

Adipose Tissue Abnormalities in Cystic Fibrosis: Noninvasive Determination of Mono- and Polyunsaturated Fatty Acids by Carbon-13 Topical Magnetic Resonance Spectroscopy

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ABSTRACT. Natural abundance *in vivo* carbon-13 topical magnetic resonance (TMR) spectroscopy was used to assess human adipose tissue stores of essential (polyunsaturated) fatty acids. TMR spectra were obtained from 17 normal volunteers and nine cystic fibrosis patients using an Oxford TMR-32 with a surface coil that sampled tissue less than 1 cm below the surface of an extremity. Spectra were taken of lower leg adipose tissue. Polyunsaturated fatty acid content was determined by comparing peak heights of the polyunsaturated peak (internal unsaturated carbons, 128 ppm) to C-1 carboxyl groups (173 ppm). Monounsaturated fatty acid content was determined by subtracting the polyunsaturated peak from the peak observed for all unsaturated carbons (external unsaturated carbon, 130 ppm) and dividing this ratio by the carboxyl peak. *In vivo* TMR of normal volunteers resulted in observed polyunsaturated fatty acid content of $17.8 \pm 2.1\%$ and a monounsaturated content of $44.8 \pm 3.8\%$. The polyunsaturated and monounsaturated fatty acid content of adipose tissue from the cystic fibrosis patients was $15.0 \pm 2.0\%$ ($p < 0.005$ versus normal volunteers) and $47.8 \pm 6.5\%$ (NS), respectively. One cystic fibrosis patient without fat malabsorption had decreased adipose polyunsaturateds, whereas another patient on high calorie gastrostomy feeds had normal levels. Carbon-13 TMR spectroscopy is a sensitive, noninvasive technique for determining essential fatty acid status in subcutaneous adipose tissue of patients with cystic fibrosis. (*Pediatr Res* 24: 243-246, 1988)

Abbreviations

TMR, topical magnetic resonance
CF, cystic fibrosis
PUFA, polyunsaturated fatty acids
MUFA, monounsaturated fatty acids
EFA, essential fatty acids
K, kilobytes

cholesterol esterification. Numerous studies have demonstrated abnormalities of EFA in circulating triglycerides and phospholipids in patients with CF (2-4). Although most investigations have focused on these circulating lipid classes, some studies have demonstrated abnormal levels of EFA stored in adipose tissue of CF patients with malabsorption (5, 6). Because percutaneous adipose tissue biopsies are invasive and not a routine pediatric procedure, the recent studies have been limited to postmortem adipose samples of both CF patients and control children (6). The relationship between abnormal EFA status and fat malabsorption remains unclear. Low levels of EFA have been noted in serum and membrane lipids in CF patients both with and without steatorrhea (3, 4, 7, 8). A recent report demonstrating abnormal *in vitro* turnover of arachidonic acid in lymphocytes from CF patients suggests the possibility of a fundamental abnormality of essential fatty acid metabolism (9).

TMR spectroscopy has been used for *in vivo* studies of human pathophysiology for several years (10, 11). The majority of these studies has used the ^{31}P nucleus, providing information on high energy phosphates, inorganic phosphate, and tissue pH (11). The ^{13}C nucleus also has paramagnetic properties, but has two major disadvantages when compared to ^{31}P . First, the low natural abundance (about 1.1%) of ^{13}C compromises sensitivity necessitating larger quantities of sample material. Second, ^{13}C - ^1H interactions produce splitting of individual carbon signals, requiring the use of proton decoupling radiofrequency signals to eliminate these interactions. An advantage of the ^{13}C nucleus is that it offers superior separation of the individual resonances, important in view of the diversity of biologically active carbon containing molecules, carbohydrates, peptides, lipids, etc. (12). This study describes ^{13}C TMR spectroscopy techniques for the noninvasive, *in vivo* determination of adipose tissue PUFA and MUFA content in patients with CF and normal controls.

