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Research Article

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The Development of Essential Fatty Acid Deficiency in Healthy Men Fed Fat-Free Diets Intravenously and Orally

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ABSTRACT The hypothesis that clinical and biochemical essential fatty acid deficiency (EFA) might occur from the feeding of eucaloric, fat-free diets was tested in two experiments in healthy men. In Study I, eight men were given fat-free, eucaloric diets containing 80% of calories as glucose and 20% as amino acid hydrolysates by a constant drip over a 24-h period. The diets were fed in succession for periods of 2 wk each, either through a superior vena cava catheter or via a nasogastric tube. EFA deficiency was detected by decreases in linoleic acid and by the appearance of 5, 8, 11-eicosatrienoic acid in lipid fractions of plasma. Linoleic acid decreased significantly during 2 wk of the fat-free diet given intravenously from 48.8 to 9.8% (percent of total fatty acids) in cholesterol esters, from 21.2 to 3.2% in phospholipids, from 9.6 to 2.0% in free fatty acids, and from 14.1 to 2.6% in triglycerides. Eicosatrienoic acid, normally undetectable, appeared 0.6% in cholesterol esters, 2.5% in phospholipids, 0.2% in free fatty acids, and 2.3% in triglycerides. EFA deficiency occurred similarly during the nasogastric feeding.

In Study II a subject received the same diet continuously by the nasogastric route for 10 days followed by a 24-h fast. He was then given the fat-free diet intermittently in three meals per day for 3 days. Finally, he was repleted with a diet containing 2.6% linoleic acid. By the 3rd day of the continuous nasogastric feeding, linoleic acid had fallen significantly and eicosatrienoic acid had appeared in plasma lipid fractions as in Study I. These findings were accentuated by day 10. Adipose tissue fatty acid composition did not change. Free fatty

acid outflow from adipose tissue was presumably suppressed during the 10 days of continuous feeding. With increased free fatty acid outflow during fasting and intermittent feeding, linoleic acid rose and eicosatrienoic acid decreased. After 13 days of repletion with dietary linoleic acid, the EFA deficiency had disappeared.

These results indicated that EFA deficiency readily develops when fat-free diets containing glucose are given intravenously or orally as constant 24-h infusions. These diets are similar to the hyperalimentation formulas now being used clinically.

INTRODUCTION

Essential fatty acid (EFA)¹ deficiency was first produced experimentally in young, growing rats by Burr and Burr in 1929 (1). This syndrome has also been described in many other species including man (2). The term "essential fatty acid" refers to linoleic acid (*cis, cis*-9,12-octadecadienoic acid). Linolenic acid (*cis, cis, cis*-9,12,15-octadecatenoic acid) will stimulate growth but will not relieve the dermal symptoms associated with EFA deficiency, while linoleic acid will effectively correct both (3). Arachidonic acid (*cis, cis, cis, cis*-5,8,11,14-eicosatetraenoic acid) is approximately three times as effective as linoleic acid in promoting growth (2), but is not an essential nutrient since linoleic acid can be converted to arachidonic acid in the body (4). Linoleic acid then is the EFA, i.e., that fatty acid which must be obtained from the diet by most species to prevent the symptoms of EFA deficiency.

The symptomatology of EFA deficiency falls into three categories: (a) gross abnormalities (e.g., cessation of growth, dermatitis, loss of hair, and increased

¹Abbreviation used in this paper: EFA, essential fatty acid.

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TABLE I
Composition of the Hyperalimentation Diet

Casein hydrolysate		20%
Dextrose		80%
Caloric density		1 kcal/ml
Electrolytes	Sodium	30 meq/liter
	Potassium	15 meq/liter
	Calcium	12.5 meq/liter
	Chloride	19.6 meq/liter
	Magnesium	4.2 meq/liter
	Phosphorus	24.6 meq/liter
Vitamins	Thiamine hydrochloride	25 mg
	Riboflavin	10 mg
	Sodium pantothenate	20 mg
	Nicotinamide	100 mg
	Pyridoxine hydrochloride	20 mg
	Ascorbic acid	500 mg

susceptibility to bacterial infections), (b) histological abnormalities (e.g., microscopic changes in the kidneys, skin, ovaries, and testes), and (c) physiological and biochemical abnormalities (2, 5). The last category includes biochemical changes in the fatty acid patterns of blood and organs such as liver and heart (6). These changes involve increased levels of 5, 8, 11-eicosatrienoic acid and decreased levels of linoleic acid and arachidonic acid. The changes in blood levels may be the earliest signs of EFA deficiency (2).

