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Choline Supplementation with a Structured Lipid in Children with Cystic Fibrosis: A Randomized Placebo-Controlled Trial

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Author Contributions to Submitted Work

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Abstract

Background—Choline depletion is seen in cystic fibrosis (CF) and pancreatic insufficiency (PI) in spite of enzyme treatment and may result in liver, fatty acid and muscle abnormalities. This study evaluated the efficacy and safety of an easily absorbed choline-rich structured lipid (LYM-X-SORB™ [LXS]) to improve choline status.

Methods—Children with CF and PI were randomized to LXS or placebo in a 12-month double blind trial. Dietary choline intake, plasma cholines, plasma and fecal phospholipids, coefficient of fat absorption (CFA), pulmonary function, growth status, body composition, and safety measures were assessed. Magnetic resonance spectroscopy for calf muscle choline and liver fat were assessed in a subgroup and compared to a healthy comparison group matched for age, sex and body size.

Results—110 subjects were enrolled (age 10.4±3.0 years). Baseline dietary choline, 88% recommended, increased 3-fold in the LXS group. Plasma choline, betaine, and dimethylglycine increased in the LXS but not placebo ($P=0.007$). Plasma lysophosphatidylcholine and phosphatidylcholine (PC) increased and fecal PC/phosphatidylethanolamine ratio decreased ($P=0.05$) in LXS only, accompanied by a 6% CFA increase ($P=0.001$). Children with CF had higher liver fat than healthy children and depleted calf muscle choline at baseline. Muscle choline concentration increased in LXS and was associated with improvement in plasma choline status. No relevant changes in safety measures were evident.

Conclusions—LXS had improved choline intake, plasma choline status and muscle choline stores, compared with placebo. The choline-rich supplement was safe, accepted by participants and improved choline status in children with CF.

Keywords

nutritional supplement; plasma choline status; phospholipids; efficacy; safety

INTRODUCTION

Suboptimal choline status with decreased plasma concentrations and related metabolic changes have been reported in children and adults with cystic fibrosis (CF) and pancreatic insufficiency (PI) treated with pancreatic enzyme medications¹⁻⁵. Factors contributing to suboptimal choline status in CF include lower dietary intake and persistent malabsorption of dietary and biliary phosphatidylcholine^{1-3;5-9}. Choline is part of cell membrane

phospholipids, plasma lipoproteins, acetylcholine neurotransmitters, and contributes to methyl-group/one-carbon metabolism¹⁰. Choline deficiency results in increased liver enzymes, development of hepatic steatosis, altered delivery of essential fatty acids to peripheral tissues, muscle abnormalities, and depleted acetylcholine^{9;11;12}. Recent reviews provide detail on choline metabolism and health¹³⁻¹⁵. The potential of choline supplementation to improve choline status and impact clinical outcomes in patients with CF has not been well studied.

The study aim was to evaluate the safety and effectiveness of LYM-X-SORB™ (LXS)¹⁶, an easily absorbed choline-rich structured lipid (BioMolecular Products, Byfield, MA; Avanti Polar Lipids, Alabaster, AL)¹⁷ to improve choline status in a randomized double-blinded placebo-controlled trial in children with CF and PI. Dietary intake, plasma choline compounds, fecal choline compounds and muscle and liver MRS were assessed, and effect on clinical outcomes was explored.

METHODS

Subjects and Protocol

Study design details have been described^{18;19}. Subjects aged 5.0 to 17.9 yrs with CF, PI were recruited from ten CF Centers. Enrollment began in March 2007, and the final protocol visit occurred in May 2011. Exclusion criteria included FEV₁ <40% predicted, residual pancreatic activity (fecal elastase >15ug/g stool), liver disease (GGT >3× range) or chronic conditions affecting growth, diet, or nutritional status. Study visits completed at The Children's Hospital of Philadelphia (CHOP) at baseline, 3, and 12 months were approved by the CHOP Institutional Review Board and each CF Center. Verbal assent was obtained from subjects 6.0 to <18.0 years and written consent from parents/legal guardians of subjects 18 years. This protocol was registered as: Study of LYM-X-SORB™ to Improve Fatty Acid and Choline Status in Children with Cystic Fibrosis and Pancreatic Insufficiency, NCT00406536.

In this double-blind study, subjects were randomized to 12 months of daily supplementation with either LXS or placebo. LXS powder was mixed with participant-selected foods and beverages and is comprised of lysophosphatidylcholine (LPC), monoglycerides and fatty acids in a 1:4:2 molar ratio. LPC was prepared from soy lecithin (Lipoid, Newark, NJ). LPC is water soluble, does not require lipase for digestion/absorption and fosters absorption of lipids¹⁷. Monoglycerides (Danisco, Madison, WI), flaxseed (Danisco, Denmark), oleic acid (Acme-Hardesty Co., Blue Bell, PA) and palmitic acid (Amsyn Inc., Stamford, CT) were complexed to sugar (American Sugar Refining, West Palm Beach, FL) and wheat flour (John R. White Co., Birmingham, AL). The placebo was a powder of similar taste and consistency, composed of trans-fat free vegetable shortening (JM Smucker Co., Orville, OH), flaxseed oil triglyceride (Barlean's Organic Oils, Ferndale, WA), and sunflower oil triglyceride (Botanic Oil Innovations, Spooner, WI). The placebo and LXS had similar calories (157 kcal/packet), total fat, and macronutrient distribution (protein 6%, carbohydrate 58%, lipid 34% kcal). LXS contained more choline than placebo (295.5 vs 39 mg/packet). Subjects aged 5.0-11.9 yrs received two packets/day (64 g powder), and aged 12.0-17.9 yrs received three packets/day (96 g powder) and continued on pancreatic enzyme

