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Serum linoleic acid status as a clinical indicator of essential fatty acid status in children with cystic fibrosis

Asim Maqbool, MD, Joan I. Schall, PhD, J. Felipe Garcia-Espana, PhD, Babette S. Zemel, PhD, Birgitta Strandvik, MD, PhD, and Virginia A. Stallings, MD

Divisions of Gastroenterology, Hepatology, and Nutrition (AM, JIS, BSZ, VAS) and Biostatistics and Epidemiology (JFGE), Department of Pediatrics, The Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia PA 19104, and the Department of Pediatrics, Institute of Clinical Sciences (BS), Göteborg University, Göteborg, Sweden

Abstract

Background—Children with cystic fibrosis (CF) and pancreatic insufficiency (PI) are at increased risk for essential fatty acid (EFA) deficiency.

Objectives—To investigate serum markers of EFA status in children with CF and PI and their association to growth, body composition and lung function.

Design—Serum phospholipid fatty acid (FA), growth and pulmonary (FEV₁, % predicted) status were assessed at baseline and 12 months in 77 children with CF and PI, 7 to 10 years old. Longitudinal mixed effect models were used for comparing associations of the triene to tetraene ratio (T:T; ratio of eicosatrienoic acid to arachidonic acid) and serum linoleic acid (LA; mol %) with the clinical outcomes. Twenty-three healthy Caucasian age- and sex- matched children served as controls for serum FA.

Results—Children with CF and PI had higher median T:T and lower LA than healthy controls. Depending on the T:T cut-off point used (0.04 or 0.02), either 17 or 52% of the children with CF had EFAD, respectively. Only LA was significantly and positively associated with z-scores for weight, height, BMI, upper arm muscle area, and FEV₁ at baseline. Children with LA ≥ 21 mol% had significantly better growth and pulmonary status than those with lower concentrations.

Conclusions—Serum phospholipid LA ≥ 21 mol% was associated with better growth, body composition and FEV₁. No clinical outcome associations were found with the T:T ratio. These findings suggest that LA concentration was a more clinically relevant biomarker of EFA status than the T:T ratio in children with CF and PI. Further research is warranted to validate this specific %LA cut-off point as a new recommendation for clinical use.

Corresponding author and reprint requests: Asim Maqbool, MD, Division of Gastroenterology, Hepatology and Nutrition, The Children's Hospital of Philadelphia, 3535 Market Street, Room#1572, Philadelphia, PA 19104, (267) 426-5746 phone, (215) 590-0604 fax, maqbool@email.chop.edu.

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Keywords

Essential fatty acid deficiency; growth; pulmonary function; triene to tetraene ratio; linoleic acid

INTRODUCTION

Humans lack the enzymes to desaturate fatty acids (FA) at the 3rd and 6th carbon from the methyl end of the molecule, and are dependent on dietary sources to prevent essential FA (EFA) deficiency. Linoleic acid (LA, 18:2w6), and alpha linolenic acid (ALA, 18:3w3) are the omega-6 and omega-3 EFA in humans. LA is an important membrane constituent; both of these EFA have multiple cellular structural and functional roles, and are precursors to long chain polyunsaturated FA, which, by themselves and through prostanoid products, are growth factors and inflammatory mediators, and also influence gene expression^{1,2}.

EFAD has been described in patients with cystic fibrosis (CF) for more than 40 years³, and many CF clinical symptoms have been shown to be influenced by EFAD⁴⁻⁷. EFA status is often clinically defined by the ratio of eicosatrienoic acid (ETA, also known as mead acid, 20:3w9) to the important tetraenoic acid, arachidonic acid (AA, 20:4w6) in tissues such as serum or plasma⁸. This ratio is known as the triene: tetraene ratio (T:T), or the EFAD index. Biochemical evidence of EFAD may precede clinical signs in children with CF⁹. LA (the omega-6 EFA) is also a biochemical indicator of EFA status. Decreased serum LA concentration is a frequently described EFA abnormality in infants, children and adults with CF^{10;11}. Our study had two aims: 1) to examine serum LA and T:T ratio status in subjects with CF and pancreatic insufficiency (PI) as compared to Caucasian age-matched healthy control subjects, and 2) to assess clinical associations of serum phospholipid LA concentration and T:T ratio with growth, body composition, and pulmonary function in subjects with CF and PI.

SUBJECTS AND METHODS

Children with CF and PI (ages 7 to 10 years) from 13 CF centers participated in a 24 month study of nutritional status and progression of pulmonary disease. A subset of these subjects elected to participate in a behavioral and nutritional educational intervention previously shown to be effective in boosting caloric intake^{12;13}, in accordance with the CF nutritional recommendations.

At the 12 and 24-month visits, serum FA and additional data were collected, and these visits are referred to as baseline and 12 month data for the purposes of this analysis. An additional anthropometric assessment occurred at 6 months. The behavioral intervention occurred in a subset of subjects with CF approximately 12 months prior to the baseline serum, pulmonary and anthropometric measurements that constitute this study. The diagnoses of CF and PI were made by the home CF Center based on clinical symptoms, and upon duplicate quantitative pilocarpine iontophoresis sweat test, with chloride values of >60mEq/L. PI was diagnosed by 72-hour quantitative fecal fat collection and analysis of dietary intake, with <93% fat absorption, and/or a stool trypsin value of <80 µg/L. Children were excluded if they had an FEV₁ <40% of predicted, significant liver disease, insulin-dependent diabetes,

B. cepacia sputum colonization, or had other medical conditions or medications known to affect growth. Age and sex matched healthy children who participated in a bone health study at The Children's Hospital of Philadelphia (CHOP) served as control subjects. These healthy children were recruited from the primary care practice at the Children's Hospital of Philadelphia and affiliated pediatric practices. They were included on the basis of similar gender and age as the subjects with CF and were all Caucasians. Exclusion criteria included use of any medication known to affect growth (e.g., thyroxin, growth hormone, current or previous oral steroid medication), height or weight <3rd percentile for age, percent of ideal body weight >130%, and significant developmental delay or impairments. None of the control subjects had pre-existing diagnosed gastrointestinal disorders or significant symptoms to suggest gastrointestinal, hepatic, or pancreatic disease. Both protocols were approved by the Committee for the Protection of Human Subjects of the Institutional Review Board at CHOP, and at the subjects' respective home institutions. Written informed consent and age-appropriate assent were obtained from the parent/legal guardian and each subject, respectively. Subjects with CF were seen at the CHOP Clinical Translational Research Center (CTRC) for two (at baseline and at 12 months) overnight admissions and at their respective home CF Centers for a day visit at 6-months, while in their usual state of good health. Evaluations at each visit included clinical status, anthropometry, dietary (food and supplement) intake and phlebotomy. Additionally, subjects with CF had phlebotomy, spirometry and fecal collections performed at the baseline and 12 month visits. The healthy control subjects had one baseline assessment performed including phlebotomy and anthropometry during a one-day study visit. For the healthy control subjects, only this baseline data was included for this study.