Experimental studies have shown that the very young animals are much more susceptible to EFA deficiency than adults (7-9). Growing animals require linoleic acid and their body stores are low, thereby making depletion easily producible. Because the dietary requirement is low and the storage of linoleic acid in adipose tissue is great, it has been extremely difficult to produce clinical EFA deficiency in adults. Most of the human studies reported to date have been in infants and children (10-14). Only two cases of linoleic acid deficiency have been reported in adult humans. Collins et al. found a deficiency secondary to short bowel syndrome in one adult patient and a deficiency resulting from a colectomy with severe malnutrition in another (15). Up to this time, EFA deficiency has not been reported in healthy adult humans.

An understanding of EFA deficiency has an immediate importance for present-day medicine because the sole source of nutrition for many severely ill patients is intravenous hyperalimentation. The infusate is fat-free, consisting of amino acid hydrolysates, glucose, minerals, and vitamins. The diet is, therefore, EFA deficient. In chronically ill, malnourished individuals it is not known whether the lack of dietary linoleic acid might have deleterious effects. The studies reported here were designed to ascertain if such a diet would cause EFA deficiency in adult humans.

METHODS

Study I. Eight healthy male volunteers^a from the Iowa State Penitentiary were admitted to the Clinical Research Center. A hyperalimentation diet was fed both intravenously and nasogastrically (Table I). This diet, "Central Venous Nutrient Solution," was 80% glucose, 20% casein amino acid hydrolysates (Amigen®, Baxter Laboratories, Morton Grove, Ill.) and was completely fat free. Appropriate electrolytes and vitamins were added to meet the daily nutritional requirements. Sterile solutions were prepared daily at a caloric equivalent of 1 kcal/ml. To minimize the effect of weight change on serum lipids, a careful dietary history was taken on admission to the Clinical Research Center, and caloric needs were estimated both from this information and from the height and weight. The men were ambulatory throughout the study and engaged in the usual ward recreational activities.

During the base-line 7-day period of the study, all subjects were fed a eucaloric general diet. The linoleic acid content of this diet was 2-3% of total calories. During the next two 14-day sequential periods each subject was fed the eucaloric, fat-free hyperalimentation diet either by nasogastric tube or intravenous catheter. The solutions were infused at a constant rate over 24 h. Four subjects in Group A received this nutrient solution for 14 days intravenously and then nasogastrically for another 14 days. The four subjects in Group B received this solution in reverse order. Each subject, therefore, was given the fat-free diet for a total of 28 days.

The formula was infused through a 17-gauge plastic catheter which had been threaded through the subclavian vein into the superior vena cava under fluoroscopic control. All infusing solutions were passed through a 44 μ m filter placed immediately proximal to the subclavian catheter. Every 3rd day all dressings were removed and skin around the catheter was defatted with acetone. All catheter tips were cultured at the termination of the study and found to be sterile.

Study II. Subject E. R., a healthy male volunteer 21 yr of age, was admitted to the Clinical Research Center for a more detailed study of the changes in serum fatty acids which occurred in Study I subjects. A eucaloric formula diet containing 2.6% of calories as linoleic acid was first fed for 2 mo. This was followed by four dietary periods: In Period A, a completely fat-free diet was administered by constant nasogastric drip for 10 days. Period B was a 24-h fast. In Period C, the fat-free diet was divided into three equal meals and was fed intermittently via nasogastric tube at 8 a.m., 12 noon, and 4 p.m. for 3 days. In Period D, a 2-wk repletion period, a formula diet containing 2.6% of calories as linoleic acid was fed.