MATERIALS AND METHODS

Nine outpatients with CF from the University of California, Davis, Cystic Fibrosis Center volunteered for this study. All CF patients had sweat chloride concentrations of more than 60 mEq/liter measured by pilocarpine iontophoresis (13). The percent ideal body weight (weight for height), triceps skinfold percentile, and Schwachman clinical scores were determined for each patient (14-16). Serum carotene (normal = 40-200 $\mu\text{g}/\text{dl}$) was measured by standard clinical laboratory techniques. Eight of the CF patients had steatorrhea for which they were taking oral pancreatic enzyme replacement therapy. One CF patient had normal 72-h coefficient of fat absorption and bentriomide-PABA pancreatic function testing. One 24-yr-old female CF patient

The PUFA stored in adipose tissue are composed primarily of EFA, linoleic acid (18:2n6) and to a much smaller extent, arachidonic acid (20:4n6) (1). EFA have three main uses: as prostaglandin precursors, components of cell membranes, and for

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required supplemental oxygen and was receiving 2500 cal/day as Isocal HCN via gastrostomy tube. Seventeen normal controls were pediatric and adult volunteers who underwent similar anthropometric evaluation. All studies were approved by the Human Subjects Committee of the University of California Davis Medical Center and informed consent was obtained from all subjects.

TMR spectra were obtained on an Oxford Research Systems TMR-32 operating at 1.9 tesla with a horizontal clear bore of 20 cm. A paired concentric surface coil probe was used with an outer ^1H decoupling coil 3 cm in diameter and an inner ^{13}C coil measuring 2.0 cm (17–19). The ^{13}C coil has an active sampling volume of about 7 ml. The probe was placed in a plastic stage protecting the coils from damage, yet allowing the tissue to be studied to be placed less than 1 mm from the plane of the coils. The patients reclined on a bed allowing the lower leg to rest over the coils in the center of the magnet. Shimming, optimizing the homogeneity of the magnetic field in the sample area, was accomplished by maximizing the integration of the H_2O signal obtained from the ^1H probe. Continuous single frequency decoupling was performed by applying a constant low power (200 MW) radiofrequency signal at the frequency of the protons attached to unsaturated carbons in order to remove ^1H - ^{13}C interactions, this also minimized any potential tissue heating. ^{13}C studies were obtained by applying a 7 μs pulse every 0.25 s for 2048 acquisitions, requiring about 8 min. The resulting free induction decay containing 2 K data points was zero-filled to 8 K and 5 Hz Gaussian line broadening was applied before Fourier transformation. All spectra were plotted from 0–200 ppm and areas of interest plotted in 10 ppm increments. We have recently described the details of these methods, including comparison to standard gas chromatographic techniques (19).

Relative PUFA content was determined by dividing the peak height of the internal polyunsaturated carbon signal (128 ppm) by the height of the carboxyl signal (173 ppm) (Fig. 1). MUFA

content was determined by subtracting the 128 ppm signal from the total unsaturated signal (external carbon, 130 ppm) and dividing by the carboxyl signal. These ratios were then multiplied by a correction factor (21.4) based on previous T1 (spin-lattice) and nuclear Overhauser effect determinations (19). All results are expressed as mean \pm SD unless otherwise indicated and results were compared by unpaired, two-tailed *t* tests.

RESULTS

The characteristics of the CF and control populations are summarized in Table 1. No significant differences were noted in age, percent ideal body weight, or triceps skinfold percentiles. The CF patient without steatorrhea had higher triceps skinfold percentiles (60%) and serum carotene (69 $\mu\text{g}/\text{dl}$) than the other CF patients. The patient receiving gastrostomy feedings had 10 percentile triceps skinfolds, 75% ideal body weight, and carotene and cholesterol levels similar to the other CF patients.

Using TMR, PUFA, and MUFA content was $17.8 \pm 2.1\%$ (14.9–21.4%) and $44.8 \pm 3.8\%$ (33.6–48.8%) in normal volunteers, whereas in CF patients values of $15.0 \pm 2.0\%$ (12.7–18.8%) ($p < 0.005$ versus normal) and $47.8 \pm 6.5\%$ (38.5–61.3%) (NS), respectively, were observed. The PUFA results of all subjects are compared to age in Figure 2. These values did not correlate with triceps skinfold percentile (Fig. 3), percent ideal body weight, age, Schwachman clinical score, or serum carotene. The CF patient without malabsorption had abnormal PUFA content of 13.7%, whereas the CF patient receiving supplemental gastrostomy feeds had PUFA content in the normal range, 18.8%. If the results from the gastrostomy-supplemented patient are removed, the differences between CF and normals become more pronounced, reaching significance levels of $p < 0.001$ and $p < 0.04$ for PUFA and MUFA.

