0031-3998/84/1812-1350$02.00/0
PEDIATRIC RESEARCH

Vol. 18, No. 12, 1984
Printed in U.S.A.

Intravenous Lipid Emulsions in the Treatment of Essential Fatty Acid Deficiency: Studies in Young Pigs

DEAN W. ANDERSEN, L. J. FILER, JR., AND LEWIS D. STEGINK

The University of Iowa, Departments of Pediatrics and Biochemistry, Iowa City, Iowa 52242

ABSTRACT. Essential fatty acid deficiency (EFAD) occurs in infants fed fat-free mixtures of glucose and amino acids. Although infusion of lipid emulsion rapidly reverses clinical symptoms, little is known about effects on tissue fatty acids. To study this question, five groups (n = 4/group) of neonatal pigs were studied. Three groups (I, II, and V) were made EFAD by feeding diets without essential fatty acids (EFA) for days 5 to 33 of life. Groups III and IV were fed a control diet. By 33 days, animals fed the deficient diet showed clinical symptoms and biochemical signs of EFAD. On days 33 to 54 of life, group I animals were fed the EFA-deficient diet and infused with lipid emulsion, providing 3.6% of energy as linoleic acid; group II animals were fed the deficient diet and infused with linoleic acid at 7.2% of energy; group V animals were fed the deficient diet with no lipid emulsion; group III and IV animals were fed the EFA-deficient diet and provided EFA intravenously. Infusion of lipid emulsion rapidly reversed clinical symptoms of EFAD and returned plasma phospholipid ω6 fatty acids levels to normal. However, erythrocyte and liver phospholipid ω6 fatty acid content and adipose tissue reserves of ω6 fatty acids normalized more slowly. Three weeks of infusion of linoleic acid at 3.6% of energy and 2 weeks of infusion at 7.2% of energy were required to return erythrocyte phospholipid fatty acids to normal. Liver phospholipid fatty acid composition still showed biochemical evidence of EFAD in animals treated with linoleic acid at 3.6% of energy for 3 wk. Adipose tissue reserves of ω6 fatty acids did not return to normal in animals treated for 3 wk with linoleic acid at 3.6% of energy. Two to 3 wk of treatment with linoleic acid at 7.2% of energy was required to return adipose tissue 6ω fatty acids reserves to normal. (*Pediatr Res* 18:1350–1355, 1984)

Abbreviations

EFAD, essential fatty acid deficiency
T/T, triene to tetraene ratio (C-20:3ω9/C-20:4ω6)

Essential fatty acid deficiency is difficult to produce in adult humans fed fat-free diets orally (3). This undoubtedly results from linoleic acid stores in adipose tissue (8 to 10%) (13). Subjects fed fat-free diets usually fast overnight, causing triglyceride mobilization from adipose tissue to supply energy needs. Essential fatty acids are released to the circulation as part of this process, supplying requirements. When a nutritional regimen that does not provide sufficient energy is fed, a similar process occurs.

During eucaloric or hypercaloric total parenteral or enteral nutrition, total energy requirements are supplied by constant infusion of glucose-amino acid mixtures in amounts equal to or greater than energy requirements. The increased insulin levels produced by such infusions decrease adipose tissue lipase activity, prevent fat mobilization and facilitate fat deposition. In this situation, a patient neither receives essential fatty acids from the diet nor has access to endogenous essential fatty acids from adipose tissue. Thus, the incidence of essential fatty acid deficiency increased as more and more medical centers adopted total parenteral and enteral nutrition as a means of patient management.

The neonate is especially susceptible to the development of EFAD. First, the content of linoleic acid stored in infant adipose tissue is less than that of adults (1 to 3% *versus* 8 to 10%). Second, due to growth, the neonate has a higher requirement for essential fatty acids. Third, the neonate fed every 3 to 4 h has a constant provision of nutrients with concomitant insulin release. This prevents mobilization of essential fatty acids from adipose tissue stores.

The treatment of EFAD involves the provision of essential fatty acids to replete body stores, often by infusion of a lipid emulsion. However, few data are available on the time course of essential fatty acid repletion in EFAD patients. The young pig provides an animal model that simulates the human infant with respect to the ease of inducing EFAD (9, 14). The newborn pig, like the low birth weight infant, has a reduced content of body fat (3.5 *versus* 7.0%) with a low content of essential fatty acids, and grows rapidly. These factors make the growing pig an ideal biomedical model for study of EFAD in human infants.

The objectives of the present study were (*a*) to demonstrate that the prophylactic use of lipid emulsion would prevent the clinical symptoms and biochemical signs of EFAD from developing; (*b*) to demonstrate that treatment of EFAD animals with lipid emulsion would reverse the clinical symptoms and biochemical signs of essential fatty acid deficiency; (*c*) to determine the effective dose for reversal of EFAD; and (*d*) to provide data on its time course.