medication as prescribed by their CF center. Choline supplement intake for LXS and placebo, respectively, was 591 and 78 mg/d in younger and 886.5 and 117 mg/d in older children. LXS is stable a minimum of 3 months at room temperature, 12 months at 5°C and 48 months at 20°C storage conditions. The absorbability of LXS was demonstrated by Lepage et al¹⁷. LXS with a triacylglycerol-based (TG) formula in five adolescents with CF compared to three adult controls, demonstrated a 10-fold higher absorption of triacylglycerols with LXS than with the TG-based formula in the children with CF, and this was comparable to absorption in the healthy controls. Therefore, LXS was highly absorbable and did not require the use of pancreatic enzyme medication to be absorbed.

Measurements

Body mass index (BMI) was calculated (kg/m^2) from weight using a digital scale (Scaletronix, White Plains, NY) and standing height using a stadiometer (Holtain, Crymych, UK). Weight, height and BMI were compared to Centers for Disease Control reference standards to generate age- and sex-specific Z scores²⁰. Total body fat mass, lean body mass, and percentage fat were measured by whole-body dual-energy X-ray absorptiometry (DXA; Delphi A, Hologic, Inc., Bedford, MA). Pubertal status was determined by self-assessment²¹.

Dietary intake was assessed using 3-day weighed food records and analyzed (Nutrition Data System, Minneapolis, MN). Energy intake was reported as kcal/day and as percent Estimated Energy Requirement (%EER) for active children^{13;22}. Choline intake was expressed as percent Adequate Intake (%AI)¹³. Subjects completed 72-hour home stool collections for coefficient of fat absorption (CFA) (Mayo Medical Laboratories, Rochester, MN)²³. For this study, a CFA of <80% is used as a cut-off for moderate fat malabsorption.

Pulmonary function was assessed and predicted percentage FEV₁ calculated^{24;25}. Complete blood count with differential, comprehensive metabolic panel, gamma glutamyl transferase (GGT), homocysteine, cysteine, methionine, alkaline phosphatase (liver and bone-specific), and urinalysis were assessed (Clinical Laboratory, CHOP). High sensitivity C-reactive protein (HS-CRP) was determined (ARUP Laboratories, Salt Lake City, UT).

Phospholipids were extracted from plasma with methanol²⁶ containing internal standards for lysophosphatidylcholine (LPC), phosphatidylcholine (PC), sphingomyelin (SM), lysophosphatidylethanolamine (LPE), phosphatidylethanolamine (PE), lysophosphatidylinositol (LPI), phosphatidylinositol (PI), lysophosphatidylserine (LPS), and phosphatidylserine (PS). Each sample extract was assayed on a Waters' Acquity ultra-performance liquid chromatograph (Waters Corporation, Milford, MA) AB Sciex 5500 mass spectrometry system (Framingham, MA). Phospholipid extracts for each class were injected on an Agilent (Santa Clara, CA) Eclipse XDB-C8 reversed phase column for separation of molecular species by gradient elution and detected by mass spectrometry. A National Institute of Standards and Technology human plasma reference material (SRM 1955)²⁷ was the control. The phospholipid class concentrations calculated as sum of molecular species per class expressed as $\mu\text{mol/L}$ and mol%.

Choline compounds were quantified from calibration curves generated from reference materials (SigmaAldrich-Fluka, St. Louis, MO) of betaine, dimethylglycine (DMG), choline chloride, cytidinediphosphocholine (CDPC) sodium salt and glycerylphosphorylcholine (GPC). Internal standard compounds were choline chloride-d9, N,N –dimethylglycine hydrochloride-d6 (SigmaAldrich-Fluka), and 44.4 uM N-carboxymethyl-trimethyl ammonium salt-d9 (CDN Isotopes, Point Claire, Quebec). Calibration standards and plasma extracts were assayed as previously reported^{2,28,29} The individual compound concentrations were expressed as $\mu\text{mol/L}$. Plasma choline concentrations $<7 \mu\text{mol/L}$ were defined as suboptimal¹⁵.

Aliquots of 72-hour stool samples were extracted for phospholipid analysis by phosphorus - nuclear magnetic resonance³⁰ (³¹P-NMR) using a combination of a modified Folch³⁰ extraction followed by cold acetone precipitation of the polar lipids. ³¹P-NMR analysis was performed on a Bruker Avance 400 MHz NMR (Bruker, Billerica, MA). Results were calculated as mol% and μg phospholipid per gm wet weight stool. When detectable, the lyso counterparts of PE, PI and PS were combined into the respective totals.