DISCUSSION

This study demonstrates significant differences in adipose tissue composition between CF patients and normal controls. Although the differences were statistically significant, some overlap was noted between normal and CF children less than 15 yr of age, and between normals and the one CF patient receiving caloric supplementation via gastrostomy tube. The significance of the abnormal EFA status found in CF patients remains unanswered. Initial studies supported the hypothesis that abnormal EFA status was secondary to malabsorption and inadequate caloric intake (20). However, further investigations did not document an improvement in EFA status in patients receiving pancreatic enzyme supplements despite a substantial decrease in steatorrhea (5, 7, 20). Abnormal EFA levels have been demonstrated in several circulating membrane lipid classes in CF patients without malabsorption and in CF heterozygotes (8, 21).

There is some evidence that abnormal EFA metabolism may be part of the underlying disorder in CF. Abnormalities in cord blood EFA in infants with CF suggest that a fundamental defect of fat metabolism may be in part responsible for the clinical manifestations of this disease (22). Several investigators have reported abnormalities of prostaglandin metabolism in CF patients (2, 4). One report has demonstrated elevation in sweat chloride concentration in a non-CF infant receiving long-term PGE_1 infusion, with normalization after cessation of PGE_1 therapy (23). *In vitro* studies have demonstrated abnormal regulation of incorporation and turnover of EFA in erythrocytes and lymphocytes taken from CF patients (4, 9). Our findings in the CF patient with normal fat absorption also suggest some form of an underlying disorder of EFA metabolism independent of fat malabsorption or a pattern of malabsorption not detectable by standard balance studies. The normal results in the CF patient receiving high calorie gastrostomy feedings demonstrate the ability to correct the reduced levels of EFA in adipose by providing sufficiently high caloric intake. This supports the hypothesis that

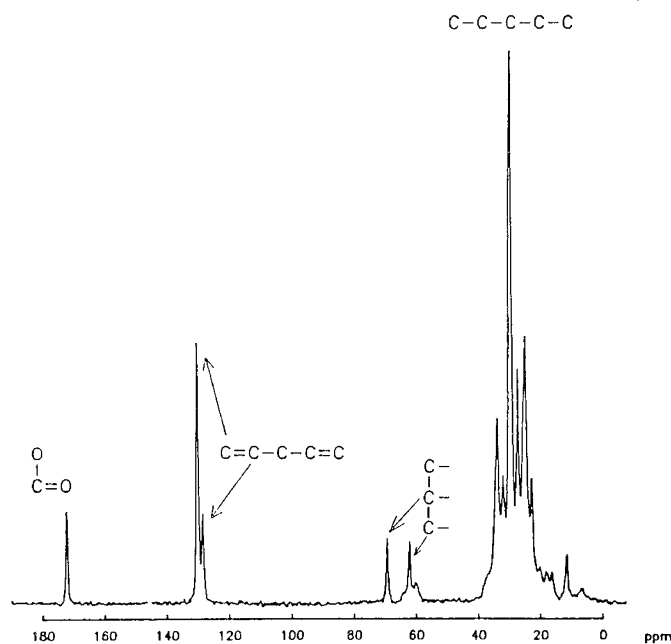


Fig. 1. Typical natural abundance ^{13}C TMR spectra of human adipose tissue with individual carbon resonances as noted. The larger unsaturated peak at 130 ppm represents external unsaturated carbons and is found in all unsaturated molecules, whereas the smaller peak at 128 ppm corresponds to only the internal unsaturated carbons, specific for polyunsaturated molecules. The peak at 69 ppm represents 2-glycerol carbon backbone and the 62 ppm peak is the 1,3-glycerol carbons. The broad shoulder at 62 ppm represents incomplete proton-carbon decoupling.

Table 1. Patient data

Patients	n	Sex	Age	Skinfold (%)	Ideal body wt (%)	Schwachman score	Carotene ($\mu\text{g}/\text{dl}$)
CF	9	3F 6M	18.0 \pm 10.5	33.6 \pm 23.1	95.7 \pm 13.2	52.5 \pm 10.3	25.9 \pm 18.6
Normals	17	8F 9M	22.2 \pm 12.8	40.2 \pm 33.7	100.4 \pm 8.5		
			NS*	NS	NS		

* Not significant at $p < 0.05$.

