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Choline Supplementation Alters Some Amino Acid Concentrations with No Change in Homocysteine in Children with Cystic Fibrosis and Pancreatic Insufficiency

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Abstract

The present study determined the plasma amino acid status in children with cystic fibrosis (CF) and pancreatic insufficiency (PI) in the modern medical and nutritional care setting and investigated the effect of choline supplementation on amino acid status. A total of 110 children, ages 5 to 18 years, with CF and PI were randomized to receive choline-enriched structured lipid (LYM-X-SORB™, LXS) or placebo with similar calorie and fat content. Plasma amino acids were measured at baseline, 3, and 12 months. We hypothesized that choline supplementation would result in lower plasma homocysteine concentrations in children with CF. At baseline, dietary protein intake was high and the amino acid profile was within laboratory reference ranges in most subjects. Alanine and cysteine were elevated in 24% and 36% of subjects, respectively. Children with baseline alanine above reference range had improved weight, body mass index, and fat-free mass. Low homocysteine was found in 62% of children 11 years and older. After 3 and 12 months, there was no effect of choline supplementation on methionine or homocysteine status. Compared to placebo, choline supplementation resulted in increased glycine and decreased threonine, histidine, valine and total branch chained amino acids at 12 months. In conclusion, daily choline supplementation with LXS did not alter methionine-homocysteine metabolism but did result in alterations in other amino acids in children with CF and PI.

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Keywords

Cystic Fibrosis; Amino Acids; Choline; Children; LYM-X-SORB™

1. Introduction

Cystic fibrosis (CF) is the most common fatal genetic disorder in the Caucasian population. Median age of survival increased significantly from 31.3 in 2002 to 41.1 years in 2012.[1] One factor contributing to clinical progress is a greater emphasis on optimizing nutritional status. Current nutritional recommendations include routine higher energy and fat intake.[2,3] However, decades of calorie and fat supplementation efforts have limited success, and have not resulted in normal growth and body composition, including muscle mass status in children and adolescents.[4] Fat-free mass (FFM) depletion was present in 25 to 30% of children with CF and has been associated with reduced lung function.[5] FFM depletion and related protein deficits occur frequently and are likely due to increased fecal nitrogen loss, protein breakdown, and decreased protein synthesis along with inadequate caloric intake to optimize growth.[6,7]

Amino acid metabolism is complex and involves various micronutrients such as choline, folic acid and vitamin B_{12}. Choline depletion and elevated vitamin B_{12} concentrations have been described in children with CF.[8,9] Little is known about the effect of choline supplementation on amino acid status, particularly in the CF care setting where the daily intake from CF-specific vitamin products results in serum vitamin B_{12} concentrations that are relatively high.[10] In a study by Innis et al [11], high dose choline supplementation for two weeks in children with CF improved choline status, reduced plasma homocysteine and increased methionine concentrations. These effects may be explained by remethylation of homocysteine to methionine in two interrelated pathways that involve choline, folic acid and vitamin B_{12}.[12] Hyperhomocysteinemia is a risk factor for cardiovascular disease, thrombosis and stroke in adults.[13] Because these conditions occur predominantly in older individuals and survival past middle-age in CF has been limited in the past, cardiovascular risk factors often have not been considered in disease management. CF survival has improved with medical advances and newborn screening. Yet, lifelong exposure to a higher fat diet, vitamin and mineral supplements and chronic malabsorption of fat, essential fatty acids and fat soluble vitamins may change this in the future. The impact of long-term supplementation of choline on homocysteine and other amino acid metabolism, and the potential effects on CF clinical outcomes have not been investigated. In this report, we hypothesized that long-term supplementation with choline would result in lower plasma homocysteine concentrations in children with CF and PI. The objectives were twofold: 1) to determine plasma amino acid status in stable children with CF and PI in the contemporary care setting; and 2) to evaluate the effect of a choline-rich easily absorbable fat supplement (LYM-X-SORB™ [LXS]) on plasma amino acid concentrations.

2. Methods and Materials

Here we report on data collected as part of a randomized, placebo controlled trial of a structured lipid (LXS), Biomolecular Products, Byfield, MA; Avanti Polar Lipids, Alabaster, AL) to evaluate changes in plasma and muscle choline status, dietary fat absorption and fatty acid status in children with CF and PI. The clinical trial registration is: Study of LYM-X-SORB™ to Improve Fatty Acid and Choline Status in Children with Cystic Fibrosis and Pancreatic Insufficiency, NCT00406536, https://clinicaltrials.govct2/show/NCT00406536. As part of a secondary aim of the study, this report describes the amino acid status of these children and explores alterations in amino acids and particularly the methionine-homocysteine pathway after LXS or placebo supplementation. LXS is a matrix of lysophosphatidylcholine (LPC), free fatty acids (FFA) and monoglycerides. It is well absorbed without pancreatic enzymes and has been shown to improve nutritional status in children with CF and PI.[14]

2.1 Study participants

Subjects ages 5.0 to 17.9 with CF and PI from ten US CF Centers were seen at The Children's Hospital of Philadelphia (CHOP). The study was approved by the CHOP Institutional Review Board and at each CF Center. Verbal assent was obtained from subjects 6.0 to <18.0 years of age with written consent from all parents/guardians for subjects <18 years. Patients with forced expiratory volume in one second (FEV_1) <40% predicted, residual pancreatic lipase activity (fecal elastase >15 μg/g stool), liver disease (serum gamma-glutamyl transferase [GGT] > 3 times reference range), or other chronic health conditions affecting gastrointestinal absorption or growth were excluded.