MATERIALS AND METHODS

Animal management. The study was divided into three phases, a 5-day pretreatment phase, a 4-wk induction phase in which essential fatty acid deficiency was produced, and a 3-wk recovery phase in which the deficient animals were treated with intravenous lipid emulsion to reverse the symptoms.

Received February 16, 1984; accepted June 27, 1984.
Correspondence and reprint requests may be addressed to L. D. Stegink, Departments of Pediatrics and Biochemistry, S-385 Hospital School, The University of Iowa, Iowa City, IA 52242.
This research was supported in part by a grant-in-aid from Abbott Laboratories, North Chicago, IL.

Pretreatment. Twenty Pitman-Moore miniature pigs, 10 male and 10 female, selected from eight litters, were randomly assigned to five feeding groups. The pigs were suckled by the sow from birth to age 5 days, and then tethered in place in a cage and bottle fed. All animals received 100 mg of intramuscular iron during the first few days of life.

Induction phase. Five groups of 4 animals each were studied. Starting on day 6, animals in groups I, II, and V were fed diet A (Table 1) to induce EFAD by 33 days of life. Groups III and IV were fed the control diet (diet B) plus fat emulsion. Both diets provide protein at 22.5% of energy and carbohydrate at 73.5% of energy. Diet A provided 4% of energy as coconut oil and was essentially devoid of essential fatty acids. The addition of lipid emulsion (Liposyn, Abbott Laboratories, North Chicago, IL) to diet B provided 4% of energy. This emulsion contained 72.6% linoleic acid (Table 2); thus, the diet provided 2.9% of energy as linoleic acid. Pigs in group I were pair-fed to pigs in group III, and pigs in group II were pair-fed to pigs in group IV. Group V animals were fed *ad libitum*.

Treatment phase. A central venous catheter was placed in pigs in groups I, II, III, and IV at 33 days of age (1), and lipid emulsion was infused over a period of 7 h daily via pump (Abbott Life Care, Abbott Laboratories). Between infusions, the catheter was flushed and filled with a heparin solution (333 units/100 ml). Group V animals were not catheterized. The lipid emulsion was infused 5 days/wk for a 3-wk period.

During this phase of the study, animals in groups I and II were fed diet B (Table 1). This diet is similar to diet A but does not contain any lipid. The animals received the lipid part of their diet intravenously. Group I animals were infused with lipid emulsion at 5% of total energy (3.6% of energy as linoleic acid). Group II animals were infused with lipid emulsion at 10% of total energy (7.2% of energy as linoleic acid). Group III animals were fed diet C while receiving lipid emulsion intravenously at 3% of energy. Diet C is similar to diet A, but contains coconut oil at 2% of energy. The coconut oil balances the fat intake of group III animals with group I animals at 5% of energy. Group IV animals were fed diet D while receiving lipid emulsion intravenously at 3% of energy. Diet D is similar to diet C but provides coconut oil at 7% of energy. The coconut oil balances the fat intake of group IV animals with that of group II animals at 10% of energy. Group V animals were fed the deficient diet (diet A) for the entire study.

Table 1. *Per cent composition of diets*

Component	Grams/100 g dry weight			
	A*	B†	C*	D*
Dried skim milk	50.00	50.00	50.00	50.00
Amigen	6.00	6.00	6.00	6.00
Dextrose	10.00	10.00	10.00	10.00
Corn starch	10.00	10.00	10.00	10.00
Corn syrup solids	16.63	16.63	18.54	13.70
Coconut oil	1.70		0.85	3.00
$CAHPO_4 \cdot 2H_2O$	1.70	1.70	1.70	1.70
NaCl	0.50	0.50	0.50	0.50
$MgSO_4$	0.15	0.15	0.15	0.15
Vitamin mixture‡	0.50	0.50	0.50	0.50
Mineral mixture§	0.50	0.50	0.50	0.50
α-Cellulose	2.32	2.32	1.26	3.95

* Diets A, C, and D provided 385 kcal/100 g; 22.5% of energy as protein. Diets were blended with water to provide a mixture containing 25% solids by weight prior to feeding.

† Diet B provided 370 kcal/100/g of diet. When diet B was blended to form the 25% solution fed during the induction phase, sufficient lipid emulsion was added to provide 4% of energy, thus making diets A and B eucaloric. During the treatment phase, diet B was blended with water alone since lipid emulsion was supplied intravenously.

‡ The vitamin mixture provided 0.04 g thiamin hydrochloride, 0.05 g riboflavin, 0.44 g nicotinic acid, 0.6 g calcium pantothenate, 0.06 g pyridoxine hydrochloride, 0.02 g folic acid, 0.44 g vitamin B_{12} (0.1% trituration with mannitol), 0.09 g of a vitamin A palmitate (500,000 units/g)-vitamin D_2 (500,000 units/g) mixture, 0.2 g DL-α-tocopheryl acetate and 98.06 g dextrose/100 g of mix. Choline chloride was added at a level of 1 g/kg of diet. Vitamin E supplement was supplied at 16 IU/day (Aquasol-E). At a vitamin E to polyunsaturated fatty acid ratio of 0.6, this amount of vitamin E will cover an intake of 17 g of linoleic acid/day.

