and decreased SAM:SAH in children with CF, which was not explained by inadequate folate or vitamin B-12 status but was

associated with a reduced plasma ratio of phosphatidylcholine to phosphatidylethanolamine (6, 28). The metabolism of choline is interrelated with the methionine-homocysteine cycle at 2 steps: 1) through the methylation of phosphatidylethanolamine to form phosphatidylcholine with the use of methyl groups from methionine by SAM with the generation of SAH and 2) through the betaine-dependent remethylation of homocysteine to methionine (Figure 1). Although methylation of phosphatidylethanolamine is an important source of phosphatidylcholine and plasma homocysteine (37–39), an increase in SAH results in inhibition of PEMT and a decrease in plasma phosphatidylcholine and choline (40, 41). In animals, choline deficiency results in a decrease in hepatic betaine synthesis and SAM, which suggests that the folate-dependant remethylation of homocysteine may not fulfill the requirements for regeneration of methionine when the betaine-dependent remethylation of homocysteine is limited by choline deficiency (42). Other studies have shown that plasma betaine concentrations are inversely associated with plasma homocysteine and increase after methionine loading (43, 44), which suggests that choline-derived betaine is important in methionine-homocysteine metabolism in humans. Our recent study showed low plasma choline and betaine in children with CF (29).

The results of the present study show that supplementation with choline, betaine, or lecithin increased the low plasma methionine and that supplementation with choline or betaine decreased the elevated SAH and increased the plasma SAM:SAH in children with CF. These results are consistent with the interdependence of the methionine-homocysteine cycle with choline metabolism (26) and the low choline status of children with CF (29). Children with CF who took choline or betaine had significantly higher plasma SAH and SAM:SAH after 14 d of supplementation. However, the plasma SAM concentrations were significantly higher after 14 d of supplementation when compared with day 0 for those children with CF who took either choline or lecithin but not in those children with CF who took betaine. Dietary phospholipid requires digestion by pancreatic phospholipase A₂ before absorption as lysophospholipid, which is followed by reacylation in the enterocyte or direct transport to the liver bound to albumin. Previously, we showed an increased fecal excretion of choline phosphoglycerides in children with CF, regardless of supplementation with pancreatic enzymes (26, 45). In addition, the phospholipid supplement used in our studies provided ≈ 0.3 g choline compared with 1.85 g choline in the choline supplement. These differences may explain in part why our results show a statistically significantly lower plasma SAH concentration and a higher SAM:SAH after 14 d of supplementation with choline but not with lecithin in children with CF.

Previously, we reported an elevated concentration of homocysteine and decreased SAM:SAH in children with CF (6, 28), which raises the possibility that inhibition of PEMT could contribute to reduced de novo choline synthesis and a subsequent low choline status, exacerbated by chronic phosphatidylcholine malabsorption and increased phosphatidylcholine turnover (6, 28, 29, 45). Plasma homocysteine concentrations depend on the rate of homocysteine formation from methionine and the rate of removal by remethylation to methionine by either methionine synthase, which requires MTHF or betaine-homocysteine methyltransferase, or the rate of entry to the transsulfuration pathway, which is regulated by cystathionine β -synthase (25–27). SAM



serves to regulate the metabolism of homocysteine through allosteric inhibition of MTHF reductase and activation of cystathionine β -synthase (25). In the presence of low SAM, as shown in children with CF, the remethylation of homocysteine is favored, and decreased cystathionine β -synthase activity serves to conserve methionine. The increase in plasma methionine and SAM in children given lecithin or choline may reflect a sparing of methionine (and SAM) for phosphatidylcholine synthesis by the PEMT pathway, which possibly also explains the lack of a statistically significant increase in plasma SAM after supplementation with betaine (Figure 1). Studies in animals, however, have shown that supplementation with betaine increases the recycling of homocysteine to methionine (46), and, in clinical practice, betaine is efficacious in reducing elevated homocysteine (47). The latter studies are consistent with the findings of the present study that show both a lower plasma concentration of homocysteine and a higher plasma concentration of methionine in children with CF in the betaine group on day 14 than on day 0 (Table 3), which suggests that supplementation with betaine was efficacious in supporting an increase in the betaine-dependent regeneration of homocysteine to methionine. However, we detected no significant difference in the plasma homocysteine concentration between day 14 and day 0 in children with CF assigned to take choline or lecithin. Possibly, the latter results are explained by greater efficacy of preformed betaine than of its choline precursor in supporting the remethylation of homocysteine.