2.2 Study design and intervention

Study design details have been previously described.[15–17] Subjects were enrolled and randomized in a 1:1 ratio to receive daily oral LXS or placebo for 12 months. LXS is coated with wheat flour and sugar for mixing with food/beverages selected by the participant.[15] The placebo had similar calories, total fat, and macronutrient distribution (protein 6%, carbohydrate 58%, lipid 34% kcal), but less choline than LXS (39 vs 295 mg/packet). Each packet had 32g powder and subjects <12 years took two packets (304 kcal/d), and those ≥ 12 years old took three packets per day (456 kcal/d). Dietary intake was assessed using 3-day weighed food records, and macro- and micronutrient content analyzed using the Nutrition Data System for Research software (National Coordinating Center, University of Minnesota, Minneapolis, MN). [18] Protein, vitamins B_6, folic acid and B_{12} intake were expressed as percent Recommended Dietary Intake (RDA) and choline as percent Adequate Intake (% AI).[19]

2.3 Nutritional Assessment and Clinical Status

Height and weight were measured using standard techniques [20], BMI derived as kg/m^2, and age- and sex-specific Z-scores (HAZ, WAZ, BMIZ) were calculated.[21] Using mid-upper arm circumference and triceps skinfold thickness, upper arm muscle and fat areas were derived, and Z scores (UAMAZ, UAFAZ) were computed.[22] Total fat-free mass (FFM), fat mass (FM), and percent body fat were obtained by whole body dual energy X-ray

absorptiometry (DXA, Delphi A, Hologic, Inc., Bedford, MA). Pubertal status was determined using Tanner stages with a validated self-assessment questionnaire.[23] Resting energy expenditure (REE) was measured by open circuit indirect calorimetry using a computerized metabolic cart (SensorMedics Spectra, Yorba Linda, CA) as previously described.[15] REE was calculated from the modified Weir equation.[24] REE (kcals/day) as a percent of predicted values (%REE) was derived from the Schofield equations that adjust for age, sex, weight, and height.[25] Pulmonary function was assessed and predicted percentage FEV_1 calculated.[26,27]

2.4 Assessment of Amino Acids and Choline

Plasma samples were obtained in the morning after an overnight fast. Amino acids were measured (Clinical Laboratory, CHOP) by ion-exchange chromatography with post-column ninhydrin derivatization (Biochrom 30 amino acid analyzer, Holliston, MA). Essentially, 500 microliters of plasma was deproteinized by the addition of 50 microliters of 35% sulfosalicylic acid, centrifuged and 120 microliters of supernatant added to 100 microliters of lithium buffer (Biochrom US, Holliston, MA) containing 200 micromolar glucosaminic acid as internal standard. Forty microliters was injected directly into the amino acid analyzer using the standard long-run protocol recommended by Biochrom. This methodology was validated for clinical use according to Clinical Laboratory Improvement Amendments (CLIA) specifications.[28] The water soluble choline was measured in EDTA-plasma by an isotope dilution LC/MS/MS technique.[29,30] The internal standard compounds were dissolved and mixed in acetonitrile to contain 12.5 μM choline chloride-d9, 5 μM of N,N –dimethylglycine hydrochloride-d6 (SigmaAldreich, St. Louise, MO) and 44.4 μM N-carboxymethyl-trimethyl ammonium salt-d9 (CDN Isotopes, Point Clare, Quebec). Samples were extracted by combining 30 μL if of plasma and 10 μL of the internal standard mixture and 200 μL of acetonitrile and 0.1%/v formic acid. This mixture was vortex mixed for 30 seconds at 2500–3000 rpm, followed by room temperature sonication and vortex mixing for 30 seconds. The samples were centrifuged at ~17,000 g for 1 minute and the clear supernatant was transferred to silanized injection inserts and vials for LC/MS/MS analysis. Choline (μmol/L) was quantified from calibration curves generated from reference materials (SigmaAldrich-Fluka, St. Louis, MO) of choline chloride (50-1.6 μM) spiked with identical amount of internal standard compound as the plasma extracts. The calibration standards and plasma extracts were assayed as previously reported [8] using a Waters AcquityTM UPLC system/AB Sciex 5500 mass spectrometer at 0.5 mL/minute flow of 81:19 (v/v) acetonitrile: 15 mM ammonium formate with the addition of a 60:40 (v/v) acetonitrile: 15 mM ammonium formate mobile phase to elute the more polar GPC and CDPC compounds from an Agilent RX-Sil (2.1 × 50 mm, 1.8 μm particle size) column.

Serum vitamin B_{12} was analyzed by quantitative chemoluminescent immunoassay (CHOP Clinical Laboratory). Plasma B_6 (pyridoxine-5-phosphate) was determined by high performance liquid chromatography (ARUP Laboratory, Salt Lake City, UT). For serum B_{12} and B_6, subjects were designated as having elevated concentrations if their B_{12} or B_6 concentrations were above versus within the established age- and sex-specific clinical laboratory reference ranges [31–33]. Whole blood was collected for red blood cell folate concentrations by chemoluminescence (ARUP Laboratory) and reported correcting for the

hematocrit. Assessment of glucose and the liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and gamma glutamyl transferase (GGT) was done using standard techniques (CHOP Clinical Laboratory).

2.5 Statistical Analyses

Descriptive statistics were presented as frequency counts and percentages for categorical variables and mean ± 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 or Fisher's tests of independence for categorical variables were used to compare characteristics at baseline. Associations at each time point between plasma amino acid concentrations and growth, body composition, and pulmonary status were assessed using Pearson or Spearman rank correlation coefficients depending upon skewness. Simple linear regression was used to correct for potential or known confounding factors including age and sex. 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 x 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. Stata 13.1 (Stata Corporation, College Station, TX) was used, and statistical significance level was set at $P < 0.05$ for all tests. The amino acid analyses were part of the exploratory aim of the larger clinical trial. The sample size for the clinical trial was determined based on testing the primary outcomes related to choline and essential fatty acid status.[17]

3. Results

3.1 Nutritional Assessment

A total of 118 subjects were randomly assigned and 110 received an intervention (Figure 1): 54 subjects in the LXS group; 56 in placebo group. Eighty-six subjects completed 3 month follow-up, 40 in LXS and 46 in placebo, and 70 subjects completed 12 month follow-up, 31 in LXS and 39 in placebo group. Enrollment began in March 2007 and the final visit occurred in May 2011. Characteristics at baseline are presented in Table 1 by randomization group. Subjects had suboptimal growth status, mild to moderate lung disease, and mildly elevated resting energy expenditure. There were no differences between placebo and LXS groups at baseline. Table 2 presents changes over time in energy, macronutrients, choline, betaine, and selected B vitamin intakes. Both LXS and placebo supplements provided 300 to 450 kcal/day depending on age. Our group has previously reported some decrease in calories from food as a compensation for the increased intake from supplements.[15] Protein intake at each observation was high (% RDA > 250%) in both groups. Choline intake increased slightly in the placebo group, and by 2.7-fold in the LXS group by 3 and 12 months ($P<0.001$).