A subsample of subjects with CF ages 10 y participated in magnetic resonance spectroscopy (MRS) study of liver and calf soleus muscle at baseline (n=26) and 12 months (n=14) to determine total liver fat and calf muscle and intra-/extra-myocellular fat and choline content. A comparison group of healthy children (n=10), matched for age, sex, and body size were recruited from Charlottesville, VA for one MRS assessment. The same protocol and model scanner was used for both healthy and CF subjects. MRI/MRS has been used to study hepatic steatosis, to quantify hepatic triglyceride content, and to determine muscle lipids and choline contents³¹⁻³⁴. The MRS scan was performed following an 11-hour fast using a 1.5T whole body scanner (Magnetom Avanto, Siemens Medical Solutions, Malvern, PA) with body array and extremity coils. Liver MRS scan without water suppression using Stimulated Echo Single Voxel Spectroscopy (STEAM/SVS) technique, with 4 sec TR and 20 msec TE, was used to acquire fat and water signals with minimal relaxation effects. A volume of interest (18cc) was selected in the right liver lobe away from major vessels. Chemical Shift Imaging (CSI) technique, (FOV = $8 \times 8 \times 1 \text{cm}$, voxel = $0.5 \times 0.5 \times 1 \text{cm}$) with and without water suppression (TE = 30msec, TR = 1690/4000 msec), centered on the calf muscle, was used to measure muscle lipid contents and choline peak. The liver lipid fraction, calf (intra- and extra-myocellular) lipid fractions were calculated as lipid divided by lipid plus water content, and muscle choline calculated as choline divided by water content.

Subjects received a 32-day supply of LXS or placebo every 28 days. Adherence was measured via packet counts by monthly telephone assessments. Adherence to LXS or placebo was estimated based upon a percentage calculated from packets used/total packets and was assessed for each 28-day period in the study to adjust LXS or placebo intake for adherence. The percentage adherence most proximate (within a month) to the time of the dietary intake assessment was used at 3 months and then again at 12 months. Average percent cumulative adherence was also calculated for two time intervals (baseline until 3 months and baseline until 12 months). Nutrient intake from LXS and placebo supplements was adjusted using proximate adherence estimate to the 3 and 12 month diet record.

Data Analysis

Descriptive statistics were presented as frequency counts and percentages for categorical variables and mean $\pm$ standard deviation (SD) or median (range) depending on skewness for continuous variables. Two-sample *t*-test or Mann-Whitney U test for continuous variables and chi-square tests of independence for categorical variables were used to compare characteristics at baseline.

Changes in outcome measures over time (baseline, 3, 12 months), the main effect of randomization groups, and randomization group (R) by time (T) interactions on outcomes were investigated on an intent-to-treat basis using mixed-effects linear regression models accounting for correlations arising from repeated-measures. Mixed models allow using all data available from each subject under the assumption of missing at random. Whether changes in outcomes over time differed by randomization groups were evaluated by examining the interaction effects of group by time ($R \times T$). If the residuals from the models were not normally distributed, log transformations were applied and reported. Sensitivity analyses were performed to examine the potential outliers and influential points. Preliminary analyses omitting these outliers did not influence results. For the analysis of the change in plasma and fecal phospholipid outcomes by randomization group from baseline to 3 months, longitudinal mixed effects models were used controlling for baseline values within each group separately and then for the group by time interaction. Associations between outcomes at each time point were also assessed using Pearson or Spearman rank correlation coefficients as appropriate depending upon skewness.

Exploratory analyses were performed using random effects models to assess associations among baseline choline and change () in choline status variables and clinical outcomes (CFA, growth status, pulmonary function) at 3 and 12 months. For prediction of MRS muscle choline at 12 months, regression models were used. Potential predictors included age, sex, adherence, dietary choline, plasma cholines, LPC and PC (both plasma and stool). Stata 13.1 (Stata Corporation, College Station, TX) was used, and statistical significance level was set at $P = 0.05$ for all tests.

RESULTS

There were 110 subjects recruited, 56 randomized to placebo and 54 to LXS supplementation (Table 1). Twenty-four subjects after baseline (10 placebo, 14 LXS) and 16 subjects after 3 months (7 placebo, 9 LXS) withdrew from the study. Subjects were 10 ± 3 y old, 53% pre-pubertal, 57% male, 57% F508 homozygous, with suboptimal growth status and mild to moderate pulmonary disease. Suboptimal plasma choline ($<7 \mu\text{mol/L}$) was seen in 45% of subjects. There were no significant differences at baseline between randomization groups.

Intake of energy, total fat, and choline, and fat absorption, plasma choline status and serum amino acids at baseline, 3 months and 12 months are presented in Table 2. Intake of calories increased modestly over 12 months. Both LXS and placebo provided 300 to 450 kcal/d depending on age. We previously documented dietary intake compensation with some decrease in calories from food with the increased intake from supplements¹⁸. Choline intake

increased slightly in the placebo (16%) while there was a nearly 3-fold increase in the LXS group (randomization group $\times$ time interaction [$R \times T$], $P < 0.001$), with participants receiving choline %AI of $>230\%$. Cumulative adherence to supplements did not differ by randomization group: 76 vs. 80% at 3 months, and 71 vs. 75% at 12 months for LXS and placebo, respectively. Proximate adherence at the time of dietary intake assessment also did not differ for LXS and placebo.

The CFA improved in both groups ($P = 0.044$), but the increase was greater (6%) in the LXS than in the placebo group (2%) (Table 2). For subjects in the LXS group, 39% had moderate fat malabsorption (CFA $<80\%$) at baseline and this proportion declined to 11% at 3 months ($P = 0.013$) and this decline was sustained through 12 months (15%, $P = 0.025$). There was no significant change over time in the proportion of subjects with CFA $<80\%$ in the placebo group.