Energy intake

Seven-day home-based weighed food records were obtained from children with CF at baseline after verbal and written instructions were provided along with measuring cups, spoons, and digital food scales. Research dietitians analyzed the diet records (Nutrition Data System, Minneapolis, MN). Details of the specific brands of supplements, frequency and dose, were recorded. Total grams of fat, kilocalories (kcal) from fat, carbohydrates and protein, total energy (kcal) were calculated. The estimated energy requirement (EER) adjusted for age, sex, height, weight, for physical activity in the "active" range as determined previously was also calculated¹⁴. LA and ALA intake in grams per day, and percent intake based on the Adequate Intake level (AI) of the Dietary Reference Intakes (DRI) were calculated and expressed as both grams per day as well as % AI¹⁵.

Serum fatty acid analysis

For the subjects with CF, fasting, serum phospholipid fatty acid (FA) status was determined at baseline and 12-month visits. A single, non-fasting serum phospholipid FA assessment for an age and sex matched sample of healthy control subjects at the baseline visit was used for comparison. Total lipids of serum were extracted according to Folch et al¹⁶. Serum phospholipids were fractionated on a single SEP-PAK aminopropyl cartridge (Waters Corp., Massachusetts, USA), subsequently separated by capillary gas-liquid chromatography as previously described¹⁷, and recorded with HP GC Chem Station software (HP GC, Wilmington, DE). The FA methyl esters were identified and quantified by comparison with

pure reference substances (Sigma Aldrich Sweden AB, Stockholm, Sweden), with heneicosanoic acid (21:0) as the internal standard. The FA's, LA, ALA, AA, ETA, and oleic acid (OA) are reported here. All values are expressed as molar percentage of total serum phospholipid FA. There is no accepted standard for defining EFA status; therefore EFA status was explored using different, previously published clinically used cut-off points in studies in subjects with CF for the T:T ratios of 0.02 and 0.04^{18–20}, and from 20 to 26 mol% for serum LA^{9–11;21–29}.

Body composition, growth and clinical status

Height and weight were measured using standard techniques³⁰ with a stadiometer accurate to 0.1 cm (Holtain, Crymych, UK), and a digital scale accurate to 0.1 kg (Scaletronix, White Plains, NY). Height was adjusted for genetic potential³¹ from measured or reported biological parent heights for the subjects with CF. Z scores for height (HAZ), adjusted height (adjHAZ), weight (WAZ) and body mass index (BMI; kg/m², BMIZ) were also computed³². Mid-upper arm circumference was measured using a flexible plastic measuring tape (Ross Laboratories, Columbus OH). A skinfold caliper (Holtain, Crymych UK) was used to measure triceps and subscapular skinfold thickness on the right side. Upper arm muscle and fat areas were derived^{33;34}, and Z scores for upper arm muscle area (UAMAZ) and upper arm fat area (UAFAZ) were computed³⁴. These determinations were made for the subjects with CF at the baseline, 6 and 12-month visits. Height, weight, and BMI measurements were analyzed for the healthy control subjects for the baseline visit only. Pulmonary function was evaluated by standard methods for spirometry^{35;36} following inhaled albuterol and chest physiotherapy in the children with CF at the baseline and 12 month visits. FEV₁ percent predicted was used as the measure of pulmonary function³⁷, and are presented using both the equations by Knudson et al³⁸ and by Wang et al³⁹.

STATISTICS

Data Analysis

Descriptive analysis was performed using means, standard deviations, medians, minimum and maximum values for continuous variables, and frequency distributions for categorical variables. Descriptive statistics and exploratory graphing techniques were used to assess the normality of the continuous variables. Demographic, growth, pulmonary status, and select serum FA comparisons between children with CF and healthy controls were assessed using student's t-tests for independent samples, or Mann-Whitney-Wilcoxon tests, because sample size are unequal, for non-normally distributed variables. Associations between dietary intake and serum FA data were measured for subjects in both groups for whom both complete dietary data and serum FA status were available. The association between dietary LA intake, serum FA and growth, nutritional and pulmonary status was measured by Pearson's correlation coefficient for normally distributed variables or Spearman's rho coefficients for non-normally distributed variables.

T:T cut-off points were derived from those published in the literature^{18–20}. The selection of the cut-off points for LA was driven by our data and by those most frequently used in the literature (26 mol%)^{10;11;27}. The LA values for our CF subjects were between 15 and 29 mol

%; 12% of the observations had values < 19 mol%, 10% had values > 26 mol%, and 78% of the observations (n=60) were within the values of 19 to 26 mol%. Therefore, we explored seven different %LA cut-off points at baseline (from 20 to 26 mol% in increments of one, i.e., < 20 vs. 20, < 21 vs. 21, etc to < 26 vs. 26 mol%) using mixed effects models for longitudinal data⁴⁰. Since this is a secondary data analysis of a data set of modest size, our analyses were exploratory. For each LA cut-off point, four models were fitted. Regression coefficients, standard errors, p values for each predictor within the models, and a measure of relative goodness of fit (Akaike's information criterion [AIC])⁴¹, were obtained. Our goal was to explore cut-off points with statistical significant p-values across the four outcomes (adjHAZ, WAZ, UAMAZ, and FEV₁). These models utilized all available information for each subject; specifically, at baseline, 6, and 12 months for growth and body composition, and at baseline and 12 months for FEV₁. The predictors were the FA status at baseline, time, and the FA status by time interaction. In addition, gender was included in all models. The data were evaluated for the cut-off point with the higher statistical significance and lower AIC value across the four outcomes (adjHAZ, WAZ, UAMAZ, and FEV₁). The Type I error rate was adjusted by using the Tukey, Ciminera and Heyse's adjustment for multiple comparisons for moderately related outcomes^{42;43}; the p value was set as p = 0.010. Agreement between the best cutoff value (< 21) of serum %LA, obtained from our mixed effects models, and two cutoff values of the T:T ratio (< 0.02 and < 0.04) was evaluated by using the Kappa coefficient. All tests were two sided and were performed with STATA 8.0 (College Station, TX) or SAS (version 9.1.3, Cary, NC).