3.2 Amino Acid Status at Baseline

Baseline concentrations of most plasma amino acids were within the laboratory reference range for healthy children (Table 3). The exceptions were alanine, cysteine, histidine, and lysine with 12 to 36% of subjects above reference ranges. Homocysteine was below the reference range in more than half of children ≥ 11 years. As presented in Table 3, the reference range for homocysteine increases with age in healthy children. In contrast, subjects with CF and PI showed no increase in plasma homocysteine as expected with age.

Further analysis of the elevated alanine was conducted and after adjusting for age and sex, subjects with alanine above the reference range had better nutritional status than those within the range, with higher WAZ (0.00 ± 0.67 vs. −0.51 ± 0.77; P= 0.001), BMIZ (0.18 ± 0.63 vs. −0.31 ± 0.8; P 0.001), and FFM (30.9 ± 12.7 vs. 24.8 ± 8.6 kg; P= 0.002). Furthermore, alanine concentrations were positively correlated with FFM (r= 0.36; p<0.001). Although all values were within the reference range, serum glucose positively predicted plasma alanine (β coef= 2.9, SE= 1.0; P= 0.009) using a linear regression model adjusted for age and sex. Four subjects had CF-related diabetes mellitus and were on insulin treatment. Alanine was higher than the reference range in only one of them.

There were no significant differences in growth, body composition, BMI or pulmonary function between those with plasma cysteine or homocysteine above vs. within the reference range. However, those with cysteine above the reference range had higher serum vitamin B_6 (above the reference range) than those within the range (35% and 9% respectively, P = 0.01). In contrast, subjects with homocysteine below the range were more likely to have high serum vitamin B_{12} (above the reference range) than subjects within the range (77% and 46% respectively, p= 0.006). These amino acid and vitamin B associations seen at baseline were also present at 3 and 12 months.

3.3 12-Month Amino Acids Evaluation

Plasma amino acids are presented by randomization group at baseline, 3 and 12 months in Table 4. Compared to placebo, plasma glycine increased over 3 and 12 months in the LXS group ($P_{0–3–12}$ =0.046). Threonine increased in placebo and slightly decreased in LXS. Total branched chain amino acids (BCAA: leucine, valine, isoleucine) decreased with LXS and did not change with placebo over 12 months ($P_{0–3–12}$≤0.01). The BCAA decrease in the LXS group was due to a significant decrease in valine and smaller decline in leucine. Total aromatic amino acids (AAA) also declined significantly in the LXS group only (P <0.001).

3.4 Other Outcomes

Table 5 presents plasma choline, serum vitamin B status and liver enzymes for placebo and LXS groups at baseline, 3 and 12 months. The changes in amino acid status were accompanied by a significant increase in plasma choline in the LXS group and decrease in the placebo group ($P_{0–3–12}$ =0.037). There were no changes in B vitamin status or liver enzymes over time in either group. Serum B_{12} concentrations were above reference ranges in > 50% of subjects as previously reported.[10] Growth status improved and REE declined in both LXS and placebo groups as previously reported.[15] FEV_1 declined over the 12

months as the subjects aged, with no differences between randomization groups (data not presented).[17]

4. Discussion

4.1 Amino Acid Status in Children with CF and PI

In the present study, most plasma amino acids were within the laboratory reference range in this sample of clinically stable children with CF and PI in the modern care and nutritional status setting. High alanine and cysteine concentrations were relatively common. Low homocysteine was prevalent in peri- and post-pubertal children. In 1981, Hubbard and Schulman described normal plasma amino acids in subjects with CF age 16–30 years with the exception of high glycine.[34] However, high glycine has been described in one third of healthy adults but was rarely seen in children.[35] The children in the current study were younger than those in the Hubbard et al. study and the clinical care, nutritional status and outcomes have changed over the two decades.[34] The higher glycine concentration found in the participants from the Hubbard and Schulman study may be unrelated to CF.

The elevated plasma alanine in 24% of subjects was an unexpected finding in this cohort of children with CF. Alanine plays a key role in gluconeogenesis. It carries ammonia and pyruvate carbon skeletons between muscle tissue and liver protein metabolism.[36] Pyruvate is used to produce glucose and returned to muscle. Thus, the positive relationship between high plasma alanine and better FFM status at baseline in our study may be partially explained. Alteration in the alanine-glucose cycle that leads to increased serum and presumably tissue alanine aminotransferase (ALT) has been linked to diabetes mellitus in non-CF adults.[37–39] The specific cystic fibrosis-related diabetes is found in 19% of adolescents and 40 to 50% of adults with CF. The elevated alanine may be of relevance to this CF comorbidity which has increased in prevalence with longer survival.[40] Enhanced gluconeogenesis and reduced suppression of gluconeogenesis by insulin leading to impaired glucose tolerance is not uncommon in CF.[41,42] The impact of early and sustained elevated alanine and glucose on the risk of CFRD is unknown and merits further investigation.

Elevated cysteine in 36% of sujects was also an unexpected finding. Cysteine is a conditionally essential amino acid and can be synthesized by adding sulfur from methionine through the formation of homocysteine.[43] Homocysteine is generally metabolized via either remethylation, which converts homocysteine back to methionine, or trans-sulfuration, which catabolizes homocysteine to cysteine.[43] Vitamin B_{12} and folate are essential for remethylation and vitamin B_6 is required for trans-sulfuration. Our study identified an inverse relationship with concentrations of homocysteine and both B_6 and B_{12}. Children with high plasma cysteine were more likely to have high serum B_6. Waskiewicz et al. [44] documented lower homocysteine in healthy adults receiving B_6, B_{12} and folate supplementation. Daily oral supplementation with as little as 0.5 mg B_{12} alone reduced homocysteine by 7% in adults.[45] High serum B_{12} has been reported in the majority of children with CF and PI and was likely related to the high vitamin B_{12} content of CF-specific vitamin and mineral products.[10] There is no clinical indication for high B_{12} doses in CF. These CF products are commonly used to provide high fat soluble vitamin doses to compensate for the chronic fat malabsorption in CF. The B_{12} supplement intake was 6 to 10

times the RDA and strongly predicted the serum B_{12} concentrations. Children in the higher serum B_{12} group were older and had poorer growth status as indicated by BMIZ and lower lung function.[10] The current findings of high cysteine and low homocysteine may be due to enhanced remethylation and trans-sulfuration related to the unusually high B_{12} and B_6 intake common in current CF clinical care.