Fatty acid analysis. The lipids of the serum were extracted with chloroform-methanol (2:1) (16). Separation of lipid classes was carried out by preparative thin-layer chromatography on Silica Gel G using hexane-ether-chloroform-acetic acid (80:10:10:1 vol/vol) as the developing solvent (17). The plates were visualized using Rhodamine 6G (Matheson, Coleman, and Bell, Norwood, Ohio) and ultraviolet light, and the bands of cholesterol esters, tri-

^a The proposed study was fully explained to each man and informed, written consent was obtained. The protocol of the study had been approved by the College of Medicine Research Committee in charge of all prison volunteer studies and by the Human Use Committee. The study was in accord with the principles of the Declaration of Helsinki.

TABLE II
Study I—The Changes in the Fatty Acid Composition of Plasma of Eight Subjects after a Fat-Free Diet*

Fatty acids expressed as percent of total fatty acids (mean ± SD)								
	Group A (IV first 14 days; NG second 14 days)				Group B (NG first 14 days; IV second 14 days)			
	CE	PPL	FFA	TG‡	CE	PPL	FFA	TG
Palmitic acid C16:0								
Base line	15.1 ± 4.1	26.6 ± 2.3	27.4 ± 7.2	31.3 ± 5.0	14.5 ± 2.8	27.8 ± 2.5	29.0 ± 2.4	29.9 ± 6.8
14 days	IV‡ 14.1 ± 0.3	30.7 ± 2.0	34.9 ± 1.2	42.3 ± 10.7	NG 17.0 ± 0.8	33.9 ± 2.6	35.4 ± 5.3§	40.2 ± 2.2
28 days	NG 20.5 ± 12.6	29.3 ± 2.2	30.9 ± 3.1	38.5 ± 2.6	IV 14.2 ± 1.7	31.3 ± 3.4	33.4 ± 4.3§	36.7 ± 2.2
Palmitoleic acid C16:1								
Base line	3.5 ± 0.8	1.0 ± 0.3	7.3 ± 4.8	1.8 ± 0.4	5.6 ± 1.3	1.1 ± 0.3	5.9 ± 0.6	1.9 ± 0.8
14 days	IV 19.5 ± 3.5¶	2.5 ± 0.5§	9.7 ± 1.4	3.5 ± 1.6	NG 18.1 ± 3.2	2.3 ± 0.7§	8.9 ± 2.6	3.8 ± 1.1
28 days	NG 17.6 ± 7.2**	2.5 ± 1.2	9.9 ± 3.2	3.6 ± 1.3§	IV 18.9 ± 5.1	2.2 ± 0.3	8.0 ± 4.0	5.1 ± 3.2
Stearic acid C18:0								
Base line	1.7 ± 0.2	18.4 ± 1.4	3.9 ± 0.4	18.4 ± 2.2	1.5 ± 0.5	18.5 ± 1.5	4.0 ± 0.2	21.4 ± 1.7
14 days	IV 0.9 ± 0.1¶	17.9 ± 1.2	4.2 ± 0.9	18.4 ± 3.7	NG 0.9 ± 0.1	14.9 ± 0.8§	5.1 ± 1.1	13.2 ± 1.5§
28 days	NG 1.1 ± 0.4¶	17.0 ± 2.8	3.5 ± 0.7	15.5 ± 4.4	IV 0.7 ± 0.2	16.7 ± 1.7	6.0 ± 5.4	12.8 ± 6.5
Oleic acid C18:1								
Base line	26.8 ± 2.0	18.4 ± 2.9	47.9 ± 9.5	25.4 ± 6.0	29.9 ± 2.1	17.3 ± 1.5	45.2 ± 3.0	20.9 ± 2.8§
14 days	IV 48.7 ± 4.0¶	30.9 ± 1.9¶	45.7 ± 2.3	22.1 ± 12.2	NG 44.6 ± 5.7	24.0 ± 2.3	43.8 ± 7.0	25.5 ± 0.8
28 days	NG 42.9 ± 3.9¶	29.0 ± 4.8	48.1 ± 4.5	29.7 ± 2.2	IV 41.6 ± 4.1	24.3 ± 2.6**	41.1 ± 8.0	30.3 ± 8.7
Linoleic acid C18:2								
Base line	48.8 ± 2.1	21.2 ± 2.8	9.6 ± 5.5	14.1 ± 2.8	40.2 ± 3.7	16.6 ± 2.4	10.1 ± 3.0	14.5 ± 3.2
14 days	IV 9.8 ± 3.6¶	3.2 ± 1.3‡‡	2.0 ± 1.0§	2.6 ± 1.8¶	NG 13.5 ± 4.2	6.6 ± 2.7	2.7 ± 1.7¶	5.0 ± 1.7¶
28 days	NG 12.7 ± 11.4**	4.3 ± 2.2‡‡	2.8 ± 2.7	3.1 ± 2.7¶	IV 11.6 ± 8.7	5.3 ± 2.1**	3.8 ± 1.4	4.1 ± 2.9**
Eicosatrienoic acid C20:3ω9								
Base line	0	0	0.1 ± 0.1	0	0	0	0	0
14 days	IV 0.6 ± 0.2**	2.5 ± 1.3§	0.2 ± 0.1	2.3 ± 1.0	NG 0.4 ± 0.2§	2.6 ± 0.8**	0.2 ± 0.1	1.9 ± 1.2§
28 days	NG 0.9 ± 0.2¶	3.5 ± 1.6	0.3 ± 0.1§	2.5 ± 1.7	IV 1.3 ± 0.5	4.2 ± 1.7	1.6 ± 1.3	2.4 ± 2.2
Arachidonic acid C20:4								
Base line	3.7 ± 1.2	9.3 ± 2.0	0.5 ± 0.4	6.5 ± 3.8	5.1 ± 2.4	11.3 ± 0.7	1.5 ± 0.9	8.2 ± 3.9
14 days	IV 3.8 ± 1.8	7.4 ± 2.0	0.7 ± 0.6	5.5 ± 1.9	NG 3.0 ± 2.0	8.3 ± 2.5§	0.6 ± 0.1	5.2 ± 1.2
28 days	NG 2.7 ± 2.0	8.1 ± 2.2	0.4 ± 0.3	3.7 ± 2.2	IV 5.6 ± 1.0	10.0 ± 1.9	3.2 ± 2.6	4.8 ± 2.3