Plasma choline increased in LXS and decreased in the placebo group (randomization group $\times$ time [$R \times T$], $P = 0.007$) (Table 2). At baseline, there was no difference between groups in suboptimal choline status (% $<7 \mu\text{mol/L}$; 38% and 52% for LXS and placebo, respectively). At 12 months, there were significantly fewer subjects with suboptimal status in the LXS group compared to placebo (29% and 59%, respectively, $P = 0.012$). Total plasma water soluble cholines, betaine and DMG also increased over time with LXS with no change in the placebo group ($R \times T$, $P < 0.001$). Homocysteine concentration increased by 12 months in the placebo group ($P = 0.012$), while methionine decreased in the LXS group ($P = 0.046$). As a result, the homocysteine/methionine ratio increased in the LXS group ($P = 0.025$) with no change in placebo.

Plasma fat soluble phospholipids, and fecal phospholipids are presented in Table 3 at baseline and 3 months. Within the LXS group only, LPC, LPE and PE all increased and the PC/PE ratio decreased ($P < 0.03$). There was a significant $R \times T$ interaction for SM which decreased in LXS and not in the placebo group. For fecal phospholipids, PC declined over 3 months and the PC/PE declined significantly in the LXS group only, resulting in less PC relative to PE eliminated. The CFA was significantly associated with improved plasma choline status at baseline in the total sample. CFA was positively correlated with plasma total water soluble cholines ($r = 0.19$, $P = 0.04$), and negatively correlated with both the PC in stool ($r = -0.50$, $P < 0.001$) and the PC/PE ratio in stool ($r = -0.57$, $P < 0.001$), indicating less loss of choline in the stool.

Safety outcomes, growth status and body composition by randomization group at baseline, 3 months and 12 months are presented in Table Supplemental Digital Content 1. Both LXS and placebo were safe with no clinically significant changes over time. Weight and BMI status improved in both groups with significant gains in both FFM and FM, and the expected decline in FEV_1 was observed as the subjects aged.

Table 4A presents the results for MRS of the calf muscle and liver in 26 children with CF (13.4 ± 2.2 y, 69% male) at baseline compared to ten healthy children of similar age, sex and growth status. Children with CF had significantly lower calf muscle choline fraction ($P = 0.011$) than the healthy subjects, and a 6 to 7 fold greater liver lipid fraction ($P = 0.007$).

Fourteen children with CF had both baseline and 12 month MRS to assess change over time with LXS supplementation (Table 4B). Calf muscle choline increased significantly after 12-month LXS supplementation ($P = 0.034$) to a level comparable with the healthy children (Table 4A). Calf muscle lipid fraction increased significantly in both groups.

We explored whether baseline choline status variables (plasma choline, betaine, LPC, PC, choline intake) and the variable status change (, increase/decrease) predicted change from baseline in clinical outcomes at 3 and 12 months using random effects models. Age, sex and baseline values for each outcome were covariates in the models. Improved CFA was negatively predicted by PC in stool (β coefficient [β]: -0.006 , 95% confidence intervals [CI]: -0.010 , -0.002 , $P = 0.002$) and by baseline PC/PE stool ratio (β : -1.40 , CI: -2.78 , -0.03 , $P = 0.046$) and PC/PE stool ratio (β : -1.99 , CI: -3.21 , -0.77 , $P = 0.001$). FEV₁% was positively predicted by LPC (β : 0.007 , CI: 0.001 , 0.012 , $P = 0.013$), and PC (β : 0.070 , CI: 0.26 , 0.114 , $P = 0.002$). In regression models, significant predictors of increased MRS muscle choline at 12 months were baseline betaine (β : 0.002 , CI: 0.001 , 0.004 , $P = 0.016$), betaine (β : 0.004 , CI: 0.002 , 0.006 , $P = 0.002$), and total plasma cholines (β : 0.003 , CI: 0.001 , 0.004 , $P = 0.008$).

DISCUSSION

This 12-month randomized, double-blind placebo-controlled trial in children with CF and PI demonstrated the safety and efficacy of LXS, a well absorbed, choline rich structured lipid, to improve choline intake, plasma choline (water soluble cholines and phospholipids), skeletal muscle choline and fecal excretion. The LXS and placebo supplements were near identical in calories and fat grams and LXS contained about eight times more choline; both powders were added by participants to foods they selected with similar adherence. LXS and placebo doses were age adjusted, and provided a mean choline of 591 or 887 mg/d in LXS and 78 or 117 mg/d in placebo in two or three supplement packets, respectively. Recommended choline intake for healthy children in our age range (5 to 18 yr) varies: 250 to 550 mg/d³⁵. Adverse events and serious adverse events were similar between groups, and none attributable to supplements. Plasma choline, betaine, DMG and total water soluble cholines significantly increased in the LXS group over 3 and 12 months. Subjects receiving LXS had increased plasma LPC, LPE, and PE and decreased SM and plasma PC/PE ratio over 3 months. Significantly less PC was lost in the stool overall and relative to PE (PC/PE stool ratio) in the LXS group with no change for placebo. The CFA improvement was greater in LXS than placebo. Both groups showed increases in weight and BMI as expected as the children aged over the years. Further analyses using the pooled sample for changes in choline status and clinical outcomes revealed that decreased PC loss in the stool was associated with improved CFA. Increased LPC and PC over time positively predicted FEV₁. Calf muscle choline content increased significantly in the LXS group, and improvement in plasma choline status significantly predicted this increase in muscle choline content.

