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ESSENTIAL FATTY ACID STATUS OF CYSTIC FIBROSIS CHILDREN - EVALUATION AND TREATMENT

by

MARGERY JANE DENEER

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A B S T R A C T

Seven children with previously diagnosed cystic fibrosis, participated in a twenty-eight day study designed to evaluate the efficacy of two treatment methods on essential fatty acid status. Seven day food records were calculated for protein, fat, carbohydrates, linoleic acid and caloric content. Children were randomly assigned to either the topical or oral treatment group, where the amount of oil to be used was determined to be 1 or 2 percent of daily calories, respectively. Blood samples taken on day 1, 15 and 29, were analyzed for total plasma, plasma phospholipid and total red cell fatty acids, using gas-liquid chromatography. Children receiving enzyme replacement therapy had fatty acid patterns indicative of EFA deficiency; a significant depression in 18:2 (20 - 28%), and increased concentrations of 16:1 and 18:1. Little alteration occurred in 20:4. The non-essential fatty acid 20:3n9 was observed in all subjects and controls; the highest concentration was 2.3%. The oral treatment improved the fatty acid status of one

subject; 18:2 increased 20% in plasma phospholipids, 20:4 increased 8.5% in total plasma lipids. In the one subject who showed some response to topical treatment, the phospholipid concentration of 20:3n9 declined by 57%, while changes in other fatty acids (16:1, 18:1, 18:2) indicated an exacerbation of the deficiency. Children not receiving enzyme therapy had fatty acid patterns comparable to control values, and demonstrated little response to either form of treatment. The results showed that despite intakes of linoleic acid within recommended amounts (mean intake 4.7%), only children receiving enzyme therapy had fatty acid patterns suggestive of EFA deficiency. This further implies that EFA deficiency is secondary to malabsorption in cystic fibrosis.

The topical treatment was poorly accepted, and limiting in the amount of oil that could be used. Oral treatment was the preferred method and resulted in improvement in fatty acid status when compliance was assurred.

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L I S T O F T A B L E S

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REVIEW OF LITERATURE

Originally considered to be an experimental condition inducible in rats, essential fatty acid (EFA) deficiency has been observed in humans with increasing frequency (Connor 1975). While a naturally occurring deficiency has not yet been reported, EFA deficiency can develop secondarily to dietary modification and surgical intervention (Prottey 1976). It has been reported in infants fed low-fat formulas, during prolonged intravenous hyperalimentation with a fat-free infusate (Collins et al 1971) and in cases of continual fecal loss of fat due to malabsorption (Rosenlund et al 1974). A deficiency in the adult may be difficult to induce, particularly if body stores of essential fatty acids are adequate. However, the onset may be fairly rapid when there is an increase in body catabolism. The ensuing complications from EFA deficiency are widespread, and without careful monitoring and treatment, can be fatal.

Elucidation of the function of essential fatty acids in human nutrition began with the observations of Burr and Burr, in 1929, that rats required unsaturated fats in the

diet for normal growth. From that point, numerous studies with animals and humans have extended the knowledge about the significance of essential fatty acids (Holman 1975). Of major importance, is the structural role which EFA plays in imparting desirable physical properties to membranes. Esterification of polyunsaturated fatty acids in the β-position of the phospholipid molecule, ensures the correct physiochemical properties for optimal functioning of the biomembranes. This vital role is essential for the integrity of all living animal tissue (Vergroesen et al 1975).

Trienoic fatty acids with chain lengths of 19, 20, 21 carbon atoms and with cis double bonds in positions 8, 11 and 14, are capable of acting as precursors for the synthesis of prostaglandins with high biological activity (van Dorp 1976). Only those fatty acids capable of this function are considered to have essential fatty acid status. Prostaglandins have many pharmacological properties; smooth muscle stimulation, constriction and dilation of blood vessels (Gurr and James 1975), and bronchial passages, (Friedman and Demers 1978) and an influence on blood platelet aggregation induced by ADP. This last effect

suggests the possibility of a regulatory function in the formation of arterial thrombi (Vergroesen et al 1975).