* Minor fatty acids not included: C14:0, C15:0, C17:0, C18:3, C20:0, C20:3ω6.

‡ CE, cholesterol esters; PPL, phospholipids; FFA, free fatty acids; TG, triglycerides; IV, intravenous feeding; NG, nasogastric feeding.

§ Change from base line significant according to paired *t* test, *P* < 0.05.

|| Change from base line significant according to paired *t* test, *P* < 0.025.

¶ Change from base line significant according to paired *t* test, *P* < 0.005.

** Change from base line significant according to paired *t* test, *P* < 0.01.

‡‡ Change from base line significant according to paired *t* test, *P* < 0.001.

glycerides, and free fatty acids were collected. The rest of the plate was developed in diethyl ether to remove impurities from the phospholipid band which was then collected. The fatty acid composition of the isolated cholesterol esters, free fatty acids, phospholipids, and triglycerides was determined by gas-liquid chromatography from methyl-esters prepared with boron fluoride methanol (18). The gas-liquid chromatograph used was a Hewlett-Packard 7610A instrument with a flame ionization detector and 6-foot dual columns (Hewlett-Packard Co., Palo Alto, Calif.). The carrier gas was helium and the stationary phase was 6% diethylene glycol succinate on 80/100 Diatoport S. The samples were run at 135–200°C with a 2 min programmed temperature rise. The helium flow was 50 ml/min. A standard prepared from a mixture of Hormel Institute GLC standards was run at the beginning of each day, and sample peaks were compared to those of the standards by the use of relative retention times for identification. Fatty acid peaks were quantitated on a weight percentage basis. Data in Study I is expressed as the mean ± the SD for one

sample in each subject at the indicated day. The linoleic acid content of the formula diet in Study II was likewise determined by analysis of an aliquot. Statistical comparisons of paired data were made by Student's *t* test (19). The overall coefficient of variation for the combined thin-layer and gas-liquid chromatography determinations of all fatty acids was 3.52%. For large peaks such as linoleic and oleic acids, the coefficient of variations was 1.0% or less.