RESULTS

Seventy-seven subjects (51% female) with CF and PI of mean age 8.4 ± 0.9 (7.0 to 10.0) years, and 23 healthy control subjects (57% female) of mean age 8.4 ± 1.1 (7.2 to 11.0) years participated. Comparisons between subjects with CF and controls were based on cross-sectional data and were restricted to the baseline visit for both groups. The subjects with CF did not differ from controls with respect to age, gender and growth status (Table 1). Subjects with CF had lower serum LA and AA, and higher serum ALA, OA and ETA mol % compared to control subjects. T:T ratio was higher for children with CF as compared to the controls (0.029 ± 0.030 v. 0.011 ± 0.004 , $p < 0.001$). All control subjects and 48% of children with CF had a T:T ratio < 0.02, and 17% of the children with CF had a T:T ratio > 0.04. Gender differences were seen for ETA and T:T status in the subjects with CF. The female subjects had lower median serum ETA mol % [0.151 (0.055 to 0.802) vs. 0.198 (0.060 to 1.560), $p = 0.017$] than the male subjects with CF. Males had higher median T:T than the female subjects with CF [0.024 (0.008 to 0.219) vs. 0.018 (0.004 to 0.137), $p = 0.015$].

Dietary data were available for 86% of subjects with CF and for 96% of the healthy control subjects. The subjects with CF had greater energy, fat and LA intake than the healthy controls. Twenty six percent of subjects with CF had LA intake less than 100% AI, and 21% of subjects had ALA intake less than 100% AI (Table 1). For both groups, LA ALA and total caloric intake were not associated with serum LA or ALA status.

Associations between serum phospholipid FA, T:T ratio and growth, body composition and FEV₁ are shown in Table 2 for the subjects with CF. The LA status was significantly and

positively associated with growth status and UAMAZ. The association of serum LA status with FEV₁ percent predicted (both Knudsen and Wang) did not reach statistical significance in this model ($p < 0.01$); however, a trend was observed ($p < 0.05$). The T:T ratio was not correlated with any of the outcomes of interest. The relationships between ALA, OA, AA, and ETA and clinical measures are shown in Table 2. ALA was positively and OA negatively associated with growth status. AA was negatively associated with FEV₁ (Wang only). Similar correlations between these clinical measures and LA, ALA, AA, OA, ETA, and T:T ratio were observed for the 12-month data, with the exception of the association between %LA and FEV₁ (not shown).

Table 3 presents the results from the longitudinal mixed effects analyses corresponding to the three selected %LA cut-off points (20, 21, and 26), and two T:T ratio cut-off points (0.02 and 0.04). There were no statistically significant associations between T:T ratio groups and growth, body composition or pulmonary function. Growth status and FEV₁ differed significantly by %LA groups. Even though some significant group differences were observed at every cut-off point, %LA associations for every clinical outcome of interest were obtained only for the cut-off point 21 %LA. Compared to the group of children with %LA < 21, children with a %LA 21 had significant better WAZ (+0.87 Z score; $p = 0.002$; AIC=301.4), better UAMAZ (+0.93 Z score; $p = 0.002$; AIC=426.0) and higher FEV₁ (+12.9 % predicted; $p = 0.006$; AIC=1172.0). Results did not reach statistical significance for the adjHAZ (+0.67 Z score; $p = 0.012$; AIC=164.0). The results using a %LA cut-off point of 20 are presented to illustrate the step-wise increased associations for growth, nutritional status and FEV₁ as serum %LA increased from 20 to 21. When children were divided using 26% LA as the cut-off point (the most frequently used cut-off point in the literature^{10;21;25–29}), those with 26% LA had the best growth status; however, the small sample size of this group limited the power to detect statistical significance. Gender effects were not seen in any of the models. Similar longitudinal mixed effects models were performed for BMIZ and UFAZ, and the results were not significant (not shown). While %LA status was associated with growth, body composition and FEV₁ at any given point in time, it did not predict change in these clinical outcomes over one year, as the group x time interactions was not significant in any of the models.

Potential differences in FA status were explored between subjects with CF and PI participating and those not participating in the behavioral intervention to boost caloric intake 12 months prior to the baseline in this study. Thirty two percent (25/77) of subjects participated in this intervention, and did not differ from those who did not participate with respect to age, gender, serum FA status (LA or T:T ratio) or pulmonary function at baseline. The associations of serum FA status with growth and pulmonary status were similar in both groups at baseline and 12 months, and in the LME models did not alter the significant effects of serum LA status predicting growth and pulmonary outcomes.

The relationship between T:T ratio and serum phospholipid %LA for the children with CF at baseline is shown in Figure 1. Using the Spearman rank correlation, %LA and the T:T ratio were significantly and negatively associated ($r = -0.623$, $P < 0.0001$). Serum LA was 26% of total serum FA in only 10% of children with CF, and 42% had serum %LA < 21. Only one of the 45 children with %LA 21 had a T:T ratio > 0.04, whereas 38% of children with

%LA <21 had a T: T ratio >0.04 (Chi-square = 16.59; df 1; p < 0.001; Kappa=0.39). When comparing %LA cut-off point of 21 to T:T ratio of 0.02, 38% of children with %LA ≥ 21 had a T:T ratio >0.02, whereas 72% of the children with %LA <21 had a T:T ratio >0.02 (Chi-square = 8.71; df 1; p < 0.0013 Kappa=0.39).

DISCUSSION

The incidence of EFAD was high in children with CF and PI; depending on the T:T ratio cutoff used (0.04 or 0.02), EFAD was present in 17 or 52% of these pre-adolescent subjects, respectively. %LA and AA were lower and ETA was higher in subjects with CF compared to the control subjects. These data are the first reported to demonstrate that %LA status was correlated with FEV₁ in children⁴⁴, in contrast to previous studies^{45;46}. LA status was correlated with important clinical CF outcomes of growth, muscle stores and pulmonary outcomes, whereas the currently recommended T:T ratio method of EFA status assessment⁴⁷ was not.