Lepage, et al¹⁷ published the initial CF LXS study and showed LXS was easily absorbed. Fat absorption without enzymes was similar in subjects receiving LXS to that of healthy subjects. The LXS water soluble LPC content did not require lipase for digestion. They showed improved caloric intake, weight, essential fatty acid and vitamin E status in LXS

compared to placebo participants. The study design, general CF health status and standards of care differ in several ways between 2002 Lepage et al¹⁷ and our current study. Lepage et al study participants had worse nutritional and pulmonary status compared with our similar aged subjects in the current study. The original study was not blinded and the placebo (commercial supplement drink) and LXS (wafer cookie) were similar in calories, but not nutrient content. Reported results excluded subjects who withdrew (8%) as well as those categorized as non-compliant (26%), and thus omitted 34% of enrolled subjects and results were reported for only the more compliant subjects¹⁷. In both Lepage et al¹⁷ and current study, LXS improved caloric intake and weight Z score. The current study showed improved intake and weight status in the placebo group as well, using a placebo that was similar in calories and composition (except choline), and both were well accepted. As recently reported, Groleau et al¹⁹ showed improved energy intake, growth, and muscle and fat mass in both groups suggesting the improved growth was the result of improved energy intake, regardless of source.

Suboptimal choline status with decreased plasma concentrations and related metabolic changes have been reported in both children and adults with CF and PI^{2-5;36}. Guerrero et al⁴ demonstrated significantly altered phospholipid status with decreased LPC and PC and increased SM species in children and young adults with CF compared to their healthy siblings. The decrease in PC species was more pronounced for those with more severe CF⁴. In our study, 45% of children with CF had suboptimal choline status at baseline (choline concentrations < 7 $\mu\text{mol/L}$), and supplementation with LXS improved choline status as indicated by increased plasma choline, betaine, DMG, and LPC and decreased SM.

Innis and colleagues^{2;3;6;37;38} have made many contributions to the understanding of CF phospholipid metabolism. Choline depletion in healthy people results in hepatocyte fat accumulation from reduced PC synthesis for VLDL production for transport of triglycerides from the liver, and may have impact on CF liver disease. Innis et al³ demonstrated the altered plasma phospholipid and methionine metabolism in children with CF and PI compared to healthy children. More dietary and biliary circulation phospholipids were lost in the stool fat, in addition to more long and medium chain fatty acids³⁷. Examining the stool⁶, they showed increased excretion of PC and LPC in CF compared to controls. Associations were seen among the excess stool phospholipid excretion and decreased plasma methionine and increased homocysteine. Dietary choline intake⁶ did not differ between the CF and control children, and plasma choline was not reported. In our study, there was a decrease in stool excretion of PC with LXS treatment accompanied by an improvement in the CFA. Providing the readily absorbable LXS choline and fat supplement contributed to normalizing choline balance. Our baseline dietary choline intake of approximately 300 mg/d was comparable to that in Chen et al⁶, increased slightly with placebo and nearly three-fold to 812 mg/d in with LXS supplementation.

In the only other CF choline supplementation study, Innis et al⁷ treated 34 children with CF and 15 control children for 14 days with: lecithin providing 0.3 g/d choline, choline at 1.85 g/d or betaine at 3 g/d. Baseline results confirmed the previous findings of choline depletion in children with CF² with plasma choline, betaine and DMG lower than controls. Decreased choline metabolites were correlated with increased homocysteine, and the authors suggested

this may contribute to CF liver complications. After supplementation at these higher doses, the abnormal methionine-homocysteine metabolites and glutathione status improved⁷. In a non-CF sample of healthy postmenopausal women, Wallace et al³⁶ investigated the effect of 1g/day choline supplementation vs. placebo over 12 weeks on choline and homocysteine status, and found that choline supplementation significantly increased plasma choline, betaine and DMG. However, there was no significant change in homocysteine or methionine concentrations, possibly due to the relatively low baseline plasma homocysteine and low risk for hyperhomocysteinemia in these healthy postmenopausal women³⁶. In our study, plasma choline, betaine and DMG all increased significantly with LXS supplementation, without normalization of methionine or homocysteine. This may be related to our lower choline dose (591 or 887 mg/day)^{7;12;39} compared to other studies, and also to the relatively low homocysteine levels of our children with CF and PI. We have previously demonstrated high supplemental vitamin B₁₂ intake in these children that resulted in high serum B₁₂ that was associated with reduced homocysteine concentrations⁴⁰.

In a recent CF-specific study, Grothe et al⁵ investigated plasma phosphatidylcholine alterations in a small sample of adults with CF compared to healthy controls, and found significantly lower choline and PC concentrations in CF. Better choline status was associated with better lung function (increased FEV₁) and inflammatory status (decreased interleukin 6 concentrations) in the CF group⁵.

Typically, healthy people absorb about 93% of their dietary fat⁴¹. People with CF and PI rarely achieve this level even with optimized enzyme replacement therapy, and typically average from 77 to 88% CFA in research studies^{23;42}. In our pediatric CF research experience, mean CFA ranges from 81 to 87% in the modern care setting^{23;43}. Improvement from a mean 84% to 90% CFA in the LXS group was a clinically significant shift toward optimizing fat absorption, and the proportion with 80% CFA increased from 61 to 85% with LXS. The loss of choline in the stool may result from enterohepatic bile PC cycle impairment and contributes to chronic poor choline status in CF^{5;6}. The improvement in CFA was significantly associated with the decreased loss of PC (both actual and relative to PE) in the stool in our study, suggesting that fat delivered in LXS was more readily absorbable and reduced excess stool fat and choline excretion.