RESULTS

Study I. During the base-line dietary period, the eight men in Study I had serum fatty acid patterns similar to results reported by others for normals as indicated in Table II (20, 21). All subjects developed biochemical EFA deficiency within 2 wk after beginning the fat-free diet. Mean linoleic acid levels fell and eicosatrienoic acid levels rose in all serum lipid frac-

TABLE III
Study II—The Changes in the Fatty Acid Composition of

	Cholesterol esters							Phospholipids						
	C16:0	C16:1	C18:0	C18:1	C18:2	C20:3 ω 9	C20:4	C16:0	C16:1	C18:0	C18:1	C18:2	C20:3 ω 9	C20:4
Base line	10.2	1.8	1.9	35.5	45.6	0	3.0	25.6	0.5	17.0	19.6	23.0	tr	8.5
Period A—fat-free														
Day 1	10.9	2.1	1.8	36.5	42.9	0	3.6	27.3	1.1	16.1	20.1	17.2	0.4	11.1
3	13.2	8.4	1.8	39.7	26.4	0	5.3	27.6	3.3	15.4	25.2	6.7	0.6	13.0
5	15.6	14.9	1.5	46.1	14.5	0	3.0	27.4	2.7	18.1	21.9	5.6	0.9	11.4
7	14.9	16.8	1.6	44.9	11.8	0.2	5.3	27.6	2.8	18.0	26.9	5.6	0.9	11.1
9	15.0	19.0	1.3	48.3	8.8	0.5	4.0	27.8	2.7	19.0	30.9	2.6	2.6	9.2
10	13.7	16.9	1.3	48.6	9.4	0.8	6.0	26.8	3.0	17.4	29.3	2.8	2.7	12.8
Period B—total fast														
0 h	13.7	16.9	1.3	48.6	9.4	0.8	6.0	26.8	3.0	17.4	29.3	2.8	2.7	12.8
12 h	12.5	16.3	1.2	47.7	10.3	1.0	6.9	26.6	2.3	16.5	26.8	5.8	3.0	12.9
18 h	12.6	16.3	1.1	47.7	11.9	0.8	6.1	25.9	1.4	17.3	27.0	8.2	2.7	11.0
24 h	12.8	16.2	1.0	45.3	13.9	0.9	6.7	26.9	1.9	14.7	23.5	10.3	3.1	13.3
Period C—intermittent feeding														
Day 3	14.2	11.0	0	39.1	21.9	1.0	8.6	30.4	2.0	15.0	20.1	10.2	2.3	13.0
4	13.4	9.2	1.2	39.6	26.0	0.7	6.3	28.2	0.8	13.9	22.8	13.8	2.6	11.9
Period D—repletion														
Day 1	13.2	7.7	1.3	39.7	31.0	0.5	3.8	23.9	0.1	19.5	22.8	17.0	1.4	9.7
3	10.5	4.6	1.2	33.4	39.5	0.6	6.5	25.4	0.8	16.6	18.6	18.2	1.6	11.5
5	9.4	2.8	1.4	32.2	43.6	0.5	6.4	26.0	0.5	17.0	18.2	19.4	0.9	11.1
13	9.6	1.8	1.6	30.7	49.2	tr	5.0	26.1	0.5	16.1	18.0	23.8	0.3	10.1

* Fatty acids expressed as percent of total fatty acids.

tions (cholesterol esters, phospholipids, free fatty acids, and triglycerides). An example of this is shown in Fig. 1 which represents the cholesterol ester linoleic and eicosatrienoic acid changes for two of the subjects in Study I. Table II contains the detailed data for all subjects divided into Groups A and B according to the sequence of administration of the diet.