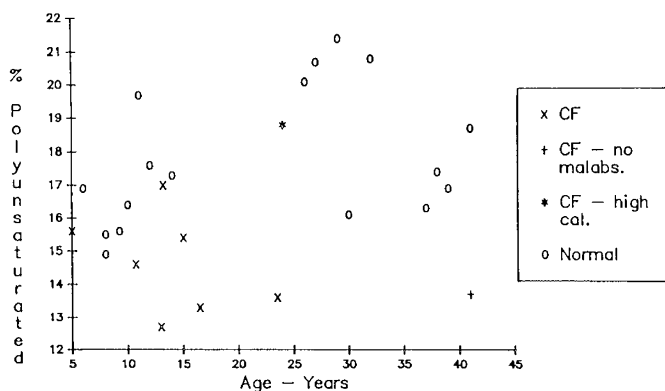


Fig. 2. Adipose tissue PUFA content compared to age. Significant overlap was noted in subjects less than 15 yr of age. The CF patient without fat malabsorption is clearly in the lower range, whereas the CF patient receiving high calorie supplemental feeds is clearly in the normal range.

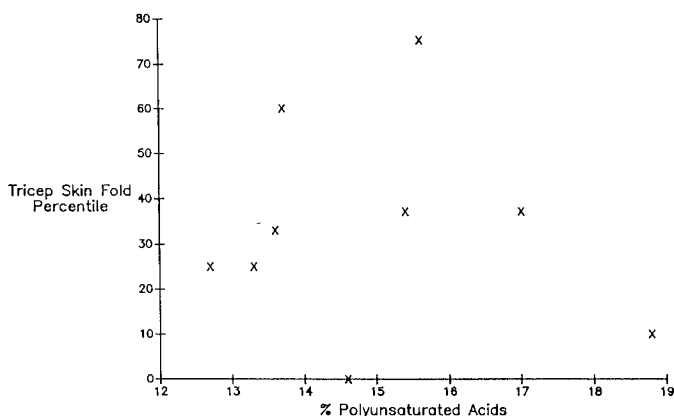


Fig. 3. Triceps skinfold percentile compared to observed adipose tissue PUFA levels. No significant correlation between these quantitative and qualitative adipose tissue measurements ($R^2 = 0.12$) was demonstrated.

sufficient caloric intake will prevent EFA from being used as an energy source, permitting their use for essential biochemical functions.

EFA deficiency is thought to play a significant role in the clinical status of CF patients. Animal models of EFA deficiency have demonstrated abnormalities in pulmonary defense mechanisms similar to those seen in patients with CF (24). Some patient studies have correlated clinical status with the degree of EFA abnormalities, but the classic clinical findings of EFA deficiency are rare in CF (3, 6). In our patient studies a correlation between clinical score and EFA status was not observed, but this may be limited by the fact that the patients under study were only mildly afflicted. Although initial studies aimed at correcting the EFA abnormalities in CF did produce biochemical and clinical improvement, further studies have not been able to reproduce all of these findings (22, 25-27). Unfortunately several of the supplementation studies (using medium-chain triglycerides, safflower oil, intravenous fat, etc.) involving CF patients may not

have provided adequate levels of caloric and/or EFA substrates. Our finding of normal adipose EFA in the one patient undergoing substantial caloric supplementation may underscore the importance of increased total caloric intake in order to improve EFA status.

In our study percent ideal body weight and triceps skinfold percentiles were used to estimate nutritional status. These measurements serve as an estimate of generalized nutritional status and total body fat content and have been used in other studies of CF patients (27). Our results demonstrate a lack of correlation between observed adipose PUFA content and triceps skinfold percentile (Fig. 3) or percent ideal body weight, indicating that these quantitative measurements alone may not provide an adequate qualitative estimate of fat stores or EFA status.

The noninvasive nature of TMR spectroscopy permits the safe evaluation of both normal children and patients with abnormalities in fat absorption and metabolism avoiding invasive techniques such as adipose biopsies. This technique may be used to optimize nutritional support of CF patients and we anticipate initiating studies to address the effects of EFA supplementation versus total caloric supplementation.

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