Although MRE (Magnetic Resonance Elastography)/MRI/MRS are research tools to assess liver disease⁴⁴, they are rarely used clinically. To the best of our knowledge, no MRS choline/lipid data are available in CF. The liver fat fraction was higher in CF subjects than in healthy controls and with little change over 12 months. Others have found that the prevalence of CF liver disease increases in childhood and into mid-adolescence, with no significant increase thereafter^{45;46}. Since our MRS subsample was 10 years old, these subjects may be in the stage of elevated but stable or slowly progressing liver steatosis, and no change was evident with supplementation. Future research may consider evaluating LXS supplementation in younger children with CF-liver disease.

Choline measurement with MRS has gained popularity as choline is a marker of cellular membrane turnover. However its metabolic role in muscle is less understood and literature is scarce. One MRS muscle study by Hsieh et al⁴⁷ showed that subjects with Duchenne

muscular dystrophy had lower choline peak and tCr (total creatine, creatine + phosphocreatine) compared to healthy controls. They found a positive correlation between the muscle choline fraction and the leg muscle function. In our study, an increase in calf muscle choline over 12 months was found only in the LXS group, associated with improved plasma choline status and may indicate improved muscle health. In a study of choline transport and fat metabolism in a choline deficient muscle cell model, choline availability was shown to affect the composition of muscle cell membrane lipids and intracellular lipid metabolism⁴⁸. Our results are intriguing and add to the general finding of improved choline status with LXS supplementation, however, additional investigation is needed to further evaluate whether changes in muscle choline status significantly impact muscle health.

In summary, LXS was an effective and safe choline, calorie and fat delivery system. Increased choline in plasma and calf muscle, and decreased stool choline loss demonstrated improved choline status for subjects taking LXS. These changes were accompanied by evidence of improved dietary fat absorption suggesting a possible role for LXS-containing products to improve nutritional and growth status and reduce malabsorption in patients with CF and PI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is Known

- Choline depletion is common in children and adults with CF and PI and may result in liver and metabolic abnormalities.

What is New

- A randomized double blind clinical trial showed supplementation with an easily absorbed, choline-rich structured lipid, LYM-X-SORB™ (LXS) was safe and effective in delivering choline, calories and fat in children with CF and PI.
- Choline supplementation increased plasma and muscle choline concentrations and decreased choline excretion in stool.
- LXS improved dietary fat absorption with positive effects on growth and nutritional status.

Table 1
Characteristics at baseline for Placebo and LXS groups

Characteristic	All n=110	Placebo n=56	LXS n=54
Sex, % males	57	59	56
F508 homozygous, %	57	55	60
Age, yr	10.4 ± 3.0	10.2 ± 2.9	10.6 ± 3.1
Pubertal Status, %			
Prepubertal	53	55	51
Pubertal	47	45	49
Growth and Body Composition			
Height for age Z score	-0.41 ± 0.92	-0.46 ± 0.94	-0.36 ± 0.90
Weight for age Z score	-0.39 ± 0.78	-0.36 ± 0.80	-0.43 ± 0.77
BMI for age Z score	-0.20 ± 0.77	-0.10 ± 0.74	-0.30 ± 0.81
Whole Body DXA			
FFM, kg	23.7 (13.3 - 65.3)	22.6 (13.3 - 61.4)	24.1 (14.1 - 65.3)
FM, kg	6.5 (3.1 - 18.9)	6.5 (3.1 - 14.7)	6.6 (3.2 - 18.9)
Fat, %	21.4 ± 5.6	21.6 ± 5.5	21.2 ± 5.7
Pulmonary Function			
FEV ₁ , % predicted	95 ± 23	97 ± 24	93 ± 21
Plasma Cholines, µmol/L			
Total water soluble choline	45.8 ± 11.5	45.8 ± 11.6	45.7 ± 11.5
Choline	7.6 ± 1.8	7.4 ± 1.8	7.8 ± 1.9
Choline suboptimal status <7, %	45	52	38
Betaine	34.0 ± 10.3	34.2 ± 10.0	33.8 ± 10.7

FFM: fat free mass; FM: fat mass; Fat: fat mass as percent total body mass; FEV₁: Forced expiratory volume at 1 second percent predicted value.

Data are presented as mean ± SD for normally distributed and median (minimum - maximum) for skewed data and frequency (percentage) for categorical data.

No significant differences between randomization groups were observed.

Table 2
Dietary Energy, Fat and Choline Intake, Fat Absorption, and Plasma Choline Status in Placebo and LXS Groups at Baseline, 3 and 12 Months