§ The mineral mixture contained 4.4 g $ZnSO_4$, 0.8 g $MnSO_4$, 0.4 g $CuSO_4$, 8.0 g $FeSO_4$, 0.8 g KI, and 85.6 g dextrose/100 g of mix.

Table 2. *Fatty acid composition of lipids in the lipid emulsion batch studied**

Fatty acid	Per cent of total
12:0	<0.05
14:0	0.2
15:0	<0.05
16:0	8.9
16:1	0.3
17:0	0.0
18:0	3.0
18:1	11.7
18:2	72.6
18:3	0.6
20:0	0.2
20:1	0.2
20:2	0.1
20:3	<0.05
20:4	0.9
20:5	0.1
NI†	0.2
22:4	0.2
22:6	0.3
22:5	<0.05
22:6	0.03
NI	0.1

* Liposyn 10% lipid emulsion, batch 63-410DH, Abbott Laboratories.

† NI, peak not accurately identified, but placed in order of elution from the column.

Body weight was determined daily and body length and girth were taken once a week (1). Feed consumption was determined daily (1). All pigs with indwelling catheters received a daily dose of 250 mg streptomycin and 200,000 units pencillin intramuscularly. Each animal received 5 mg of vitamin K_1 intramuscularly once a week.

Laboratory procedures. Blood samples (10 ml) for lipid analyses were obtained at 5, 26, 33, 40, 47, and 54 days of age. Back fat adipose tissue samples were obtained by biopsy at 5, 26, 33, 40, 47, and 54 days of age.

Methods for extracting lipids from tissues, red blood cells, and plasma for fatty acid analysis and the methods for determination of carcass composition have been described previously (6–8). Adipose tissue biopsy was carried out using the method described by Baker *et al.* (2).

Statistical analyses used analysis of variance with orthogonal contrasts.

RESULTS

Clinical signs and symptoms of EFAD. Clinical symptoms of EFAD—scaly skin, scabby tails, and exudative lesions—developed in all group I and II pigs at approximately 28 to 30 days of age. This was 23 to 25 days after weaning from the sow to the deficient diet. During the treatment phase, administration of lipid emulsion for 5 to 12 days at 5% of total energy (3.6% of energy as linoleic acid), or 3 to 6 days at 10% of total energy (7.2% of energy as linoleic acid) corrected the clinical symptoms

of EFAD. Pigs in groups III and IV did not develop clinical symptoms of EFAD.

Three of the four pigs in group V did not show clinical symptoms of EFAD over 82 days of observation, but did develop biochemical signs of EFAD. Unlike animals in groups I and II, these pigs were not restrained and had free access to water. It has been reported by Burr (4) that EFAD is associated with an increase in insensible water loss, and that the clinical signs of EFAD are often dependent upon the relative humidity of the animal quarters. This may account for the differences between these groups.

Biochemical indices of EFAD. Three biochemical indices used by Holman and co-workers (10–12) to characterize EFAD states in humans and animals were selected as a means for early detection of EFAD and its repair. These indices are based on the synthesis and accumulation of a specific eicosatrienoic acid (C-20:3ω9) during EFAD and the simultaneous decrease that occurs in the concentration of linoleic acid (C-18:2ω6) and arachidonic acid (C-20:4ω6). These indices were the triene to tetraene ratio (C-20;3ω9/C-20:4ω6), the level of C-20:3ω9, and the sum of the ω6 fatty acids.

Changes in these indices were studied in three lipid fractions known to be sensitive to EFAD: plasma phospholipid fatty acids, erythrocyte stromal phospholipid fatty acids, and adipose tissue triglyceride fatty acids. Heart and liver phospholipid fatty acid composition was measured at the end of the study.

Plasma phospholipid fatty acids. The biochemical indices of essential fatty acid deficiency as determined by changes in fatty acid composition of plasma phospholipids are summarized in Figure 1. During the induction phase of the study (days 5 to 33 of age), neonatal pigs fed the control diet (groups III and IV) had normal levels of total ω6 fatty acids (*upper panel*), normally low triene/tetraene ratios (*middle panel*), and normally low levels of C-20:3ω9 (*lower panel*). By contrast, animals fed the deficient diet (groups I, II, and V) showed clear signs of EFAD as early as 26 days of age (21 days on the fat-free diet). Plasma phospholipid levels of ω6 fatty acids, the T/T ratio, and total levels of C-20:3ω9 were significantly altered from 26 to 33 days of age. Hill and co-workers (9), consider a triene/tetraene ratio in excess of 0.4 as the most sensitive biochemical indicator of EFAD in the pig. By these criteria, all animals in groups I, II, and V were EFAD by 26 days of age.