EFAD has been frequently described in subjects with CF^{3;10;48–50}. The most commonly reported PUFA abnormality reported in CF is decreased LA status compared to healthy control subjects^{51;52}. The classical clinical manifestations of EFAD include desquamating skin rashes, alopecia, easy bruising, clotting disorders and impaired growth^{53–55}. FA abnormalities and EFAD have been reported in infants with newly diagnosed CF^{11;56}, malnourished children with CF⁵⁶, and in well-nourished children^{8;9} with CF. The T:T ratio (ETA: AA) has also been reported to be abnormal (i.e., elevated) in studies in subjects with CF^{51;52}. Our aim was to identify a biochemical indicator of EFAD that was related to important clinical indicators in children with CF, such as growth, body composition and/or pulmonary status. While these outcomes are influenced by many factors in CF, they also may be additional subtle, subclinical signs of EFAD.

Studies conducted in subjects with CF have identified multiple factors that influence serum LA status, including dietary fat intake and absorption^{3;27;57}, energy intake and energy balance^{24;25;58}, and increased FA turnover. When caloric intake is inadequate to meet energy needs (negative energy balance), LA may be utilized for energy, and may result in decreased LA body stores and status⁵⁹. Low LA status has been reported in subjects with CF with normal fat absorption as well as in subjects without clinical signs of EFAD^{60;61}. Increased FA turnover has been shown to be associated with greater AA membrane release^{62–65}, with LA deficiency resulting in increase of pro- inflammatory eicosanoids^{66;67}. In contrast, EFAD in non-CF subjects has been associated with decreased eicosanoid production^{68–71}. These derangements in FA metabolism in CF may relate to the primary defect^{8;72}, and underscore the complexities of FA abnormalities in subjects with CF, which cannot be entirely explained by dietary intake and fat malabsorption alone. FA metabolism abnormalities observed in CF may be of additional biological significance for this specific population.

In healthy populations, the incidence of EFAD is rare, and the DRI¹⁵ defines LA adequacy based on typical LA intake in these populations. LA and EFAD have been traditionally described by the presence of the physical findings in deficiency states and/or by biochemical

evidence (i.e., an abnormal T:T ratio). The physical findings of EFAD in children and adults are from enteral and parenteral caloric supplementation studies in which provision of fat was either very limited or withheld^{53–55;73}. The biochemical approach to defining EFAD is by an abnormal T:T (ETA:AA) ratio, which represents a shift in the biosynthetic desaturation and elongation pathways for the omega 3, 6, and 9 FA⁷⁴. These changes occur when serum ETA is increased and/or when serum AA is decreased⁴⁹, resulting from diminished LA and ALA as compared to other FA such as OA in healthy subjects. The data from the current study and from the literature illustrate this shift in metabolism resulting in elevated T:T ratio with decreased LA status (Figure 1).

The T:T ratio varies by geographical location and by dietary intake patterns across healthy human populations. What constitutes typical, or within the normal range FA status can change over time for a population and/or across populations, and is exemplified by the evolution of the definition of a normal T:T ratio. The original T:T ratio of >0.04 denoting deficiency was based on the work of Holman from 1960²⁰. Subsequent T:T ratio investigations in healthy populations with normal omega-6 FA status by Holman and others demonstrated a T:T ratio of 0.010 ± 0.008 (mean $\pm$ SD), leading to the recommendation that T:T >0.02 be considered abnormal^{18;23;28;75}. This threshold has been adopted by some CF investigators²⁷. The T:T ratio from our healthy control subjects was consistent with this revised T:T ratio; none had a T:T ratio >0.02 . Similarly, geographical and population-specific dietary variations in LA intake may influence LA status^{76;77}, thereby influencing what is considered normal %LA for different healthy groups.

The challenge in defining normal FA status in subjects with CF is in determining if CF recommendations should be limited to a comparison with a healthy population, or should reflect a CF-specific clinically meaningful cut-point. The associations of LA status with growth and nutritional status as previously reported and as presented in the current study suggest the cut-off point to define adequate EFA status in CF could be developed on the basis of health outcomes, as opposed to the methodology used to define EFA adequacy in healthy populations detailed above.

The manner in which biochemical PUFA status is reported is relevant to this discussion. LA status is most often reported in mol%, and conveys information regarding the physical properties and functional aspects of cell membranes⁷⁸. Reporting FA status by absolute amounts (such as $\mu\text{g/dL}$) has been used less frequently in the CF research and clinical care literature. In addition, absolute FA concentration varies by age and does not consistently relate to mol%⁷⁹. In the CF literature, reference values for serum phospholipid %LA varies from 20 to 27 mol % total FA, with %LA 26 the most frequently used value^{17;21;22;24–28}. In the current study, %LA 21 was an informative cut-off point for growth and pulmonary status associations in children, with a threshold effect observed. In addition T:T and %LA are biochemically related, and %LA 21 conveyed similar biochemical EFA status information as T:T 0.04.

This current study explored the relationship between serum EFA status and clinical measures as a secondary analysis of two studies, and this was a limitation. Since longitudinal data were available for the subjects with CF and not for the healthy controls, the

predictive value of EFA status at baseline on future growth status was explored only in the subjects with CF. The possible differences in phospholipid FA profiles in fed/fasted states and with respect to diurnal variation are, in part, mitigated by the absence of reports suggesting such variations occur. In addition, an extensive body of literature suggests children with CF have lower serum LA status and EFA deficiency than healthy children. The methodology-based potential differences in serum FA status between these two groups does not alter the key findings of the relationship of serum FA status to clinically relevant outcomes in the children with CF. Lastly, since model building and hypothesis testing need to be conducted on separate data sets for hypothesis testing to be unbiased, further research is warranted to validate this specific %LA cut-off point as a new recommendation for clinical use at this time.

In summary, these findings suggest that serum LA status was a better indicator of EFA status in children with CF and PI as compared to the T:T ratio. Depending on the T:T ratio used (0.04 or 0.02), 17 or 52% of the subjects with CF has EFAD, respectively. While an abnormal T:T ratio is indicative of a shift in FA metabolism due to EFA insufficiency or deficiency, it was not related to the selected outcomes including growth and pulmonary status. In the current study, LA status was associated with these CF-specific clinically important outcomes. LA 21% was an informative marker. Prospective studies are required to confirm and expand these findings prior to making recommendations for use of this specific %LA cut point as a change in standards of clinical care. As an interim step, we suggest that LA status be determined in addition to the T:T ratio for assessment of EFA status in children with CF and PI, in the development of optimal nutritional care of patients with CF.