Plasma homocysteine concentrations typically increase with age in healthy children. In this CF cohort, homocysteine was low in 62% of children 11years and older. In the presence of high B_{12} and B_6, this expected increase in homocysteine did not occur in the children in this study. While high plasma homocysteine is a risk for coronary heart disease in otherwise healthy adults, individuals with low homocysteine may also be at risk for other conditions due to limited capacity to respond to oxidative stress, including the production of glutathione, a free-radical scavenger, and also taurine and sulfate. Low homocysteine was indicated in the malnutrition-inflammation complex that predicted lower survival in patients on hemodialysis.[46] The long-term effect of sustained low homocysteine concentrations in children with CF during the adolescent growth phase is unknown. These current results suggest that there may be a need to reduce vitamin B_{12} intake in these children with high B_{12} to support a more normal homocysteine status. Further investigation of the long-term health impact of chronically low homocysteine in children with CF is warranted.

4.2 Effect of Choline on Amino Acids

Choline supplementation over 12 months did not alter methionine-homocysteine metabolism as indicated by plasma concentrations, thus rejecting the hypothesis that long term choline supplementation lowers plasma homocysteine concentrations in children with CF and PI. Innis and colleagues have shown that supplementations with much higher dose of choline for 14 days in children with CF resulted in a significant decrease in homocysteine.[11] The average dose of choline in LXS supplementation (591 mg/day) was considerably lower than that used by Innis et al (1850 mg/day).[11] The homocysteine concentration also was lower at baseline in our sample (5.4 ± 1.5 μmol/L) compared to Innis et al (7.94 ± 0.45 μmol/L). Furthermore, serum vitamin B_{12} (1040 ng/ml) was higher in our subjects compared to Innis et al (594 ± 35 ng/mL).[11,47] The remethylation of homocysteine to methionine using choline-betaine pathway acts as an alternate pathway to remethylation by the B_{12} and folate-dependent pathway.[12] Increased activity of choline-betaine for remethylation of homocysteine has been well described in animals and humans with B_{12} or folate deficiency.[48,49] The effect of higher choline intake on the choline-remethylation process in the presence of high serum B_{12} has not been described previously. The magnitude of the effect of choline supplementation may be decreased based on the competitive interrelation between the two remethylation pathways.

LXS supplementation resulted in lower plasma BCAA, especially valine and to a lesser extent leucine. These findings were unlikely related to the dietary protein intake which was high and sustained in both groups throughout the study. The reduction in plasma valine and trend toward lower leucine indicated that LXS may increase BCAA catabolism or increase uptake by muscle. BCAA catabolism is unique, as it is controlled by a common flux generating step that occurs largely in skeletal muscle.[50] No relationship between the

common BCAA metabolic pathway and plasma choline has been previously reported. This study demonstrated higher glycine and lower threonine in children receiving the choline-rich LXS. This finding can be explained, at least in part by the fact that glycine is synthesized from choline and threonine in humans.[51] The choline supplementation may result in greater conversion of threonine to glycine in these subjects.

Potential limitations of the study were that these findings for amino acids status were part of a secondary and exploratory analyses of the original clinical trial and the lack of a healthy control group for comparison of response to choline supplementation. A major strength of the study is the collection of high quality longitudinal amino acid data that provides updated information on amino acid status for children with CF and PI receiving clinical care in the current environment. There has been no comprehensive update of amino acid status within the past 25 years.

In conclusion, clinically stable children with CF and PI had an amino acid profile that was within the reference range with the exception of high plasma alanine and cysteine, and low homocysteine. High alanine was likely due to increased endogenous gluconeogenesis. The altered cysteine and homocysteine concentrations may be related to excessive vitamin B_6 and B_{12} intake. In this CF cohort, choline supplementation at the daily doses used here in a setting of high serum B_{12} did not alter methionine-homocysteine metabolism. Supplementation resulted in lower total BCAA suggesting reduced skeletal muscle catabolism or improved anabolism in children with CF and PI. Further studies are needed to confirm these findings and consider the clinical implications of altered plasma amino acid concentrations and high B_{12} during childhood growth for risk of complications such as CF-related diabetes and cardiovascular disease, as patients with CF now will commonly live decades into adult life.

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Abbreviations

AAA	Aromatic amino acids
AI	Adequate Intake

ALT	Serum alanine aminotransferase
AST	Serum aspartate aminotransferase
BCAA	Branch chain amino acids
BMIZ	BMI-for-age Z score
CF	Cystic Fibrosis
CHOP	The Children's Hospital of Philadelphia
DRI	Dietary Reference Intake
DXA	Dual energy x-ray absorptiometry
FEV_1	Forced expiratory volume at one second, % predicted
FFA	Free fatty acids
FFM	Fat-free mass kg
FM	Fat mass, kg
GGT	Serum gamma-glutamyl transferase
HAZ	Height-for-age Z score
LPC	Lysophosphatidylcholine
LXS	LYM-X-SORB™
PI	Pancreatic Insufficiency
RDA	Recommended dietary allowance
REE	Resting energy expenditure
WAZ	Weight-for-age Z score