The subjects in Group A received the fat-free formula by intravenous catheter for 14 days immediately

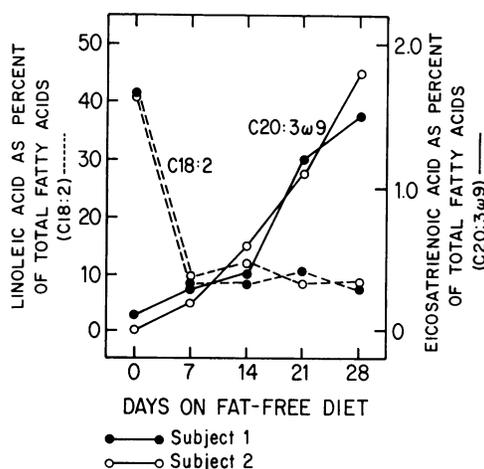


FIGURE 1 The changes in linoleic acid and eicosatrienoic acid as percent of the total fatty acid composition of cholesterol esters in two subjects of Study I.

after the base-line period. The first remarkable change in the fatty acid composition was the profound lowering of linoleic acid in each lipid class (Table II). Linoleic acid decreased from 48.8 to 9.8% in the cholesterol ester fraction, from 21.2 to 3.2% in the phospholipid fraction, from 9.6 to 2.0% in the free fatty acid fraction, and from 14.1 to 2.6% in the triglyceride fraction during the 14 days of the fat-free diet. These differences were all statistically significant ($P < 0.005$).

During the second 14-day period in which Group A received the diet by nasogastric feeding, the mean linoleic acid levels did not change significantly from the values at the end of the intravenous feeding.

The second change of interest seen in the serum fatty acid pattern was the appearance of eicosatrienoic acid in each lipid fraction. During the intravenous feeding, it increased significantly ($P < 0.05$) in each fraction from a base line of zero or trace amounts to 0.6% in cholesterol esters, 2.5% in phospholipids, 0.2% in free fatty acids, and 2.3% in triglycerides. Levels of eicosatrienoic acid rose still higher after 28 days on a fat-free diet, the last 2 wk of which was administered nasogastrically. These values were not statistically different, however, from those after the 14 days of intravenous fat-free feeding.

Although there were reciprocal changes in linoleic acid and eicosatrienoic acid in all of the lipid classes,

Plasma of a Single Subject (E. R.) after a Fat-Free Diet*

Free fatty acids							Triglycerides						
C16:0	C16:1	C18:0	C18:1	C18:2	C20: 3ω9	C20:4	C16:0	C16:1	C18:0	C18:1	C18:2	C20: 3ω9	C20:4
26.1	1.8	8.6	52.5	9.0	0	1.1	27.5	0.2	17.0	22.5	22.5	0	6.6
28.9	4.4	3.0	47.3	11.5	0	1.8	28.8	1.3	17.0	21.2	15.5	0.3	10.1
31.2	11.9	1.9	42.1	5.6	0.3	2.6	29.0	4.2	14.5	27.1	7.0	1.2	9.1
34.6	10.6	3.2	41.8	4.2	0.3	1.9	28.8	4.2	14.2	27.4	5.9	1.4	11.4
34.6	12.1	2.9	43.8	2.4	0.3	1.5	29.9	5.8	14.1	30.8	3.7	1.6	9.1
35.1	11.0	4.1	44.2	1.8	0.4	1.0	31.4	4.8	14.7	30.7	2.8	2.9	8.0
34.5	10.7	3.9	46.3	1.8	0.4	1.1	28.1	4.3	15.0	32.5	2.9	2.9	10.3
34.5	10.7	3.9	46.3	1.8	0.4	1.1	28.1	4.3	15.0	32.5	2.9	2.9	10.3
29.1	8.7	2.4	49.7	5.5	0.5	0.9	27.5	3.8	14.0	30.9	6.4	3.0	9.6
28.0	7.1	2.4	49.7	6.7	0.6	1.9	35.8	5.3	13.0	37.5	—	—	—
28.4	6.2	2.7	50.6	7.6	0.9	1.4	31.2	3.2	10.6	27.0	10.3	3.5	9.2
34.8	8.6	3.0	41.4	6.3	0.4	1.3	38.4	1.8	14.3	20.9	8.4	1.3	10.7
27.7	5.4	6.9	49.5	6.0	0.3	1.2	27.2	2.4	15.6	27.9	12.0	1.6	7.7
27.5	4.5	7.4	50.6	6.2	0.3	1.0	26.1	1.7	16.9	26.1	14.1	1.7	8.0
27.3	3.5	5.6	51.8	8.2	0.4	0.9	29.4	1.2	15.3	22.1	17.0	1.2	8.3
26.9	2.8	5.7	51.6	9.9	0.2	1.1	25.3	0.9	13.7	21.9	19.2	1.5	11.1
29.8	3.2	5.3	49.6	12.4	0	0.9	28.1	0.7	15.4	20.2	22.8	0.3	8.4