Variable	n	Baseline	n	3 months	n	12 months	P1	P2
Dietary Intake								
Energy Intake, kcal/d								
Placebo	54	2438 ± 798	37	2609 ± 563	35	2618 ± 677	0.386	0.954
LXS	44	2423 ± 618	31	2567 ± 580	23	2705 ± 644	0.435	
EER, %								
Placebo	54	119 ± 35	37	123 ± 29	35	123 ± 31	0.940	0.951
LXS	44	119 ± 26	31	123 ± 23	23	124 ± 27	0.849	
Fat, g/d								
Placebo	54	91 (35 - 227)	37	97 (51 - 180)	35	101 (55 - 203)	0.273	0.869
LXS	44	92 (55 - 188)	31	93 (41 - 173)	23	88 (59 - 183)	0.906	
Fat, % kcal								
Placebo	54	36 ± 6	37	35 ± 6	35	36 ± 4	0.158	0.355
LXS	44	36 ± 5	31	34 ± 7	23	35 ± 5	0.139	
Choline, mg								
Placebo	54	308 (144 - 736)	37	358 (193 - 773) ^{††}	35	361 (183 - 765) ^{††}	0.001	<0.001
LXS	44	291 (129 - 735)	31	831 (444 - 1691) ^{††}	23	812 (244 - 1727) ^{††}	<0.001	
Choline, % AI								
Placebo	54	88 (37 - 288)	37	92 (52 - 309) [†]	35	108 (49 - 292) ^{††}	0.014	<0.001
LXS	44	87 (34 - 221)	31	248 (134 - 524) ^{††}	23	235 (61 - 350) ^{††}	<0.001	
Coefficient of Fat Absorption, %								
Placebo	41	89.7 (51.9 - 96.7)	34	91.3 (71.0 - 99.6)	30	91.8 (54.2 - 97.2)	0.044	0.652
LXS	36	83.9 (58.3 - 97.4)	27	89.5 (72.1 - 98.9) [†]	20	90.3 (69.4 - 95.8) ^{††}	0.001	
Plasma Choline, umol/L								
Total Water Soluble								
Placebo	56	43.4 (27.6 - 78.1)	46	42.1 (30.9 - 70.9)	39	41.1 (28.2 - 70.9)	0.357	<0.001
LXS	52	44.7 (26.7 - 80.9)	40	55.7 (19.4 - 127) ^{††}	31	51.8 (30.8 - 105) ^{††}	<0.001	

Variable	n	Baseline	n	3 months	n	12 months	P1	P2
Choline								
Placebo	56	6.9 (4.2 - 12.8)	46	7.1 (4.9 - 11.5)	39	6.6 (3.6 - 11.4) [‡]	0.014	0.007
LXS	52	7.6 (4.6 - 12.2)	40	9.3 (3.9 - 15.6) ^{††}	31	7.9 (4.1 - 12.8)	0.001	
Betaine								
Placebo	56	31.5 (19.6 - 61.5)	46	31.5 (20.1 - 53.3)	39	31.8 (20.7 - 59.8)	0.405	<0.001
LXS	52	32.6 (15.8 - 72.1)	40	42.8 (13.1 - 108) ^{††}	31	39.5 (19.6 - 86.1) ^{‡‡}	<0.001	
Dimethylglycine (DMG)								
Placebo	56	2.4 (1.0 - 6.0)	46	2.3 (1.0 - 5.7)	39	2.4 (0.6 - 6.7)	0.751	<0.001
LXS	52	2.7 (1.2 - 5.0)	40	3.1 (1.5 - 7.9) ^{††}	31	2.9 (1.2 - 5.4) [‡]	<0.001	
Choline/Betaine Ratio								
Placebo	56	0.23 ± 0.06	46	0.23 ± 0.07	39	0.21 ± 0.06	0.066	0.012
LXS	52	0.25 ± 0.08	40	0.21 ± 0.07 ^{††}	31	0.21 ± 0.07 ^{‡‡}	<0.001	
Serum Amino Acids								
Homocysteine, µmol/L								
Placebo	56	4.9 ± 1.3	46	4.9 ± 1.4	39	5.3 ± 1.5 [‡]	0.012	0.306
LXS	54	5.4 ± 1.3	40	5.3 ± 1.4	31	5.9 ± 1.3	0.105	
Cysteine, nmol/mL								
Placebo	56	45.3 (25.0 - 66.3)	46	45.6 (21.6 - 62.8)	39	44.2 (27.1 - 102)	0.933	0.307
LXS	54	43.0 (12.4 - 79.0)	40	46.2 (29.2 - 70.0)	31	43.5 (31.1 - 63.6)	0.066	
Methionine, nmol/mL								
Placebo	56	25.2 (14.3 - 65.7)	46	24.1 (15.2 - 63.2)	39	25.1 (16.4 - 67.3)	0.619	0.069
LXS	54	23.9 (11.1 - 56.3)	40	24.3 (8.00 - 67.6)	31	22.2 (12.0 - 40.1)	0.046	
Homocysteine/Methionine Ratio								
Placebo	56	0.18 (0.07 - 0.60)	46	0.20 (0.05 - 0.50)	39	0.20 (0.08 - 0.48)	0.475	0.061
LXS	54	0.21 (0.10 - 0.51)	40	0.20 (0.09 - 0.53)	31	0.26 (0.10 - 0.49) [‡]	0.025	

EER: Energy intake as percent Estimated Energy Requirement at the active physical activity level.

Data are presented as mean ± SD for normally distributed and median (minimum - maximum) for skewed data.

P1 is testing for Time effect within the randomization groups; P2 is testing for Randomization group by Time interaction effect.

No significant differences between randomization groups at baseline.