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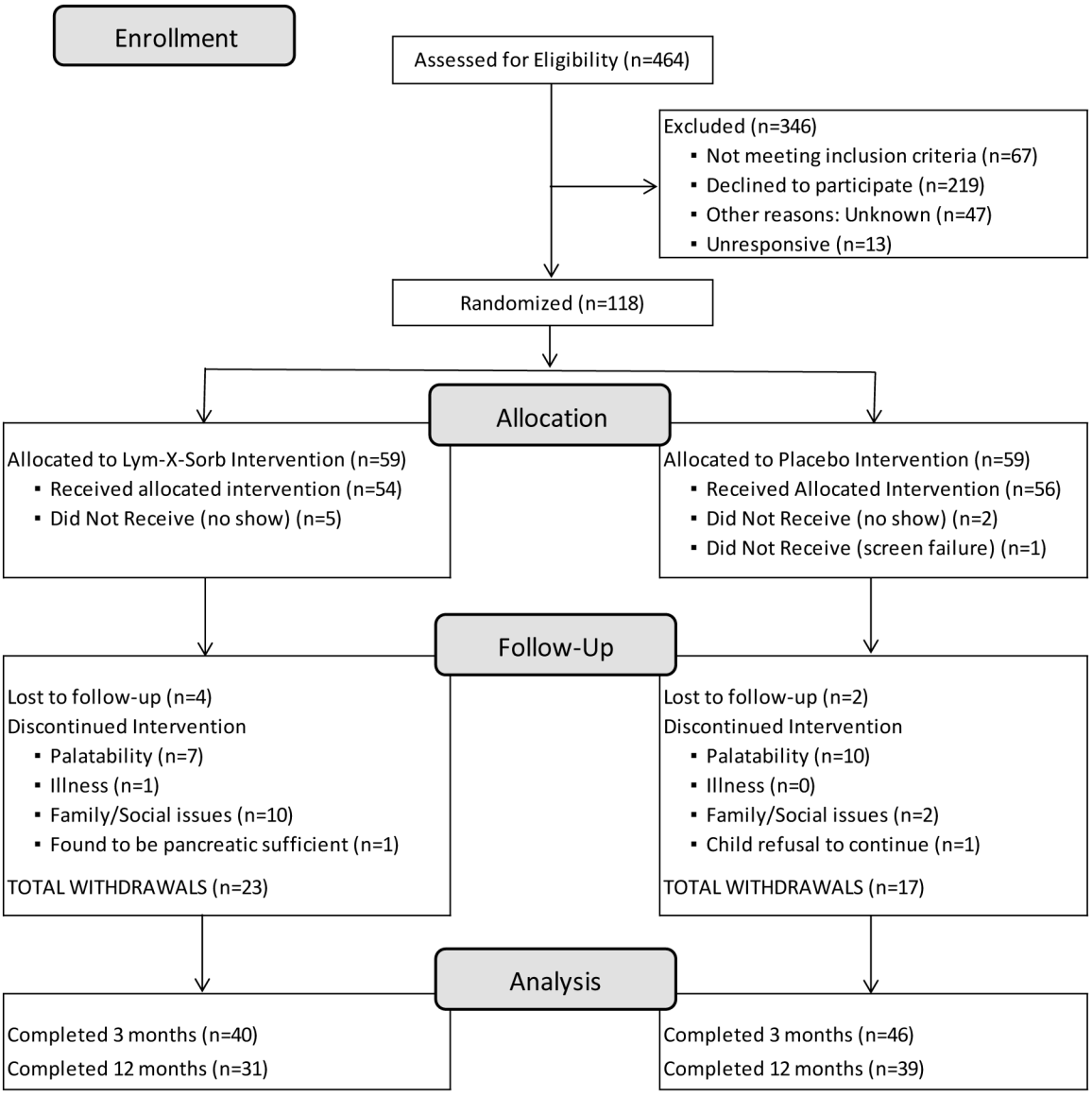

Figure 1.

Flow diagram for subjects randomized, drop-outs and completers in the placebo-controlled 12-month trial of daily LXS supplementation in children with cystic fibrosis and pancreatic insufficiency.

Table 1

Characteristics at baseline for placebo and LYM-X-SORB™ (LXS)

	All (110)	Placebo (56)	LXS (54)
Age (y)	10.4 ± 3.0 [a]	10.2 ± 2.9	10.6 ± 3.1
Sex, male	63 (57%) [b]	33 (59%)	30 (56%)
Genotype, ΔF508 homozygous [d]	62 (57%)	30 (55%)	32 (60%)
Pubertal status, pre–pubertal	58 (53%)	31 (55%)	28 (52%)
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) [c]	22.6 (13.3 – 61.4)	24.1(14.1 – 65.3)
FM (kg)	6.5 (3.0 – 18.9)	6.5 (3.0 – 14.7)	6.6 (3.2 – 18.9)
Percent Fat (%)	21.4 ± 5.6	21.6 ± 5.5	21.2 ± 5.7
UAMAZ [e]	−0.53 ± 0.82	−0.49 ± 0.83	−0.56 ± 0.81
UAFAZ [e]	−0.29 ± 0.80	−0.26 ± 0.84	−0.32 ± 0.77
Pulmonary Function and Energy Expenditure			
FEV_1 % predicted [f]	95 ± 23	97 ± 24	93 ± 21
REE, Kcal/day [g]	1248 (862 – 2243)	1211 (885 – 2201)	1323 (862 – 2243)
REE % predicted [g]	107 ± 9	107 ± 9	108 ± 9

FFM, fat free mass (kg); FM, fat mass (kg); UAMAZ, upper arm muscle area Z score; UAFAZ, upper arm fat area Z score; REE, resting energy expenditure (kcal/day); FEV_1 % predicted, forced expiratory volume at 1 second, percent predicted value; REE % predicted, resting energy expenditure percent predicted value using Schofield equations. No differences between LXS and placebo randomization groups at baseline, by unpaired student's t test or Mann-Whitney U test for continuous variables, and chi-squared or Fisher's exact test for categorical variables.