The *right side* of Figure 1 shows the changes occurring in plasma phospholipid fatty acid composition during the treatment

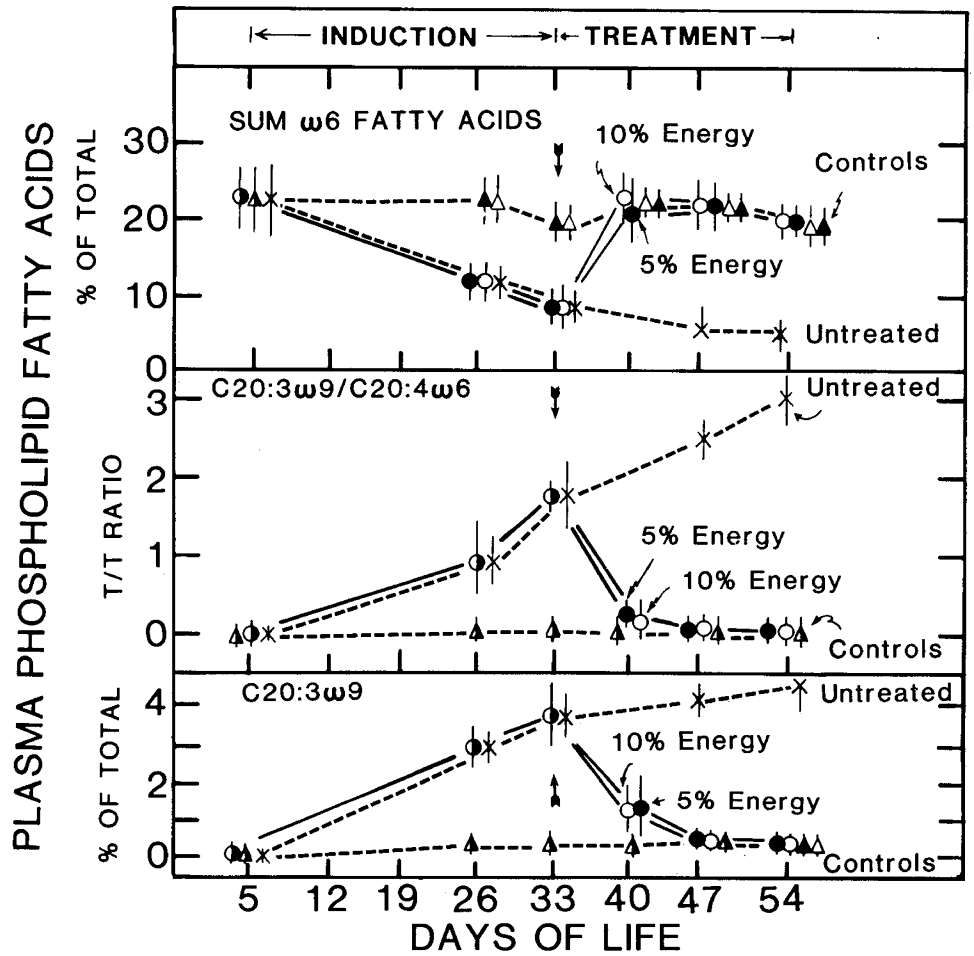

Fig. 1. Fatty acid composition of plasma phospholipids during the induction phase (days 5 to 33 of age) and treatment phase (days 33 to 54 of age) of the study. The values are expressed as the sum of all ω6 fatty acids in the *top panel*, as the triene to tetraene ratio in the *middle panel*, and as levels of C-20:3ω9 in the *bottom panel*. Group I animals (●——●) were fed an essential fatty acid-deficient diet during the induction phase of the study and were infused with lipid emulsion at 5% of energy (3.6% of energy as linoleic acid) during the treatment phase. Group II animals (○——○) were also fed the essential fatty acid-deficient diet during the induction phase of the study but were infused with lipid emulsion at 10% of energy (7.2% of energy as linoleic acid) during the treatment phase. Control animals in groups III (▲---▲) and IV (△---△) were provided essential fatty acids orally during the induction phase and intravenously (3% of energy as lipid emulsion) during the treatment phase of the study. During the treatment phase of the study, group III animals also received 2% of total energy as saturated fat orally, while group IV animals received 7% of total energy as saturated fat orally. Group V animals (×---×) were fed the essential fatty acid-deficient diet during both the induction and treatment phases of the study. Data are graphed as the mean ± SD.

phase of the study (days 33–54 days of age). Infusion of lipid emulsion at 5 to 10% of total energy requirement rapidly decreased the triene to tetraene ratio (*middle panel*) to normal after 7 days of treatment. Animals not infused with lipid emulsion but fed the deficient diet for the entire 54-day study period (group V) continued to show elevated levels of C-20:3ω9 and a high triene to tetraene ratio. Similarly, infusion of lipid emulsion returned total ω6 fatty acids (*upper panel*) to control levels. These changes also occurred within the 1st wk of treatment. However, levels of C-20:3ω9 (*lower panel*) returned to control values more slowly and 2 to 3 wk of treatment were required for normalization.