[‡] 3-mo value significantly different from baseline within randomization group, p<0.05,

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12-mo value significantly different from baseline within randomization group, $p < 0.05$
 $p < 0.01$
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Table 3
Plasma and Fecal Phospholipids in Placebo and LXS Groups at Baseline and 3 Months

Variable	n	Baseline	n	3 Months	P1	P2
Plasma Fat Soluble Phospholipids, $\mu\text{mol/L}$						
LPC						
Placebo	56	168 $\pm$ 70	46	175 $\pm$ 79	0.371	0.274
LXS	51	165 $\pm$ 59	40	180 $\pm$ 65 [†]	0.026	
PC						
Placebo	56	1915 $\pm$ 457	46	1978 $\pm$ 513	0.244	0.979
LXS	51	1882 $\pm$ 449	40	1979 $\pm$ 422	0.272	
SM						
Placebo	56	325 $\pm$ 120	46	330 $\pm$ 124	0.318	0.037
LXS	51	356 $\pm$ 155	40	334 $\pm$ 139	0.064	
LPE						
Placebo	56	42 (18 - 100)	46	39 (19 - 78)	0.942	0.068
LXS	51	39 (17 - 71)	40	45 (19 - 71) ^{††}	0.001	
PE						
Placebo	56	421 (179 - 973)	46	397 (248 - 1253)	0.516	0.220
LXS	51	415 (209 - 857)	40	470 (211 - 1144) [†]	0.023	
PC/PE Ratio						
Placebo	56	4.48 (1.48 - 9.72)	46	4.42 (1.81 - 8.12)	0.907	0.198
LXS	51	4.44 (0.77 - 10.5)	40	3.90 (1.58 - 9.94) [†]	0.019	
Fecal Phospholipids, $\mu\text{g/g}$ stool						
LPC						
Placebo [†]	52	52 (1 - 259)	37	37 (0 - 575)	0.724	0.260
LXS	44	65 (2 - 1271)	30	53 (3 - 565)	0.356	
PC						
Placebo	52	107 (0 - 1096)	37	73 (4 - 946)	0.901	0.074
LXS	44	212 (2 - 1609)	30	118 (4 - 1293)	0.035	
SM						
Placebo	52	14 (0 - 176)	37	14 (0 - 89)	0.277	0.691
LXS	44	22 (1 - 113)	30	18 (0 - 178)	0.854	
PE						
Placebo	52	64 (13 - 242)	37	58 (9 - 326)	0.909	0.308
LXS	44	82 (9 - 425)	30	69 (16 - 238)	0.190	
PC/PE Ratio						
Placebo	52	1.33 (0.00 - 4.51)	37	0.90 (0.06 - 6.21)	0.645	0.062
LXS	44	2.12 (0.05 - 10.6)*	30	1.16 (0.10 - 5.97) ^{††}	0.002	

Definitions: LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin; LPE, lysophosphatidylethanolamine; PE, phosphatidylethanolamine.

Data are presented as mean $\pm$ SD for normally distributed and median (minimum - maximum) for skewed data

P1 is testing for Time effect within the randomization groups; P2 is testing for Randomized by Time interaction effect.

[†] 3-mo value significantly different from baseline within randomization group, $p < 0.05$,

^{††} $p < 0.01$

* Randomization groups significantly different within visit by student's unpaired t-test or Wilcoxon rank sum test for continuous variables and chi-square test or Fisher's exact test for categorical variables, $p < 0.05$,

** $p < 0.01$

[‡] One subject randomized to the placebo group was removed from the analyses of fecal phospholipids as an extreme outlier in loss of phospholipids.

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Table 4A
Magnetic Resonance Spectroscopy (MRS) of the Liver and Calf Muscle. Children with CF Compared to Healthy Controls at Baseline

Characteristic	Subjects with CF N=26	Healthy Subjects N=10
Age, yr	13.4 ± 2.2	14.0 ± 3.0
Sex, % males	69	70
Height-for-age Z score	-0.4 ± 0.8	-0.4 ± 1.3
Weight-for-age Z score	-0.5 ± 0.8	-0.4 ± 0.7
BMI-for-age Z score	-0.3 ± 0.7	-0.2 ± 0.3
MRS		
Calf Choline, % ^	0.09 ± 0.03	0.12 ± 0.03*
Calf Lipid, % ^	4.10 ± 1.46	3.64 ± 1.29
Liver lipid fraction, %	6.14 ± 6.12	0.53 ± 0.40**

Note: Calf Choline %: fraction of choline/water as %; Calf Lipid %: fraction of calf lipid/total lipid+water as %; Liver lipid %: fraction of liver lipid/total lipid+water as %.

P1 is testing for Time effect within the randomization groups; P2 is testing for Randomization group by Time interaction effect.

^ For the Calf MRS measures, n=25 for subjects with CF.

* Healthy subjects significantly different from subjects with CF at p<0.05

** p<0.01

Table 4B
Placebo and. LXS Groups at Baseline and 12 Months

Characteristics	n	Baseline	12 Months	P1	P2
Age, yr					
Placebo	6	13.3 ± 1.1	14.3 ± 1.1		
LXS	8	12.6 ± 1.7	13.7 ± 1.8		
MRS					
Calf Choline, %					
Placebo	6	0.09 ± 0.01	0.09 ± 0.02	0.785	0.034
LXS	8	0.09 ± 0.02	0.12 ± 0.04	0.005	
Calf Lipid fraction, %					
Placebo	6	4.87 ± 1.97	5.83 ± 2.80	0.001	0.636
LXS	8	3.87 ± 1.02	5.01 ± 1.08	<0.001	
Liver lipid, %					
Placebo	6	5.01 ± 5.57	6.60 ± 6.52	0.100	0.993
LXS	8	7.98 ± 8.54	9.60 ± 4.66	0.591	