Recent studies have led to an increased understanding of the importance of oxidative stress and glutathione system dysfunction in many diseases (21), including many of the complications of CF (13–18), particularly those associated with the immune system (13–18, 48, 49). Glutathione plays a pivotal role in detoxifying reactive molecules generated by mitochondria and microsomal CYP-450 enzymes and in maintaining reduced sulfhydryl groups on molecules involved in cell proliferation, apoptosis, energy production, and calcium homeostasis (50). Glutathione is also exported to the extracellular spaces, such as the epithelial lining fluid of the lung, and to immune cells where it serves an important antioxidant function (21, 22). The intracellular glutathione status depends on precursor availability, the rate of glutathione oxidation to GSSG, and the capacity to recycle GSSG back to glutathione at the expense of NADPH (21, 22). In our study, we measured the plasma glutathione, most of which is derived by export from the liver; GSSG, however, is exported from cells as an important mechanism to maintain a high intracellular glutathione:GSSG (36). The glutathione:GSSG is often used as an indicator of the cellular redox status and is >10 under most normal physiologic conditions (21). In our study, the glutathione:GSSG in children with CF was <5 and $\approx 25\%$ that of the control children, consistent with numerous reports of oxidative stress in CF (13–18). We found that glutathione:GSSG was ≈ 45 – 60% higher after 14 d of supplementation than before supplementation with choline-related metabolites, which was statistically significant in those children with CF given choline or betaine. Because SAM activates cystathionine β -synthase activity (21) and because our results showed that supplementation with methyl groups from choline increased SAM and SAM:SAH, it is possible that entry of homocysteine to the transsulfuration pathway and glutathione synthesis was also increased. Supplementation with betaine of children with CF, however, was associated with a significant decrease in the elevated plasma concentrations of adenosine and homocysteine, both of which

when elevated are associated with increased oxidative stress. Whether a reduction in oxidative stress secondary to reduced adenosine and homocysteine is related to the increased glutathione:GSSG in the children in our study given supplements of betaine is unclear, as is the reason for the accompanying decrease in the plasma cysteine, which is the metabolic precursor to glutathione (21).

The results of this study provide evidence that supplementation with choline-related metabolites (choline, lecithin, and betaine) may improve the abnormal membrane lipid composition in children with CF. Supplementation with choline, possibly by providing increased choline to support phosphatidylcholine synthesis, was associated with a significant increase in the RBC membrane ratio of phosphatidylcholine to phosphatidylethanolamine. However, neither the physiologic significance nor the relevance to other cell membranes can be addressed by our results.

In summary, our studies provide evidence that the metabolism of methionine, homocysteine, and choline are interrelated in humans. We have shown that choline or betaine supplementation of children with mutations in CFTR, resulting in the clinical spectrum of CF, results in an increased plasma concentration of methionine and increased SAM:SAH and glutathione:GSSG. Possibly, chronic malabsorption of choline-containing phospholipids in CF results in depletion of choline, which may be further compromised by a decrease in de novo choline synthesis resulting from a low SAM:SAH in children with CF (6, 28, 29, 45) (Table 2). Supplementation with methyl groups as choline may provide an effective intervention through conservation of methionine and increased SAM, which could have beneficial clinical effects related to choline availability and oxidative stress in patients with CF. Alternatively, supplementation with betaine could provide an effective intervention to reduce homocysteine and oxidative stress and to increase the recycling of homocysteine to methionine. Further studies are needed to consider the efficacy of different supplements, such as choline and betaine, and to identify the appropriate doses in studies of sufficient duration to consider the clinical relevance of these interventions.

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SMI was the principal investigator and helped with grant funding, with the study concept and design, and with the manuscript and data preparation. AGFD participated as the clinician scientist in patient selection, patient enrollment, and collection of clinical information. SJJ and SM measured the plasma thiols. All of the authors contributed to the review and revision of the manuscript. None of the authors had a conflict of interest.

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