The erythrocyte phospholipid fatty acids. Changes in erythrocyte phospholipid fatty acid composition (Fig. 2) should provide a better measure of diet-induced changes in tissue fatty acid pools than changes in plasma levels. During the EFAD induction phase of the study (days 5 to 33 of age), the fatty acid composition of red cell phospholipids changed more slowly than that of plasma phospholipids. This was expected, since this pool reflects red cells of differing ages.

The percentage of total ω6 fatty acids in red cell phospholipids (*upper panel*) did not decrease significantly in any group, including animals fed the EFAD diet (groups I, II, and V) during the induction period. However, the T/T ratio (*middle panel*) in erythrocyte phospholipid fatty acids increased significantly by 33 days of age, indicative of essential fatty acid deficiency. The increased T/T ratio in erythrocytes was less than that noted in plasma. Although marked changes in the T/T ratio of plasma phospholipid fatty acids were noted after 21 days on the deficient diet (26 days of age), the phospholipid fatty acids of red cells did not show marked changes in the T/T ratio until 28 days on the diet (33 days of age). At that age, all pigs in groups I, II, and V (with the exception of one pig in group II and one pig in group V) had a red cell phospholipid T/T ratio of 0.4 or greater. Control pigs in groups III and IV had T/T ratios for erythrocyte phospholipid fatty acids of less than 0.1 throughout the study. Similar changes were noted in the levels of C-20:3ω9 (*lower panel*).

The *right side* of Figure 2 shows changes in the erythrocyte phospholipid fatty acid composition during the treatment phase of the study (days 33 to 54 of age). Red cell phospholipid levels of ω6 fatty acids (*upper panel*) in animals fed the deficient diets (groups I, II, and V) did not decrease significantly during the EFAD induction phase of the study; thus, treatment had little effect upon their levels. However, the level of energy infused as linoleic acid did alter the rate at which other biochemical signs of EFAD were reversed. The T/T ratio (*middle panel*) and the quantity of C-20:3ω9 (*lower panel*) returned to normal more rapidly in animals infused with lipid emulsion at 10% of total