[a] Continuous normally distributed variables given as means ± SD

[b] Categorical variables are given as numbers and percentages

[c] Continuous variables with significant skewness are given as medians (range)

[d] Sample sizes were 108, 55 and 53 for All, Placebo and LYX groups, respectively

[e] Sample sizes were 109, 55, and 54 for All, Placebo and 54 LYX groups, respectively

[f] Sample sizes were 107, 56 and 51 for All, Placebo and 51 LYX groups, respectively

[g] Sample sizes were 103, 54 and 49 for All, Placebo and 49 LYX groups, respectively

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Table 2

Total daily energy, macronutrients, choline, betaine and B vitamin intake in placebo and LYM–X–SORB™ (LXS) groups at baseline, 3, and 12 months.

Nutrient	n	Baseline	n	3 months	n	12 months	$P_{0–3}$	$P_{0–3–12}$
Energy (kcal)								
Placebo	54	2438 ± 798 [a]	37	2609 ± 563	35	2618 ± 667	0.76	0.95
LXS	44	2423 ± 618	31	2567 ± 580	23	2704 ±643		
Protein (gm/kg)								
Placebo	54	2.7 ± 1.0	37	2.6 ± 1.0 *	35	2.5 ± 1.0 **	0.92	0.86
LXS	44	2.6 ± 0.8	31	2.4 ± 0.7	23	2.5 ± 0.8		
Protein as % RDA								
Placebo	54	282 ± 108	37	273 ± 108 *	35	265 ± 109 **	0.97	0.83
LXS	44	274 ± 79	31	260 ± 76	23	265 ± 89		
Protein as % kcal								
Placebo	54	13.6 ± 2.9	37	12.9 ± 2.6 *	35	12.9 ± 3.1	0.77	0.78
LXS	44	13.3 ± 2.6	31	12.5 ± 2.2 *	23	13.0 ± 2.3		
Fat as % kcal								
Placebo	54	35.9 ± 6.1	37	34.9 ± 5.8	35	36.3 ± 4.5	0.79	0.36
LXS	44	36.1 ± 4.6	31	34.4 ± 6.9	23	34.6 ± 5.1		
Carbohydrate as % kcal								
Placebo	54	51.8 ± 7.2	37	52.6 ± 6.2	35	51.1 ± 5.3	0.59	0.44
LXS	44	52.7 ± 6.0	31	53.5 ± 8.2 *	23	53.0 ± 6.4		
Choline (mg/day)								
Placebo	54	308 (144 – 736) [b]	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) ***		
Choline as % AI								
Placebo	54	88 (37 – 288)	37	92 (52 – 309) **	35	108 (49 – 292) **	<0.001	<0.001
LXS	44	87 (34 – 221)	31	248 (134 – 524) ***	23	235 (61 – 350) ***		
Betaine (mg/day)								
Placebo	54	135 (25 – 359)	37	124 (73 – 339)	35	112 (31 – 734)	0.006	0.021

Nutrient	n	Baseline	n	3 months	n	12 months	$P_{0–3}$	$P_{0–3–12}$
LXS	44	135 (47 – 344)	31	106 (9 – 300) ***	23	121 (15 – 392) *		
Total B_6 (µg/day)								
Placebo	54	4.6 (1.9 – 32.4)	37	5.5 (1.4 – 40.7)	35	4.5 (1.1 – 8.9)	0.29	0.38
LXS	44	4.7 (2.4 – 78.2)	31	5.0 (1.9 – 12.0)	23	4.6 (2.6 – 11.5)		
Total B_6 as % RDA								
Placebo	54	554 (291 – 2496)	37	572 (228 – 4073)	35	529 (148 – 1221) *	0.37	0.64
LXS	44	542 (243 – 7827)	31	547 (188 – 1526)	23	497 (265 – 945)		
Total folic acid (ug/day)								
Placebo	54	800 (340 – 1872)	37	744 (250 – 1954)	35	631 (230 – 1307) **	0.96	0.78
LXS	44	747 (335 – 1684)	31	738 (262 – 1546)	23	681 (338 – 1471)		
Total folic acid as % RDA								
Placebo	54	280 (113 – 840)	37	251 (99 – 800) *	35	227 (106 – 497) ***	0.73	0.94
LXS	44	286 (128 – 562)	31	263 (97 – 674)	23	247 (118 – 423) **		
Total B_{12} (µg/day)								
Placebo	54	18.7 (5.3 – 109.8)	37	19.3 (6.1 – 111.6)	35	15.2 (3.0 – 36.4) ***	0.62	0.37
LXS	44	17.4 (5.6 – 158.9)	31	18.6 (4.7 – 40.3)	23	17.4 (9.1 – 36.8)		
Total B_{12} as % RDA								
Placebo	54	1163 (442 – 4575)	37	1093 (354 – 4649)	35	893 (250 – 2271) ***	0.73	0.63
LXS	44	1168 (310 – 8826)	31	1035 (319 – 2519)	23	966 (508 – 2043)		

AI: adequate intake. RDA: recommended dietary intake

$P_{0–3}$ is testing for partial randomization group x time interaction between baseline and 3 month from a mixed effects linear regression model that included baseline, 3, and 12 months data (i. randomization group x time interaction was used).

$P_{0–3–12}$ is testing for randomization group x time interaction between baseline and 3 and 12 month.

*(P≤0.05)

**(P≤0.01)

***(P≤0.001) difference from baseline within randomization groups.

[a]Continuous normally distributed variables given as means ± SD

[b]Continuous variables with significant skewness are given as medians (range). Log transformation was applied for skewed variables in the mixed models.

Table 3

Plasma amino acids at baseline in subjects with CF compared to reference ranges (RR).