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Abbreviations

AI	Adequate Intake, g/day
ALA	Alpha-linolenic acid, mol% total fatty acids

AdjHAZ	Height for age Z score, adjusted for genetic potential
AA	Arachidonic acid, mol% total fatty acids
BMIZ	Body Mass Index (kg/m ²) Z score
CHOP	Children's Hospital of Philadelphia
CF	Cystic Fibrosis
CTRC	Clinical Translational Research Center
DHA	Docosahexaenoic Acid, mol% total fatty acids
DRI	Dietary Reference Intake
EER	Estimated energy requirement, %
EFA	Essential fatty acid
EFAD	Essential fatty acid deficiency
ETA	Eicosatrienoic (Mead) acid, mol% total fatty acids
FA	Fatty Acids
%FEV₁	Forced expiratory volume at 1 second, % predicted value
FFM	Fat free mass, kg
LA	Linoleic acid, mol% total fatty acids
OA	Oleic acid, mol% total fatty acids
PI	Pancreatic Insufficiency
PUFA	Polyunsaturated fatty acid
T:T	Triene to tetraene ratio (FA ratio of ETA; AA)
UAFAZ	Upper arm fat area Z score
UAMAZ	Upper arm muscle area Z score
WAZ	Weight for age Z score

Reference List

1. Stubbs CD, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim Biophys Acta*. 1984; 779:89–137. [PubMed: 6229284]
2. Clarke SD. The multi-dimensional regulation of gene expression by fatty acids: Polyunsaturated fats as nutrient sensors. *Curr Opin Lipidol*. 2004; 15:13–8. [PubMed: 15166803]
3. Kuo PT, Huang NN, Bassett R. The fatty acid composition of the serum chylomicrons and adipose tissue of children with cystic fibrosis of the pancreas. *J Pediatr*. 1962; 60:394–403. [PubMed: 14460644]
4. Strandvik B, Berg U, Kallner A, et al. Effect on renal function of essential fatty acid supplementation in cystic fibrosis. *J Pediatr*. 1989; 115:242–50. [PubMed: 2754552]
5. Strandvik B, Hultcrantz R. Liver function and morphology during long-term fatty acid supplementation in cystic fibrosis. *Liver*. 1994; 14:32–6. [PubMed: 8177027]

6. Strandvik, B. Polyunsaturated Fatty Acid in Human Nutrition. New York: Raven Press; 1992. Long chain fatty acid metabolism and essential fatty acid deficiency with special emphasis on cystic fibrosis; p. 159-67.
7. Strandvik B. Relation between essential fatty acid metabolism and gastrointestinal symptoms in cystic fibrosis. *Acta Paediatr Scand Suppl.* 1989; 363:58-63. discussion 63-5:58-63. [PubMed: 2701926]
8. Strandvik B, Gronowitz E, Enlund F, et al. Essential fatty acid deficiency in relation to genotype in patients with cystic fibrosis. *J Pediatr.* 2001; 139:650-5. [PubMed: 11713441]
9. Roulet M, Frascarolo P, Rappaz I, et al. Essential fatty acid deficiency in well nourished young cystic fibrosis patients. *Eur J Pediatr.* 1997; 156:952-6. [PubMed: 9453380]
10. Farrell PM, Mischler EH, Engle MJ, et al. Fatty acid abnormalities in cystic fibrosis. *Pediatr Res.* 1985; 19:104-9. [PubMed: 3918287]
11. Marcus MS, Sondel SA, Farrell PM, et al. Nutritional status of infants with cystic fibrosis associated with early diagnosis and intervention. *Am J Clin Nutr.* 1991; 54:578-85. [PubMed: 1877513]
12. Stark LJ, Knapp LG, Bowen AM, et al. Increasing calorie consumption in children with cystic-fibrosis - Replication with 2-year follow-up. *Journal of Applied Behavior Analysis.* 1993; 26:435-50. [PubMed: 8307828]
13. Stark LJ, Mulvihill MM, Powers SW, et al. Behavioral intervention to improve calorie intake of children with cystic fibrosis: Treatment versus wait list control. *Journal of Pediatric Gastroenterology and Nutrition.* 1996; 22:240-53. [PubMed: 8708877]
14. Trabulsi J, Ittenbach RF, Schall JJ, et al. Evaluation of formulas for calculating total energy requirements of preadolescent children with cystic fibrosis. *Am J Clin Nutr.* 2007; 85:144-51. [PubMed: 17209190]
15. Institute of Medicine. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. Washington, DC: National Academy Press; 2002. Energy; p. 5-1-5-114.
16. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem.* 1957; 226:497-509. [PubMed: 13428781]
17. Gronowitz E, Mellstrom D, Strandvik B. Serum phospholipid fatty acid pattern is associated with bone mineral density in children, but not in adults, with cystic fibrosis. *Br J Nutr.* 2006; 95:1159-65. [PubMed: 16768839]
18. Holman RT, Smyth RL, Johnson S. Effect of sex and age on fatty acid composition of human serum lipids. *Am Journal Clin Nutr.* 1979; 32:2390-9. [PubMed: 506962]
19. Holman RT, Johnson SB, Ogburn PL. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. *Proc Natl Acad Sci U S A.* 1991; 88:4835-9. [PubMed: 2052562]
20. Holman R. The ratio of trienoic: Tetraenoic acids in tissue lipids as a measure of essential fatty acid requirement. *J Nutr.* 1960:405-10. [PubMed: 14402760]
21. Chase HP, Cotton EK, Elliott RB. Intravenous linoleic acid supplementation in children with cystic fibrosis. *Pediatrics.* 1979; 64:207-13. [PubMed: 382082]
22. Decsi T, Koletzko B. Fatty acid composition of plasma lipid classes in healthy subjects from birth to young adulthood. *Eur J Pediatr.* 1994; 153:520-5. [PubMed: 7957371]
23. Jeppensen PB, Hoy CE, Mortensen P. Essential fatty acid deficiency in patients receiving home parenteral nutrition. *Am J Clin Nutr.* 1998; 68:126-33. [PubMed: 9665106]
24. Landon C, Kerner JA, Castillo R, et al. Oral correction of essential fatty acid deficiency in cystic fibrosis. *J Parenter Enteral Nutr.* 1981; 5:501-4.
25. Parsons HG, Oloughlin EV, Forbes D, et al. Supplemental calories improve essential fatty-acid deficiency in cystic-fibrosis patients. *Pediatr Res.* 1988; 24:353-6. [PubMed: 3211621]
26. Rettammel AL, Marcus MS, Farrell PM, et al. Oral supplementation with a high-fat, high-energy product improves nutritional status and alters serum lipids in patients with cystic fibrosis. *J Am Diet Assoc.* 1995; 95:454-9. [PubMed: 7699188]
27. Shoff SM, Ahn HY, Davis L, et al. Temporal associations among energy intake, plasma linoleic acid, and growth improvement in response to treatment initiation after diagnosis of cystic fibrosis. *Pediatrics.* 2006; 117:391-400. [PubMed: 16452358]