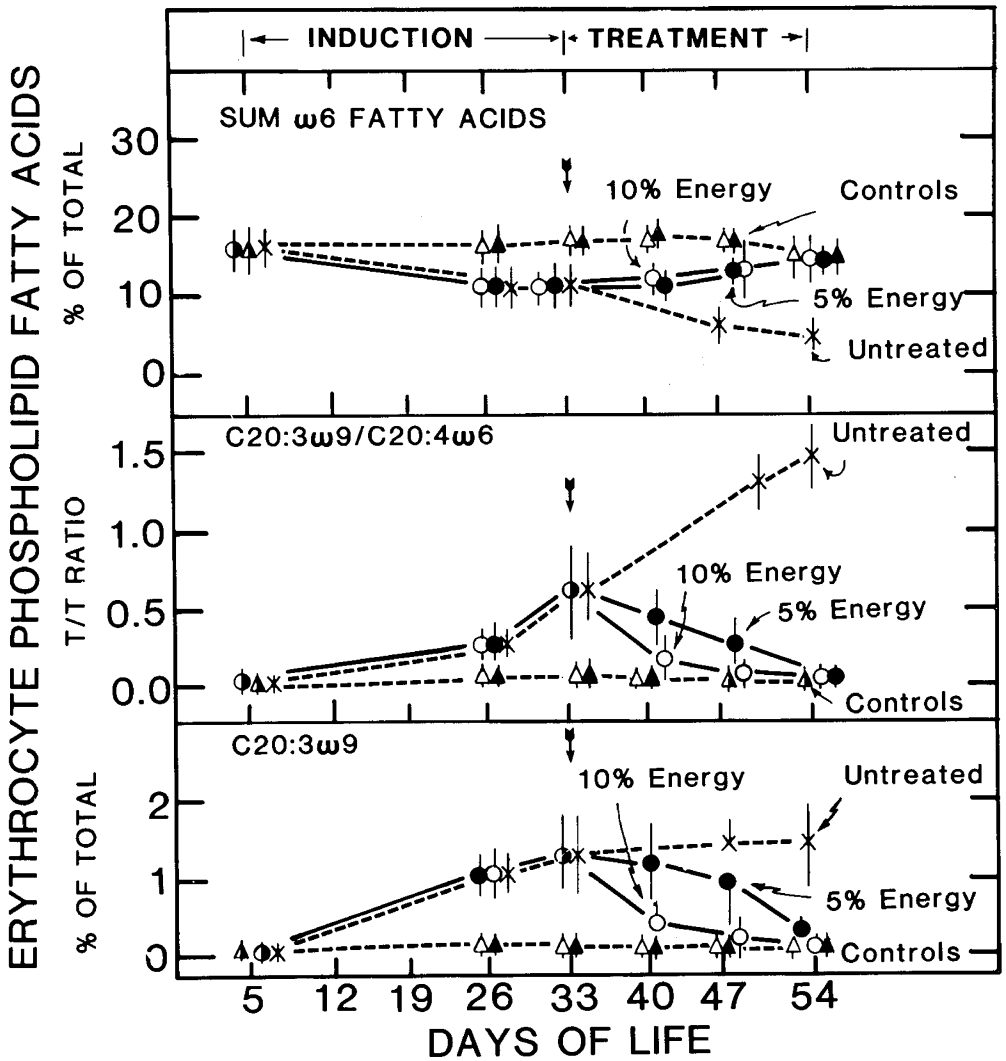

Fig. 2. Fatty acid composition of erythrocyte phospholipids during the induction phase (days 5 to 33 of age) and treatment phase (days 33 to 54 of age) of the study. The values are expressed as the sum of all ω6 fatty acids in the *top panel*, as the triene to tetraene ratio in the midde panel, and as levels of C-20:3ω9 in the *bottom panel*. The values for group I (●——●), group II (○——○), group III (▲---▲), group IV (△---△), and group V animals (×---×) are shown graphed as the mean ± SD. Additional details are found in the legend to Figure 1.

energy (7.2% of energy as linoleic) than in animals infused with lipid emulsion at 5% of total energy (3.6% of energy as linoleic). Two weeks of treatment were required to return the T/T ratio and C-20:3ω9 levels to normal in animals infused with lipid at 10% of energy, while 3 wk were required at the 5% infusion level. Pigs in group II received twice as much energy as linoleic acid as pigs in group I (7.2 *versus* 3.6% of total energy). The slower response of pigs in group I to therapy reflects the fact that these animals received only 3.6% of the total energy as linoleic acid. Hill and co-workers (9) estimate the pig's requirement for linoleic acid at 2% of total energy. Thus, the EFAD pigs in group I were provided with an intake of linoleic acid only 1.7 times greater than requirement.

Heart and liver fatty acids. Upon autopsy, phospholipids were extracted from heart and liver of all animals. It was anticipated that no pigs, other than those maintained on the deficient diet (group V), would show biochemical evidence of EFAD. The data for heart phospholipids (Table 3) support this hypothesis. Only trace quantities of C-20:3ω9 were detected in heart phospholipids of any animal in groups I, II, III, and IV. Neither the triene to tetraene ratio nor the total ω6 fatty acid values differed significantly between animals in groups I and II and controls (groups III and IV). However, the T/T value was significantly increased, and the total ω6 fatty acid percentage significantly decreased in group V animals fed the deficient diet for the entire study period.

The fatty acid composition of liver phospholipids showed residual effects of EFAD despite 3 wk of therapy with lipid emulsion (Table 4). The triene to tetraene ratio in group I and II animals remained significantly ($p < 0.05$) elevated as compared to values in group III and IV animals, although the elevation was small for group II animals. By contrast, total ω6 fatty acids did not differ significantly ($p > 0.05$) between groups I, II, III, and IV, and appear to be close to normal values. However, group V animals fed the deficient diet for the entire study period had significantly elevated T/T values and significantly decreased levels of ω6 fatty acids as expected. These data suggest that treatment of deficient pigs with intravenous linoleic acid at 3.6% of energy (group I) for 3 wk is not sufficient to reverse biochemical evidence of EFAD in liver tissue, despite the return to normal of biochemical indices in plasma and the disappearance of clinical symptoms.

Table 3. *Heart phospholipid fatty acid composition*

Group	Triene-tetraene ratio	Sum of ω6 fatty acids
I	0.01 ± 0.01*	41.0 ± 2.75*†
II	0.02 ± 0.01	43.5 ± 3.44
III	0.02 ± 0.01	41.4 ± 1.91
IV	0.01 ± 0.01	44.1 ± 1.56
V‡	1.25 ± 0.30§	19.4 ± 2.81§
Sow-fed‡	0.00 ± 0.00	44.8 ± 5.41

* Data shown as mean ± SD.
†-Data expressed as percentage of total.
‡ Animals killed at 63 days, rather than at 54 days.
§ Differ significantly from values in all other groups.

Table 4. *Liver phospholipid fatty acid composition*

Group	Triene-tetraene ratio	Sum of ω6 fatty acids
I	0.21 ± 0.05*	25.7 ± 3.12*†
II	0.04 ± 0.04	30.4 ± 2.72
III	0.00 ± 0.00	27.3 ± 3.73
IV	0.00 ± 0.00	29.8 ± 3.29
V‡	1.37 ± 0.20	12.6 ± 3.60
Sow-fed‡	0.00 ± 0.00	36.5 ± 4.60

* Data shown as mean ± SD.
† Data expressed as percentage of total.
‡ Animals killed at 63 days, rather than at 54 days.

Adipose tissue lipid fatty acids. The fatty acid composition of lipids extracted from back fat adipose tissue was also determined. These fatty acids are derived primarily from the triglycerides of depot fat rather than from membrane phospholipids. The amount of arachidonic acid (C-20:4ω6) and eicosatrienoic acid (C-20:3ω9) found in adipose tissue is less than 0.1%. Thus, a calculated triene to tetraene ratio based on such low levels is of questionable value in determining EFAD status. Since adipose tissue serves as a reservoir of essential fatty acids, the data are better expressed as a sum of all ω6 fatty acids present.