Amino acid	Reference range (RR: nmol/mL)	n	Plasma concentration (nmol/mL)	Proportion of subjects (%)		
				within RR	< RR	> RR
Alanine	88 – 440	110	384 ± 109 [a]	84 (76%) [b]	0	26 (24%)
Arginine	15 – 120	110	76 ± 25	107 (97%)	0	3 (3%)
Asparagine	19 – 106	110	55 ± 12	110 (100%)	0	0
Aspartic Acid	0 – 31	110	6.2 ± 2.5	110 (100%)	0	0
Cysteine	2 – 47	110	44 ± 10	71 (65%)	0	39 (36%)
Glutamic Acid	24 – 163	110	54 ± 24	105 (96%)	4 (4%)	1 (1%)
Glutamine	285 – 832	110	551 ± 89	109 (99%)	0	1 (1%)
Glycine	103 – 424	110	321 ± 78	102 (93%)	0	8 (7%)
Histidine	38 – 132	110	101 (60 – 176) [c]	98 (89%)	0	12 (11%)
Isoleucine	17 – 106	110	63 ± 13	109 (99%)	0	1 (1%)
Leucine	42 – 188	110	118 ± 21	109 (99%)	0	1 (1%)
Lysine	71 – 217	109	177 ± 30	99 (90%)	0	11 (10%)
Methionine	8 – 49	110	26 ± 9	107 (97%)	0	3 (3%)
Phenylalanine	23 – 95	110	56 ± 10	110 (100%)	0	0
Proline	83 – 346	110	166 (82 – 396)	107 (97%)	1 (1%)	2 (2%)
Serine	50 – 215	110	113 ± 19	110 (100%)	0	0
Threonine	86 – 321	110	142 ± 38	108 (98%)	2 (2%)	0
Tyrosine	22 – 102	110	63 (32 – 134)	108 (98%)	0	2 (2%)
Valine	39 – 321	110	221 (152 – 353)	109 (99%)	0	1 (1%)
Ornithine	13 – 151	110	82 ± 23	110 (100%)	0	0
α-aminobutyric	0 – 43	108	21 ± 8	105 (97%)	0	3 (3%)
Taurine	55 – 204	109	78 ± 22	103 (94%)	6 (6%)	0
Homocysteine [d]						
5 – 10.9 y	0.8 – 6.5	68	4.9 ± 1.2	63 (93%)	0	5 (7%)
11–16.9 y	5.7 – 11.7	39	5.5 ± 1.5	16 (41%)	23 (59%)	0
≥17 y	10.5 – 16.5	3	6.0 ± 0.6	0	3 (100%)	0
BCAA	-	110	403 ± 67	-	-	-

Amino acid	Reference range (RR: nmol/mL)	n	Plasma concentration (nmol/mL)	Proportion of subjects (%)		
				within RR	< RR	> RR
AAA	-	110	121 ± 21	-	-	-
BCAA:AAA ratio	-	110	3.41 ± 0.62	-	-	-

BCAA, branch- chained amino acids (Valine, Leucine, Isoleucine). AAA, aromatic amino acids (Tyrosine, Phenylalanine).

[a] Continuous normally distributed variables given as means ± SD

[b] Categorical variables are given as numbers (percentages)

[c] Continuous variables with significant skewness are given as medians (range)

[d] The reference ranges for homocysteine are given by age group.

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Table 4

Plasma amino acids in placebo and LYM–X–SORB™ (LXS) groups at baseline, 3, and 12 months.

Amino acids (nmol/mL)	n	Baseline	n	3 months	n	12 months	$P_{0–3}$	$P_{0–3–12}$
Alanine								
Placebo	56	384 ± 99 [a]	46	405 ± 120	39	401 ± 106	0.50	0.43
LXS	54	383 ± 119	40	423 ± 127 [*]	31	392 ± 104		
α–aminobutyric								
Placebo	56	22 ± 9	45	20 ± 7	38	22 ± 8	0.70	0.82
LXS	52	20 ± 7	40	17 ± 8	31	18 ± 7		
Arginine								
Placebo	56	81 ± 26	46	81 ± 26	39	77 ± 24	0.15	0.13
LXS	54	70 ± 23 [¥]	40	77 ± 21 [*]	31	64 ± 20 [*]		
Asparagine								
Placebo	56	54 ± 10	46	58 ± 28	39	58 ± 9	0.35	0.51
LXS	54	55 ± 13	40	55 ± 13	31	56 ± 10		
Aspartic Acid								
Placebo	56	6.1 ± 2.6	45	5.7 ± 2.4	39	5.0 ± 2.7 [*]	0.41	0.65
LXS	54	6.4 ± 2.3	38	5.7 ± 2.7	31	5.6 ± 4.1		
Cysteine								
Placebo	56	45 ± 10	46	46 ± 9	39	46 ± 11	0.33	0.31
LXS	54	43 ± 10	40	46 ± 8	31	44 ± 9		
Glutamic Acid								
Placebo	56	54 ± 25	46	55 ± 26	39	55 ± 22	0.27	0.51
LXS	54	55 ± 24	40	50 ± 17	31	55 ± 28		
Glutamine								
Placebo	56	568 ± 87	46	571 ± 107	39	593 ± 100	0.12	0.27
LXS	54	533 ± 88 [¥]	40	564 ± 120	31	555 ± 105		
Glycine								
Placebo	56	321 ± 81	46	309 ± 87	39	320 ± 60	0.017	0.046
LXS	54	320 ± 76	40	340 ± 88	31	336 ± 73		

Amino acids (nmol/mL)	n	Baseline	n	3 months	n	12 months	P_{0-3}	P_{0-3-12}
Histidine								
Placebo	56	102 (60 – 176) [b]	46	91 (60 – 148) *	39	93 (59 – 179)	0.052	0.005
LXS	54	99 (71 – 150)	40	107 (65 – 159)	31	88 (58 – 192) *		
Homocysteine								
Placebo	56	4.8 ± 1.3	46	4.9 ± 1.4	39	5.3 ± 1.5 **	0.17	0.31
LXS	54	5.4 ± 1.3 ¥	40	5.3 ± 1.4	31	5.9 ± 1.3		
Isoleucine								
Placebo	56	64 ± 10	46	64 ± 14	39	66 ± 10	0.43	0.23
LXS	54	62 ± 14	40	59 ± 10	31	59 ± 11		
Leucine								
Placebo	56	119 ± 17	46	119 ± 24	39	126 ± 20	0.62	0.059
LXS	54	117 ± 24	40	113 ± 19	31	109 ± 24		
Lysine								
Placebo	55	179 ± 27	46	185 ± 38	39	179 ± 25	0.23	0.13
LXS	54	174 ± 33	39	171 ± 37	31	160 ± 30 *		
Methionine								
Placebo	56	27 ± 9	46	26 ± 9	39	27 ± 9	0.29	0.069
LXS	54	25 ± 8	40	27 ± 10	31	23 ± 6		
Ornithine								
Placebo	56	81 ± 19	45	82 ± 24	39	81 ± 18	0.064	0.17
LXS	54	82 ± 26	39	73 ± 22 *	31	78 ± 27		
Phenylalanine								
Placebo	56	57 ± 9	45	59 ± 13	39	56 ± 10	0.09	0.14
LXS	54	55 ± 11	40	52 ± 7	31	50 ± 9 *		
Proline								
Placebo	56	165 (82 – 396)	45	165 (9 – 380)	39	172 (115 – 325)	0.10	0.17
LXS	54	168 (87 – 294)	40	187 (120 – 378) **	31	178 (93 – 347)		
Serine								
Placebo	56	114 ± 17	46	109 ± 19	39	111 ± 21	0.32	0.26
LXS	54	111 ± 22	40	110 ± 23	31	104 ± 25		