28. Siguel EN, Chee KM, Gong JX, et al. Criteria for essential fatty acid deficiency in plasma as assessed by capillary column gas-liquid chromatography. *Clin Chem*. 1987; 33:1869–73. [PubMed: 3665042]
29. van Egmond AW, Kosorok MR, Kosciuk R, et al. Effect of linoleic acid intake on growth of infants with cystic fibrosis. *Am J Clin Nutr*. 1996; 63:746–52. [PubMed: 8615359]
30. Lohman, T.; Roche, AR.; Martorell, R. *Anthropometric Standardization Reference Manual*. Champaign, IL: Human Kinetics; 1988.
31. Himes JH, Roche AF, Thissen D, et al. Parent-specific adjustments for evaluation of recumbent length and stature of children. *Pediatrics*. 1985; 75:304–13. [PubMed: 3969331]
32. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, et al. CDC Growth Charts: United States. *Adv Data*. 2000:1–27. [PubMed: 11183293]
33. Frisancho, AR. *Anthropometric Standards for Assessment of Growth and Nutritional Status*. Ann Arbor, MI: University of Michigan Press; 1990.
34. Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. *Am J Clin Nutr*. 1981; 34:2540–5. [PubMed: 6975564]
35. Morris, A.; Kanner, RE.; Crapo, R., et al. *Clinical Pulmonary Function Testing: A Manual of Uniform Laboratory Procedures*. 2. Salt Lake City, UT: Intermountain Thoracic Society; 1984. p. 1-24.
36. Gardner RM. Report of snowbird workshop on standardization of spirometry. *Am Rev Resp Dis*. 1979; 119:831–8. [PubMed: 453705]
37. Zapletal, A. Lung function in children and adolescents - methods, reference values. In: Herzog, H., editor. *Progress in Respiration Research*. Pratteln, Switzerland: Offsetdruck; 1987.
38. Knudson RJ, Lebowitz MD, Holberg CJ, et al. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis*. 1983; 127:725–34. [PubMed: 6859656]
39. Wang X, Dockery DW, Wypij D, et al. Pulmonary function between 6 and 18 years of age. *Pediatr Pulmonol*. 1993; 15:75–88. [PubMed: 8474788]
40. Laird N, Ware JH. Random-effects models for longitudinal data. *Biometrics*. 1982; 38:963–74. [PubMed: 7168798]
41. Akaike H. New Look at Statistical-Model Identification. *IEEE Transactions on Automatic Control*. 1974; AC19:716–23.
42. Tukey JW, Ciminera JL, Heyse JF. Testing the statistical certainty of a response to increasing doses of a drug. *Biometrics*. 1985; 41:295–301. [PubMed: 4005384]
43. Zhang J, Quan H, Ng J, et al. Some statistical methods for multiple endpoints in clinical trials. *Controlled Clinical Trials*. 1997; 18:204–21. [PubMed: 9204221]
44. Maqbool A, Schall J, Zemel B, et al. Essential fatty acid status and growth and pulmonary function in children with cystic fibrosis. *Pediatric Pulmonology*. 2004; 337
45. Gibson RA, Teubner JK, Haines K, et al. Relationships between pulmonary function and plasma fatty acid levels in cystic fibrosis patients. *J Pediatr Gastroenterol Nutr*. 1986; 5:408–15. [PubMed: 3723260]
46. Thompson GN. Relationships between essential fatty acid levels, pulmonary function and fat absorption in pre-adolescent cystic fibrosis children with good clinical scores. *Eur J Pediatr*. 1989; 148:327–9. [PubMed: 2707278]
47. Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 2002; 35:246–59. [PubMed: 12352509]
48. Bennett MJ, Medwadowski BF. Vitamin A, vitamin E, and lipids in serum of children with cystic fibrosis or congenital heart defects compared with normal children. *Am J Clin Nutr*. 1967; 20:415–21. [PubMed: 6067223]
49. Holman RT. Nutritional and metabolic interrelationships between fatty acids. *Fed Proc*. 1964; 23:1062–7. [PubMed: 14209800]
50. Underwood BA, Denning CR, Navab M. Polyunsaturated fatty acids and tocopherol levels in patients with cystic fibrosis. *Ann N Y Acad Sci*. 1972; 203:237–47. [PubMed: 4512105]
51. Cawood AL, Carroll MP, Wootton SA, et al. Is there a case for n-3 fatty acid supplementation in cystic fibrosis? *Curr Opin Clin Nutr Metab Care*. 2005; 8:153–9. [PubMed: 15716793]