Fatty acid analyses of the lipids extracted from back fat biopsy specimens are summarized in Figure 3. By 26 days of age, pigs in groups I, II, and V fed the EFAD diet had a significantly lower content of adipose tissue ω6 fatty acids. Total ω6 fatty acid composition was only 5% of total fatty acids at 33 days in animals fed the deficient diet during the induction phase. This value was lower than that noted in control animals fed the diet providing lipid emulsion orally at 5% of energy (14.5%) or values noted in sow-fed controls (16%). Total levels of ω6 fatty acids continued to decrease in pigs fed the deficient diet throughout the entire 54-day period (group V). Total ω6 levels were only 3.5% on day 54 of the study. Infusion of lipid emulsion at 5% of energy (3.6% of linoleic acid as energy), starting at day 33 and continuing until day 54, prevented this decrease in ω6 fatty acid levels, but did not significantly increase total ω6 fatty acid levels above values noted at 33 days. However, levels of total ω6 fatty acids did increase to control levels after 2 wk of treatment in animals infused with lipid emulsion at 10% of total energy (7.2% of energy as linoleic acid). These data, like the liver phospholipid data, indicate that a significant time period of treatment and high levels of ω6 fatty acids (7.2% of energy as linoleic acid) are required to return adipose tissue levels of essential fatty acids to normal in deficient animals, despite normalization of clinical symptoms, plasma, and erythrocyte phospholipid fatty acid concentrations, and the triene to tetraene ratio at lower levels of intake. Lower levels of linoleic acid infusion (3.6% of energy, group I) did not normalize adipose tissue reserves even after 3 wk of treatment.

Growth and feed efficiency. Body weights (Table 5) for animals in groups I through V did not differ significantly at birth, weaning (5 days), surgery (33 days), or at the end of the feeding trials (54 days).

Weight gain from day 6 to day 33 of feeding (the induction phase for EFAD in groups I, II, and V) was significantly less for

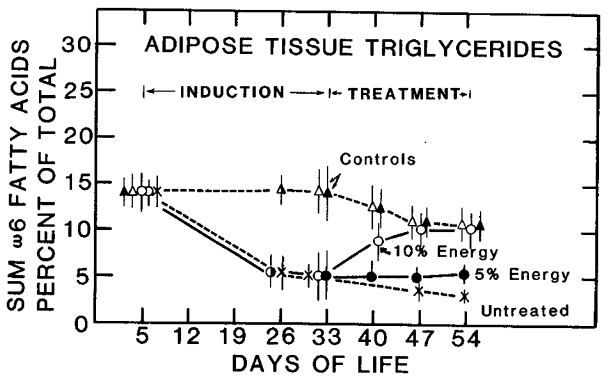

Fig. 3. Fatty acid composition of adipose tissue triglycerides during the induction phase (days 5 to 33 of age) and treatment phase (days 33 to 54 of age) of the study. The data are expressed as the mean ± SD of the sum of all ω6 fatty acids present. The values for group I (●——●), group II (○——○), group III (▲- - -▲), group IV (△- - -△), and group V animals (×- - -×) are shown graphed as the mean ± SD. Additional details are found in the legend to Figure 1.

Table 5. *Body weights (g) at different ages**

Group	Age (days)			
	0	5	33	54
I	895 ± 91	1,566 ± 411	4,635 ± 874	8,843 ± 1,480
II	1,019 ± 130	2,023 ± 455	4,910 ± 595	9,093 ± 1,182
III	898 ± 142	1,612 ± 472	4,922 ± 902	8,740 ± 1,272
IV	919 ± 127	1,726 ± 451	4,745 ± 411	8,770 ± 861
V	1,061 ± 255	1,870 ± 296	5,618 ± 794	10,140 ± 1,196

* Data expressed as the mean ± SD. No significant differences in body weights at birth, 5, 33, and 54 days were noted using analysis of variance with orthogonal contrasts.

groups I and II combined *versus* group V ($p = 0.02$, analysis of variance). Pigs in groups I and II were restrained and nipple-fed, while pigs in group V were unrestrained and pan-fed. This difference in housing and feeding probably accounted for the differential in weight gain.

Feed efficiency for group V animals from 34 to 54 days of study was significantly less ($p = 0.02$) than that observed for animals in groups I, II, III, and IV. Group V animals were severely deficient in EFA during this time period while other animals were either free of EFAD (groups III and IV) or were being treated for EFAD (groups I and II). The decreased feed efficiency thus correlates with the known association of EFAD with reduced food efficiency.

No differences in carcass composition were noted among pigs in groups I, II, III, and IV. Carcass composition of group V animals cannot be compared to animals in the other groups because of the difference in age at time of slaughter.