It is not surprising on the basis of the two major functions of essential fatty acids that clinical symptoms are extensive in a deficiency state. Essential fatty acid deficiency in man is characterized by dermatitis, growth failure, increased susceptibility to infection, increased permeability of the skin to water, and a decreased synthesis of prostaglandins (Vergroesen et al 1975) which have been implicated in the pathophysiology of pulmonary function (Friedman and Demers 1978), and in abnormal thrombocyte aggregation. However, prior to the onset of clinical signs of deficiency, abnormalities can be identified at a biochemical level. A characteristic series of changes in the fatty acid pattern of serum and tissues occurs in individuals with EFA deficiency. The concentration of the essential fatty acids, linoleic (18:2) and arachidonic acid (20:4) decrease, while there is a concurrent increase in the endogenous monoenoic fatty acids, palmitoleic (16:1) and oleic (18:1). The presence of a non-essential fatty acid 5, 8, 11 - eicosatrienoic acid (20:3n9) is considered indicative of a deficiency (Rivers and Hassam 1975). This

fatty acid arises from the desaturation of oleic acid, utilizing the same enzyme system as is required for the conversion of linoleic acid to arachidonic acid (Sprecher 1975). Linoleic acid acts to competitively inhibit the formation of 20:3n9. In a deficiency state, 20:3n9 is substituted for arachidonic acid in the phospholipid structure to maintain the required degree of unsaturation. This substitution however, does not allow for normal cell functioning. As the level of 20:4 decreases in EFA deficiency, the production of 20:3n9 increases. Holman (1960) described a triene : tetraene ratio (20:3n9 : 20:4) as a criterion to explain adequacy of EFA in the diet. A normal value of 0.4 or less indicates that the minimum requirement for 18:2 has been met, while in a deficiency state, the ratio may be elevated to a value of 5 to 6.

The actual requirement for essential fatty acids has been estimated from results of experimental work with animals and humans. It is often expressed as a percentage of calories and refers to an amount of linoleic acid (18:2). In man, this fatty acid can be converted to arachidonic acid (20:4) in vivo. Long-chain saturated triglycerides

increase the requirement for linoleic acid (Kaunitz et al 1960) as do medium chain triglycerides (Hirono et al 1977). In human subjects, 1% of total kilocalories was found to prevent dermal lesions in infants (Vergroesen et al 1975), while 4% was necessary for optimal caloric efficiency of infants (Adam et al 1958). When body catabolism is increased, an intake of 1% of kcals as linoleic acid may be inadequate for new tissue synthesis (Connor 1975). Vergroesen et al (1975) recommended a dietary linoleic acid intake of 13 - 16% of total kcals. At these levels, platelet aggregation was significantly decreased, coronary blood flow was increased and serum cholesterol levels were lowered. All of which may prove to be significant for patients with cardiovascular complications.

Because of the absence of a naturally occurring EFA deficiency in man, it is assumed that a mixed diet contains an adequate amount of linoleic acid. The concern therefore, is for the optimal intake necessary to correct or prevent EFA deficiency in those individuals considered to be at risk. Attention has recently been focused on cystic fibrosis patients in whom essential fatty acid deficiency has been routinely identified (Caren and Corbo 1966; Kuo et al 1962; Kuo and Huang 1965; Robinson 1975; Rosenlund et al 1974; Watts et al 1975).

Cystic fibrosis (CF) is a lethal hereditary disease of unknown etiology. Clinically, its main features include elevation of sweat electrolytes, involvement of the respiratory system and pancreatic insufficiency (Crozier 1974). Of concern nutritionally, is the presence and severity of the pancreatic insufficiency, which occurs in 80% of all patients resulting in steatorrhea and generalized malabsorption. These features either separately, or in combination with other factors, are being implicated in the etiology of the EFA deficiency in CF children.

While EFA deficiency has been observed primarily at the biochemical level in cystic fibrosis (i.e., a decrease in 18:2, 20:4; an increase in 16:1, 18:1) the concern is for the effects of a lifelong subclinical depletion and the possible consequence on the course of the CF. The exact etiology of EFA deficiency in cystic fibrosis has not been clearly defined, though most researchers agree that the intake of fat and the efficiency of absorption of each child are intimately involved in the onset of the deficiency. There is little support for the hypothesis of Rivers and Hassam (1975) that EFA deficiency may be the primary defect in cystic fibrosis.

Traditional diet therapy for CF patients involved restriction of fat intake to reduce persistent steatorrhea and its associated nutrient loss, particularly nitrogen. The validity of this practise was questioned by Chung et al (1951). These investigators were able to demonstrate that fat absorption improved in proportion to a three to four fold increase in fat intake. Although steatorrhea also increased, there was no change in the excretion of nitrogen. The restricted fat diet is still often used because of individual intolerance.