Amino acids (nmol/mL)	n	Baseline	n	3 months	n	12 months	P_{0-3}	P_{0-3-12}
Taurine								
Placebo	56	74 ± 22	45	73 ± 22	38	72 ± 19	0.053	0.13
LXS	53	82 ± 21 ¥	40	72 ± 17 *	31	81 ± 33		
Threonine								
Placebo	56	144 ± 35	46	155 ± 51	39	165 ± 40 **	0.14	0.029
LXS	54	140 ± 41	40	133 ± 48	31	129 ± 38		
Tyrosine								
Placebo	56	67 (32 – 134)	45	67 (41 – 132)	39	59 (33 – 121)	0.71	0.18
LXS	54	60 (39 – 95)	40	64 (39 – 122)	31	53 (25 – 92)		
Valine								
Placebo	56	221 (169 – 307)	46	224 (154 – 340)	39	229 (181 – 337)	0.88	0.043
LXS	54	219 (152 – 353)	40	212 (151 – 279)	31	200 (130 – 318) *		
BCAA								
Placebo	56	408 (318 – 570)	46	387 (278–628)	39	414 (314–594)	0.81	0.047
LXS	54	386 (278 – 606)	40	388 (286–533)	31	377 (245–547)		
AAA								
Placebo	56	126 (77–194)	44	129 (83–232)	39	119 (80–179)	0.63	0.21
LXS	54	113 (80–172)	40	117 (74–190)	31	103 (65–152) ***		
BCAA: AAA ratio								
Placebo	56	3.38 ± 0.59	44	3.26 ± 0.62	39	3.69 ± 0.99	0.99	0.68
LXS	54	3.44 ± 0.64	40	3.35 ± 0.55	31	3.67 ± 0.62		

BCAA, branch- chained amino acids (Valine + Leucine + Isoleucine); AAA, aromatic amino acids (Tyrosine + Phenylalanine).

P_{0-3} is testing for partial randomization group x time interaction between baseline and 3 month from a mixed effects linear regression model that included baseline, 3, and 12 months data (i. randomization group x time interaction was used).

P_{0-3-12} is testing for randomization group x time interaction between baseline and 3 and 12 month.

*(P≤0.05)

**(P≤0.01)

***(P≤0.001) difference from baseline within randomization groups.

¥(P≤0.05) differences between LYX and Placebo randomization groups at baseline.

[a] Continuous normally distributed variables given as means ± SD

[b] Continuous variables with significant skewness are given as medians (range). Log transformation was applied for skewed variables in the mixed models.

Table 5

Plasma choline, serum B vitamins and liver enzymes in placebo and LYM–X–SORB™ (LXS) groups at baseline, 3, and 12 months.

Variable	n	Baseline	n	3 months	n	12 months	$P_{0–3}$	$P_{0–3–12}$
Plasma Choline								
Choline (μmol/L)								
Placebo	56	6.9 (4.2 – 12.8) [a]	46	7.1 (5.0 – 11.5)	39	6.6 (3.6 – 11.4) **	0.011	0.037
LXS	52	7.6 (4.6 – 12.2)	40	9.3 (3.9 – 15.6) **	31	7.9 (4.1 – 12.8)		
Serum B Vitamins								
B_6 (ng/mL)								
Placebo	56	17.4 (5.2 – 91.7)	46	16.1 (4.7 – 93.8)	38	18.7 (5.8 – 74.4)	0.89	0.39
LXS	50	16.9 (5.0 – 55.5)	38	17.2 (3.2 – 77.8)	31	18.1 (4.2 – 72.9)		
RBC folate (ng/mL)								
Placebo	53	507 ± 157 [b]	46	515 ± 167	39	479 ± 152	0.84	0.85
LXS	47	534 ± 181	37	546 ± 145	31	482 ± 182		
B_{12} (pg/mL)								
Placebo	55	1142 (387 – 2861)	46	1259 (387 – 2861)	39	1296 (387 – 2861) *	0.22	0.26
LXS	51	1040 (371 – 3455)	39	1073 (457 – 3455)	31	1099 (457 – 2257)		
Liver Enzymes								
ALT (U/L)								
Placebo	56	41 ± 25	46	42 ± 29	39	46 ± 60	0.59	0.86
LXS	54	37 ± 17	40	35 ± 15	31	44 ± 69		
AST (U/L)								
Placebo	56	44 ± 26	46	49 ± 51	39	47 ± 45	0.47	0.57
LXS	54	41 ± 18	40	40 ± 17	31	57 ± 116		
GGT (U/L)								
Placebo	56	29 ± 23	46	33 ± 38	39	31 ± 25	0.39	0.68
LXS	54	24 ± 15	40	24 ± 17	31	28 ± 28		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase;

Log transformation was applied for skewed variables in the mixed models.

P_{0-3} is testing for partial randomization group x time interaction between baseline and 3 month from a mixed effects linear regression model that included baseline, 3, and 12 months data (i. randomization group x time interaction was used).

P_{0-3-12} is testing for randomization group x time interaction between baseline and 3 and 12 month.

*(P≤0.05)

**(P≤0.01) difference from baseline within randomization groups.

[a]Continuous variables with significant skewness are given as medians (range)

[b]Continuous normally distributed variables given as means ± SD

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