DISCUSSION

The availability of sequential values for adipose tissue fatty acid content altered our perception of the time course and the amount of linoleic acid required for complete recovery of the essential fatty acid-deficient animal.

The data demonstrate that plasma phospholipid fatty acid composition does not adequately reflect overall essential fatty acid status of either tissue phospholipids or adipose tissue reserves. Erythrocyte phospholipid fatty acid composition appears to be more representative of tissue levels of essential fatty acids during EFAD. However, the data suggest that even red cell phospholipid fatty acid composition returns to normal more rapidly than tissue composition. For example, the T/T ratio in liver phospholipids was still significantly elevated in group I animals after 3 wk of therapy with linoleic acid supplied at 3.6% of energy.

The fatty acid composition of the total red cell phospholipid pool probably reflects both essential fatty acid status at the time of red cell formation and current dietary status. The latter influences red cell phospholipid composition due to a slow rate of fatty acid exchange between plasma and red cell fatty acid pools. Adipose tissue triglyceride reserves of ω6 fatty acids return to normal more slowly than either plasma or red cell phospholipid values and may more accurately reflect overall essential fatty acid status. For example, it was the only parameter to mimic the abnormality in liver phospholipids found in group I animals. The ω6 fatty acid pool in adipose tissue is also important since it is the source of essential fatty acids during periods of fasting or when animals are fed a diet providing an energy intake below requirement.

The failure of adipose tissue ω6 fatty acid stores and liver phospholipid composition to normalize when ω6 fatty acids were provided at 3.6% of energy explains why single infusions of lipid emulsions failed to consistently reverse symptoms of essential fatty acid deficiency in the early use of lipid emulsions for the treatment of human EFAD. Hill *et al.* (9) estimated the linoleic acid requirement of the growing pig at 2% of total energy. The recommended intake of linoleic acid for the human infant is 3% of total energy (5). Thus, requirements are similar. Our data suggest that infants with EFAD should be treated with increased levels of essential fatty acids for significant periods of time beyond the disappearance of clinical signs of EFAD and normalization of the T/T ratio in plasma phospholipids. These data suggest that at least 3 wk of treatment is required when linoleic acid is provided at 7.2% of total energy. Longer periods of time will be required at lower intakes of linoleic acid.

REFERENCES

1. Andersen DW, Filer LJ Jr, Wu-Rideout MY-C, White LB, Stegink LD 1982 Utilization of intravenously administered glucose-oligosaccharides in growing miniature pigs. Pediatr Res 16:304
2. Baker GL, Andersen DW, Eash SA 1970 Effect of diet on fatty acid composition of miniature swine adipose tissue lipids. Am J Clin Nutr 23:426
3. Brown WR, Hansen AE, Burr GO, McQuarrie I 1938 Effects of prolonged use of extremely low-fat diet on an adult human subject. J Nutr 16:511
4. Burr GO 1942 Significance of the essential fatty acids. Fed Proc 1:224
5. Committee on Nutrition 1976 Commentary on breast feeding and infant formulas, including standards for formulas. Pediatrics 57:278
6. Filer LJ Jr, Fomon SJ, Anderson TA, Andersen DW, Rogers R, Jensen RL 1973 Growth, serum chemical values and carcass composition of Pitman-Moore miniature pigs during the first eight weeks of life. J Nutr 103:425
7. Filer LJ Jr, Fomon SJ, Anderson TA, Nixt TL, Andersen DW 1974 Effects of age, sex and diet upon carcass and liver fatty acid composition of Pitman-Moore miniature pigs. Lipids 9:536
8. Filer LJ Jr, Fomon SJ, Anderson TA, Andersen DW, Rogers R, Jensen RL 1974 Effect of age, sex and diet on growth, serum chemical values and carcass composition of Pitman-Moore miniature pigs during the first four weeks of life. Growth 38:197
9. Hill EG, Warmanen EL, Silbernick CL, Holman RT 1961 Essential fatty acid nutrition in swine. 1. Linoleate requirement estimated from triene/tetraene ratio of tissue lipids. J Nutr 74:335
10. Holman RT 1968 Essential fatty acid deficiency. In: Holman RT (ed) Progress in the Chemistry of Fat and Other Lipids, vol 9. Pergamon Press, Inc., New York, pp 275–348
11. Holman RT, Caster WO, Wiese HF 1964 The essential fatty acid requirement of infants and the assessment of their dietary intake of linoleate by serum fatty acid analysis. Am J Clin Nutr 14:70
12. Sinclair HM 1957 Effects of deficiency of essential fatty acids. Deficiency of essential fatty acids in lower animals. In: Sinclair HM (ed) Essential Fatty Acids (4th International Conference on Biochemical Problems of Lipids, Oxford). Academic Press, New York, pp 249–255
13. Wene JE, Connor WE, DenBesten L 1975 The development of essential fatty acid deficiency in healthy men fed fat-free diets intravenously and orally. J Clin Invest 56:127
14. Witz W, Beeson WM 1951 The physiological effects of a fat-deficient diet on the pig. J Animal Sci 10:112