52. Coste T, Armand M, Lebacqz J, et al. An overview of monitoring and supplementation of omega 3 fatty acids in cystic fibrosis. *Clinical Biochemistry*. 2007; 40:511–20. [PubMed: 17316592]
53. Hans AE, Wiese HF, Boelsche AN, et al. Role of linoleic acid in infant nutrition. Clinical and chemical study of 428 infants fed on milk mixture varying in kind and amount of fat. *Pediatrics*. 2006; 21:171–92.
54. Hansen AE, Haggard ME, Boelsche AN, et al. Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. *J Nutr*. 1958; 66:565–76. [PubMed: 13621281]
55. Paulsrud JR, Pensler L, Whitten CF, et al. Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. *Am J Clin Nutr*. 1972; 25:897–904. [PubMed: 4626563]
56. Lai HC, Kosorok MR, Laxova A, et al. Nutritional status of patients with cystic fibrosis with meconium ileus: a comparison with patients without meconium ileus and diagnosed early through neonatal screening. *Pediatrics*. 2000; 105:53–61. [PubMed: 10617704]
57. Goyens PL, Spilker ME, Zock PL, et al. Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of alpha-linolenic acid and linoleic acid in the diet and not by their ratio. *Am J Clin Nutr*. 2006; 84:44–53. [PubMed: 16825680]
58. Cunnane SC, Belza K, Anderson MJ, et al. Substantial carbon recycling from linoleate into products of de novo lipogenesis occurs in rat liver even under conditions of extreme dietary linoleate deficiency. *J Lipid Res*. 1998; 39:2271–6. [PubMed: 9799813]
59. Hubbard VS. What is the association of essential fatty acid status with cystic fibrosis? *Eur J Pediatr*. 1983; 141:68–70. [PubMed: 6662144]
60. Brown AJ, Pang E, Roberts DC. Persistent changes in the fatty acid composition of erythrocyte membranes after moderate intake of n-3 polyunsaturated fatty acids: Study design implications. *Am J Clin Nutr*. 1991; 54:668–73. [PubMed: 1832813]
61. Hubbard VS, McKenna MC. Absorption of safflower oil and structured lipid preparations in patients with cystic fibrosis. *Lipids*. 1987; 22:424–8. [PubMed: 3613873]
62. Berguerand M, Klapisz E, Thomas G, et al. Differential stimulation of cytosolic phospholipase A2 by bradykinin in human cystic fibrosis cell lines. *Am J Respir Cell Mol Biol*. 1997; 17:481–90. [PubMed: 9376123]
63. Carlstedt-Duke J, Bronnegard M, Strandvik B. Pathological regulation of arachidonic acid release in cystic fibrosis: The putative basic defect. *Proc Natl Acad Sci U S A*. 1986; 83:9202–6. [PubMed: 3097647]
64. Levistre R, Lemnaouar M, Rybkine T, et al. Increase of bradykinin-stimulated arachidonic acid release in a delta F508 cystic fibrosis epithelial cell line. *Biochim Biophys Acta*. 1993; 1181:233–9. [PubMed: 7686400]
65. Miele L, Cordella-Miele E, Xing M, et al. Cystic fibrosis gene mutation (deltaF508) is associated with an intrinsic abnormality in Ca²⁺-induced arachidonic acid release by epithelial cells. *DNA Cell Biol*. 1997; 16:749–59. [PubMed: 9212168]
66. Chase HP, Dupont J. Abnormal levels of prostaglandins and fatty-acids in blood of children with cystic-fibrosis. *Lancet*. 1978; 2:236–8. [PubMed: 79031]
67. Strandvik B, Svensson E, Seyberth HW. Prostanoid biosynthesis in patients with cystic fibrosis. *Prostaglandins Leukot Essent Fatty Acids*. 1996; 55:419–25. [PubMed: 9014220]
68. Friedman Z. Essential fatty acids revisited. *Am J Dis Child*. 1980; 134:397–408. [PubMed: 6768287]
69. Horrobin DF, Manku MS, Huang YS. Effects of essential fatty acids on prostaglandin biosynthesis. *Biomed Biochim Acta*. 1984; 43:S114–S120. [PubMed: 6517895]
70. Lerner R, Lindstrom P, Berg A, et al. Development and characterization of essential fatty acid deficiency in human endothelial cells in culture. *Proc Natl Acad Sci U S A*. 1995; 92:1147–51. [PubMed: 7862650]
71. Mathias MM, Dupont J. Quantitative relationships between dietary linoleate and prostaglandin (eicosanoid) biosynthesis. *Lipids*. 1985; 20:791–801. [PubMed: 4068908]
72. Bhura-Bandali FN, Suh M, Man SFP, et al. The Delta F508 mutation in the cystic fibrosis transmembrane conductance regulator alters control of essential fatty acid utilization in epithelial cells. *Journal of Nutrition*. 2000; 130:2870–5. [PubMed: 11110839]

73. Wene JD, Connor WE, DenBesten L. The development of essential fatty acid deficiency in healthy men fed fat-free diets intravenously and orally. *The Journal of Clinical Investigation*. 1975; 56:127–34. [PubMed: 806609]
74. Peter, JH.; Kubow, JK.; Shils, M.; Moshe, S.; Ross, AC., et al., editors. *Modern Nutrition in Health and Disease*. 10. Philadelphia, PA: Lippincot Williams & Wilkins; 2006. p. 92-122.
75. Mascioli E, Lopes S, Champagne C, et al. Essential fatty acid deficiency and home total parenteral nutrition patients. *Nutrition*. 1996; 12:245–9. [PubMed: 8862529]
76. Ailhaud G, Massiera F, Weill P, et al. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res*. 2006; 45:203–36. [PubMed: 16516300]
77. Kris-Etherton PM, Taylor DS, Yu-Poth S, et al. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr*. 2000; 71:179S–88S. [PubMed: 10617969]
78. Lloyd-Still JD, Johnson SB, Holman RT. Essential fatty acid status and fluidity of plasma phospholipids in cystic fibrosis infants. *Am J Clin Nutr*. 1991; 54:1029–35. [PubMed: 1957818]
79. Lagerstedt SA, Hinrichs DR, Batt SM, et al. Quantitative determination of plasma c8-c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. *Mol Genet Metab*. 2001; 73:38–45. [PubMed: 11350181]

Figure 1.
The relationship between T:T and %LA

Table 1

Baseline clinical characteristics and selected fatty acid profiles

	Subjects with CF (n=77) Mean $\pm$ SD	Healthy Controls (n=23) Mean $\pm$ SD
Age, y	8.4 $\pm$ 0.9	8.4 $\pm$ 1.1
Sex, %female	51	57
F508 homozygous genotype, %	55	—
WAZ	-0.4 $\pm$ 1.2	-0.1 $\pm$ 0.9
BMIZ	-0.1 $\pm$ 1.1	-0.1 $\pm$ 1.0
HAZ	-0.5 $\pm$ 1.1	-0.1 $\pm$ 0.9
Adjusted HAZ	-0.8 $\pm$ 1.1	—
UAMAZ	-0.0 $\pm$ 1.2	0.5 $\pm$ 1.0 _—
UAFMZ	-0.3 $\pm$ 1.0	-0.1 $\pm$ 1.0 _—
FEV1, (Knudsen) ¹ % predicted	97 $\pm$ 15	—
FEV1, (Wang) ² % predicted	100 $\pm$ 15	—
Dietary Intake	(n=66)	(n=22)
Estimated Energy Requirement ³ , %	115 $\pm$ 35	91 $\pm$ 12 [†]
Total fat, grams/day	90 $\pm$ 31	62 $\pm$ 12 [†]
Fat intake, % of kcals/day	37 $\pm$ 5	32 $\pm$ 4 [†]
Linoleic Acid		
grams/day	13 $\pm$ 6	10 $\pm$ 4 [*]
%Adequate Intake	130 $\pm$ 55	103 $\pm$ 36 [*]
Alpha Linolenic Acid		
grams/day	1.3 $\pm$ 0.5	0.8 $\pm$ 0.2 [†]
%Adequate Intake	135 $\pm$ 60	89 $\pm$ 20 [†]
Oleic Acid, grams/day	31 $\pm$ 11	21 $\pm$ 4 [‡]
Select Serum Fatty Acids, mol % total FA ⁴		
Linoleic acid	21.9 [15.2, 29.3]	24.4 [18.5, 29.1] [†]
Alpha Linolenic Acid	0.19 [0.10, 0.41]	0.16 [0.10, 0.39] ^{**}
Oleic Acid	10.9 [7.9, 14.2]	9.1 [8.2, 11.3] [‡]
Arachidonic Acid	8.1 [4.2, 12.7]	11.1 [8.4, 12.6] [‡]
Eicosatrienoic Acid	0.17 [0.05, 1.56]	0.11 [0.07, 0.22] [†]
Ratio		
Triene:Tetraene ⁵	0.030 [0.004, 0.219]	0.009 [0.005, 0.019] [‡]