the percent increases seen in eicosatrienoic acid were not enough to make up for the great decrease in linoleic acid. The monounsaturated fatty acids, palmitoleic (C16:1) and oleic (C18:1), increased to make up this difference (Table I). Arachidonic acid levels decreased significantly only in the phospholipid fraction during the first 14 days of intravenous feeding ($P < 0.05$). Dermatitis, which is usually the first clinical sign of EFA deficiency was not detected at any time in the study.

The subjects in Group B received the fat-free diet for 14 days by constant drip nasogastric feeding immediately after the base-line period. During this period, linoleic acid declined significantly ($P < 0.025$) in all lipid classes (Table II). The greatest drop relative to base line was in the free fatty acid fraction where it decreased from 10.1 to 2.7% of total fatty acids. Linoleic acid in the cholesterol ester fraction fell from 40.2 to 13.5%, in phospholipids from 16.6 to 6.6%, and in triglycerides from 14.5 to 5.0%. The subsequent intravenous feeding sustained but did not alter significantly the changes which had already occurred during the first 14 days.

After 14 days of nasogastric fat-free feeding, all subjects of Group B had significantly increased ($P < 0.025$) levels of eicosatrienoic acid: 2.6% in phospholipid fatty acids, 1.9% in triglyceride fatty acids, 0.4% in cholesterol fatty acids, and 0.2% in free fatty acids. After 28 days on the fat-free diet, 14 days of nasogas-

tric feeding followed by 14 days of intravenous feeding, eicosatrienoic acid levels rose significantly ($P < 0.05$) in each fraction above the values seen after the 14 day fat-free nasogastric period.

The percentages of palmitoleic acid and oleic acid again increased to counterbalance the great decrease seen in linoleic acid. For example, in the cholesterol ester fraction, palmitoleic rose from 5.6 to 18.9% and oleic from 29.9 to 44.6% during the first 14 days of nasogastric feeding. Arachidonic acid levels decreased significantly ($P < 0.05$) from 11.3 to 8.3% in the phospholipid fraction during the first 14 days of nasogastric feeding, but not during the second 14 days of intravenous feeding.

Study II (subject E. R.). This second study was undertaken in Subject E. R. to obtain more detailed data of the metabolic processes which occurred during Study I. During four dietary periods, he received in sequence a continuously fed fat-free formula, a 24-h fast, an intermittently fed fat-free formula, and a repletion formula. Linoleic acid decreased markedly in all plasma lipid fractions by the 3rd day of the fat-free diet of Period A (Table III). At this time the amount of linoleic acid in the cholesterol ester and free fatty acid fractions was about half of base line and in phospholipid and triglyceride about one-third of base line. All fractions appeared to have leveled off after days 9 and 10 of the fat-free formula.