It is imperative that one understand the mechanism of effective absorption of fat, to comprehend the malabsorption syndrome of CF and the implications for EFA deficiency. Physical characteristics of the fatty acid molecule play an important role in absorption. Saturated fatty acids are less well absorbed than the unsaturated homologue, and the polyunsaturated molecule is more completely absorbed than the monounsaturated compound. With an increase in molecular weight, absorption decreases. The absorption of any one fatty acid can be affected by the fat with which it is simultaneously ingested (Fernandes et al 1962). From these observations, it has been concluded that the feeding of

unsaturated fatty acids would be beneficial to patients with steatorrhea. Similar findings were reported by Pinter et al (1964) in a study designed to evaluate the effectiveness of different fats on decreasing steatorrhea. Steatorrhea was described as "marked" for subjects receiving butter or glyceryl monostearate; "less severe" for those receiving cottonseed oil or glyceryl monolinoleate; and "much improved" to "no steatorrhea" for those receiving medium chain triglycerides (MCT). All subjects were given known amounts of fat (approximately 40% of kcals) based on an isocaloric substitution of previously established intakes.

Because of the relatively short fatty acid chain lengths in MCT (C8:0, C10:0), it appears to be absorbed intact and transported directly to the portal vein without prior hydrolysis. While the effectiveness in treating steatorrhea with MCT is well noted, the use of MCT results in fatty acid changes characteristic of essential fatty acid deficiency. Kuo and Huang (1965) observed changes in the fatty acid composition of plasma and depot fats after feeding MCT to CF children for a three to five month period, at amounts comparable to a normal fat intake. Prior to treatment, fatty

acid patterns were characteristic of endogenous lipogenesis which resulted from impaired fat digestion and absorption, i.e., increased proportions of saturated and monoenoic fatty acids of 16 and 18 carbons and depressed levels of linoleic and arachidonic acids. Feeding with MCT magnified these changes and produced sharp depressions of the polyenoic fatty acids. Despite the biochemical appearance of EFA deficiency, subjects did not demonstrate any clinical signs. Similar results have been reported by Hirono et al (1977) in infants fed MCT. Thus it can be seen that the type of fat ingested can affect the efficiency of absorption by the intestine.

The presence of fat together with pancreatic lipase in the small intestine, is not sufficient to ensure optimal absorption. The action of lipase itself can be altered by changes in the pH of the gut contents. Investigations related to the efficacy of pancreatic extracts, have shown that lipase is inactive at pH 4 or less. This is of major importance for patients with pancreatic insufficiency because the intragastric and intraduodenal pH have been reported as consistently less than 4, possibly due to reduced bicarbonate secretion (Di Magno et al 1977). The

routine use of enzyme supplementation for cystic fibrosis patients does not totally relieve steatorrhea. Graham's (1977) investigation of commercially available extracts demonstrated that a wide range of enzyme activity could be found in commercial preparations. Again, the persistently low gastric pH greatly reduced the effectiveness of all compounds.

After cleavage of the triglyceride by pancreatic lipase, the resulting monoglyceride and free fatty acids are absorbed into the mucosa in the form of a micelle, containing bile salts (Desnuelle 1973). It has been shown that excretion of bile acids in the feces of children with cystic fibrosis may be seven times greater than losses in normal control subjects. Excretion was substantially higher in those not taking pancreatic supplements (Weber et al 1973). While there is no explanation for this finding, it is postulated that the presence of unhydrolyzed fat and other nutrients may impair bile acid reabsorption. The concern over such continual losses is for depletion of the bile acid pool, thereby further compounding the problems of fat malabsorption because of intraluminal bile acid deficiency.

The long-term implications of a sub-clinical deficiency of essential fatty acids in CF are related to the functions of EFA, but at best, any discussion in this area is strictly speculative when based on animal and human studies where EFA deficiency has developed to life-threatening severity. Of particular interest, is the possible effect of an EFA deficiency on the severity of pulmonary complications. In essential fatty acid deficiency, the proportion of oleic acid to linoleic acid has been altered. Under these conditions, Campbell et al (1976) reported an interference in oxygen uptake by hemoglobin in erythrocyte membranes. They hypothesized that this could account for the digital clubbing observed in cystic fibrosis and for the severity of the respiratory problems. Investigations by Godinez and Longmore (1973) using perfused rat lung, demonstrated a direct correlation between the concentration of palmitic acid in the blood and the rate of surfactant formation. At a stable concentration of palmitic acid, an increase in oleic acid reduced the incorporation of the former into surfactant. A decrease (of approximately 30%) in palmitic acid concurrent with an increase in oleic acid (about 14%) has been observed in the lecithin fraction of mucus in CF children, and may

relate to severity of respiratory problems (Sanjurjo et al 1977). A respiratory distress syndrome has also been identified in EFA deficient chickens (Hopkins et al 1963).