Based on the equations of ¹Knudsen et al (38) and ²Wang et al (39). Estimated energy requirement for the subjects with CF (12). ⁴Median [range] values presented, as all fatty acids (except for linoleic acid) and the triene: tetraene ratio are not normally distributed. ⁵ Ratio of eicosatrienoic (mead) acid to arachidonic acid. Normally distributed variables are compared using means $\pm$ sd and t-tests; non-normally distributed variables for which medians are compared by Wilcoxon rank sum.

* p<0.05;

** p<0.005;

[†]p<0.001;

[‡]p<0.0001.

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Table 2

Baseline correlations (p values) between selected serum fatty acids and growth and pulmonary status in children CF and PL.

	Subjects with CF (n=77)							
	WAZ	HAZ	AdjHAZ ¹	BMIZ	UAMAZ	UAFAZ	FEV ₁ ^{2,3}	FEV ₁ ^{2,4}
Select Fatty Acids								
Linoleic acid	0.344 ^{**}	0.309 [*]	0.290 [†]	0.267 [†]	0.297 [*]	0.109	0.267 [†]	0.286 [†]
Alpha linolenic acid	0.241 [†]	0.109	0.121	0.310 [*]	0.353 ^{**}	0.151	0.147	0.184
Oleic acid	-0.234 [†]	-0.291 [*]	-0.242 [†]	-0.171	-0.166	-0.145	0.139	0.073
Arachidonic acid	-0.082	-0.047	-0.065	-0.096	-0.130	0.034	-0.216	-0.331 ^{**}
Eicosatrienoic acid	-0.108	-0.161	-0.121	-0.084	-0.139	0.022	-0.136	-0.187
Ratio								
Triene: Tetraene⁵	-0.099	-0.167	-0.132	-0.070	-0.111	-0.018	-0.051	-0.061

Data available for ¹76/77 subjects (99%) and ²75/77 subjects (97%), respectively; FEV₁ based on the equations of ³Knudsen et al (38), ⁴Wang et al (39), ⁵Ratio of eicosatrienoic (mead) acid to arachidonic acid.

* p<0.01;

** p<0.005; statistical significance defined as p<0.01.

[†] p<0.05 shown for trend information.

Table 3

The relationship between serum LA status or T:T ratio with growth, body composition, and FEV₁ for the subjects with CF

Z scores													FEV ₁ %	
adjHAZ			WAZ			UAMAZ			Wang					
	Coeff	SE	p	Coeff	SE	p	Coeff	SE	p	Coeff	SE	p		
LA cut points, mol %														
Group (20)	0.83	0.35	.017	0.82	0.37	.024	0.74	0.38	.052	5.62	6.21	.366		
Time	0.12	0.04	.007	0.05	0.07	.455	0.33	0.11	.002	-2.81	2.51	.263		
Group X Time	-0.11	0.05	.039	-0.07	0.08	.363	-0.21	0.12	.092	-2.01	2.80	.472		
Gender	0.05	0.26	.855	0.30	0.27	.254	0.20	0.26	.437	1.68	3.37	.619		
Constant	-1.54	0.36	.000	-1.19	0.37	.001	-0.89	0.38	.019	98.46	5.98	.000		
n= 63 ; n=14			AIC=165.4			AIC=305.7			AIC=432.9			AIC=1181.1		
Group (21)	0.67	0.27	.012	0.87	0.28	.002	0.93	0.30	.002	12.94	4.72	.006		
Time	0.04	0.03	.183	0.03	0.05	.543	0.33	0.08	.000	-3.30	1.69	.051		
Group X Time	-0.00	0.04	.913	-0.06	0.07	.365	-0.29	0.10	.005	-2.00	2.24	.372		
Gender	0.08	0.25	.745	0.35	0.26	.171	0.23	0.25	.367	2.99	3.18	.348		
Constant	-1.27	0.25	.000	-1.05	0.26	.000	-0.83	0.27	.002	94.88	4.03	.000		
n= 45 ; n=32			AIC=164.0			AIC=301.4			AIC=426.0			AIC=1172.2		
Group (26)	0.71	0.42	.090	1.27	0.46	.005	1.14	0.48	.018	13.65	7.77	.079		
Time	0.07	0.07	.362	-0.15	0.11	.182	0.12	0.17	.480	-8.39	3.44	.015		
Group X Time	-0.03	0.08	.712	-0.16	0.12	.171	-0.06	0.18	.764	-4.40	3.63	.225		
Gender	-0.08	0.25	.750	0.17	0.26	.512	0.10	0.24	.688	1.03	3.33	.757		
Constant	-0.16	0.42	.708	0.69	0.46	.132	0.78	0.48	.105	115.59	7.64	.000		
n=8; n=69			AIC=168.5			AIC=303.2			AIC=430.4			AIC=1178.9		
T:T cut points														
Group (0.02)	0.20	0.28	.471	0.42	0.30	.168	0.20	0.32	.524	2.62	4.92	.594		
Time	0.02	0.03	.536	-0.12	0.05	.019	0.16	0.08	.047	-4.14	1.63	.011		
Group X Time	-0.03	0.04	.438	-0.20	0.07	.002	-0.02	0.11	.816	0.51	2.22	.818		

statistical significance noted for **p 0.010 in bold**. AIC is the Akaike Information Criterion, a method employed to assess relative goodness of fit (41).