To further substantiate the evidence implicating EFA deficiency in respiratory function, one must examine the metabolic behaviour of the prostaglandins. The lungs have been identified as a major site for the synthesis, release and degradation of prostaglandins. Prostaglandins of the E and F series exert potent physiological effects on smooth muscle of blood vessels and the tracheobronchial tree; the E series facilitates dilation while the F series facilitates constriction. Plasma samples analyzed by radioimmunoassay, showed significantly increased levels of both E and F series prostaglandins in newborns with respiratory distress syndrome (Friedman and Demers 1978). It is not known whether synthesis and release of the prostaglandins are enhanced during the acute phase of the disease or if there is merely an interference in the degradation by the lungs because of the disease. The elevated levels of both E and F series declined to normal values as the respiratory status of the children improved.

Prostaglandin synthesis is determined by the relative amounts of 20:4, 18:2 and 20:3n9 available. The non-essential

fatty acid 20:3n9 has been shown to be inhibitory to the synthesis of the E series, but can enhance synthesis of the F_{α} series (Dodge and Hamdi 1978). A study by Chase and Dupont (1978) revealed that levels of the $F_{2\alpha}$ prostaglandin were elevated above normal in children with cystic fibrosis who demonstrated biochemical signs of essential fatty acid deficiency. Correction of the EFA deficiency resulted in a decline in the $F_{2\alpha}$ levels towards normal values.

Results from investigations by Hubbard et al (1977) do not support the hypothesized involvement of essential fatty acid deficiency in respiratory status. They examined the fatty acid composition of plasma lipids in CF children with and without pancreatic insufficiency, and found the characteristic alterations only in those children with malabsorption requiring enzyme replacement therapy. However, progressive chronic pulmonary disease is found in CF children regardless of pancreatic function. These results suggest EFA deficiency is associated with pancreatic function, not respiratory status. The work of Galabert et al (1978) substantiates these observations. It is obvious that further research is required to clarify the relationship between

prostaglandins and EFA in the etiology of lung disease. Similarly, the extent to which the abnormal substitution of 20:3n9 in the phospholipids will affect membrane integrity, growth and infection, is not known and requires further investigation.

Intravenous administration of a soya oil emulsion (Intralipid) has been used successfully in the treatment of essential fatty acid deficiency in surgical patients. Collins et al (1971) found that administration of 22.5 grams of linoleic acid daily (6.4% of kcals) improved the 20:4 level of serum phospholipids and decreased the proportion of 20:3n9, suggesting a requirement of at least this amount. Fatty acid supplementation by regular intravenous infusions of Intralipid improved the fatty acid status of seven children with cystic fibrosis (Elliott 1976) though details of the lipid profiles were not given. The authors also claim improvement in the clinical course of CF; decreased sweat sodium concentrations, weight gain and improvement in pancreatic function (in two subjects). The small sample size plus the possibility of a placebo effect must be considered as influences on these findings.

Ideally, a treatment for EFA deficiency in CF should be simple, convenient and readily adaptable to the lifestyle of a child. Intravenous administration of fat does not equate with any of these criteria. While the effectiveness of Intralipid has been established, use should be restricted to the critical cases not responding to other therapeutic measures.

Press et al (1974) demonstrated successful treatment of essential fatty acid deficiency in adult men using cutaneously applied sunflower oil. Approximately 120 mg of linoleic acid (2-3 mg/kg/day) applied daily to one forearm, resulted in improvement in the fatty acid profiles of the serum lecithin fraction within one to two weeks. Similar results are reported in newborn infants, using 1-4 gm/kg/day of sunflower oil (approximately 0.9 gm/kg/day of 18:2) (Friedman et al 1976). It has been suggested that linoleic acid might be directly incorporated into circulating lipoproteins without first passing through the liver. This hypothesis would explain the greater efficiency observed using cutaneous oil versus one orally administered (Press et al 1974). In 1977, Rosenlund et al investigated the effects of orally

administered corn oil on the fatty acid status of cystic fibrosis children, using 1 gm/kg/day of oil over a one year period (approximately 0.5 gm/kg/day 18:2). Lipid profile data are given for the initial and final blood samples. After a one year period, the concentration of linoleic and arachidonic acids in the phospholipid fraction were shown to have risen by 106% and 96% respectively. These results suggest that oral administration of a source of EFA can be used to treat EFA deficiency in cystic fibrosis.