TABLE IV
*Study II (subject E. R.)—The Percent Fatty Acid Composition of Adipose Tissue
before and after a Fat-Free Diet*

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C20: 3 ω 9	C20:4
Base line	2.2	2.5	23.7	3.9	7.2	47.6	12.3	0.7	0	0.1
After 2 wk of the fat-free diet	1.0	2.9	21.4	5.7	5.2	49.7	12.8	0.9	0	0.3

Eicosatrienoic acid was not present in measurable quantities in any fraction at base line. It appeared on the 1st day of the fat-free diet in the phospholipid and triglyceride fractions and increased in these two fractions to the end of Period A to values of 2.7 and 2.9%, respectively. The eicosatrienoic acid of the cholesterol ester fraction did not appear until day 7 and reached only 0.8% by day 10. Free fatty acid eicosatrienoic acid appeared at day 3 and reached only 0.4% by day 10. Arachidonic acid did not change significantly.

During the 24-h fast, Period B, linoleic acid levels rose in all fractions. The greatest relative rise was a fourfold increase in free fatty acid linoleic acid from 1.8 to 7.6%. Linoleic acid in the phospholipid and triglyceride fractions increased similarly from 2.8 to 10.3% and 2.9 to 10.3%, respectively. Cholesterol ester linoleic acid increased also from 9.4 to 13.9%.

The intermittent formula feeding of Period C resulted in a further increase in cholesterol ester linoleic acid, from 13.9 to 21.9%. Phospholipid linoleic acid did not change. The linoleic acid of the free fatty acid and triglyceride fractions fell from 7.6 to 6.3% and 10.3 to 8.4%, respectively.

Eicosatrienoic acid did not decrease during Period B, the 24-h fast. Intermittent feeding produced no change in the cholesterol ester eicosatrienoic acid, slight decreases in free fatty acid and phospholipid eicosatrienoic acid, and a marked decrease in triglyceride eicosatrienoic acid from 3.5 to 1.3%.

After administration of linoleic acid as 2.6% of calories in the repletion diet, Period D, the serum linoleic acid reached base-line levels in all fractions by day 13. Eicosatrienoic acid had practically disappeared by day 13 being present as 0.3% of phospholipids and triglycerides and in a trace amount in cholesterol esters.

No clinical signs of EFA deficiency were detected in this subject.

Adipose tissue biopsies were done at the beginning and end of Study II (Table IV). The fatty acid composition was similar in the two samples which both contained linoleic acid as 12% of the total fatty acids.

Neither sample contained any detectable eicosatrienoic acid.

DISCUSSION

Two important conclusions emerged from these studies. First, EFA deficiency invariably occurred in healthy, well-nourished adult humans continuously fed a eucaloric fat-free diet made up of glucose and amino acids. This state of deficiency, as manifested by great decreases of linoleic acid in the plasma lipid classes and the appearance of eicosatrienoic acid, became detectable in 1 day and was fully manifested by 3 days. No real difference was noted between the intravenous and nasogastric routes of administration. Secondly, short periods of fasting or of intermittent feeding seemed to replete partially an EFA-deficient individual, presumably from EFA stores in the adipose tissues.

To be emphasized is that there were two requirements for the development of the EFA deficiency: first, a fat-free diet and, secondly, the continuous infusion of glucose which prevented the mobilization of the large stores of linoleate in the adipose tissues (12% of the total fatty acids in our subject of Study II). A feast of glucose calories led to a famine of linoleate for the viscera and peripheral tissues despite the presence of 700 or more g of linoleate in the adipose tissues, enough to supply the EFA needs of the body for 233 days or more if it could be but mobilized.

Although linoleic acid decreased rapidly for the first 2 wk of the deficiency state, it did not decline further with time. In neither of these studies nor in those reported in the literature has linoleate ever disappeared completely from the serum. This may be due to the release of linoleate via tissue catabolism or the hydrolysis of biliary phospholipids, or perhaps to the substitution of isomers of linoleic acid, including C18:2 ω 10, C18:2 ω 9, and C18:2 ω 7, which can be biosynthesized and which were not resolved by the gas chromatographic methods used. These isomers have been shown to occur in EFA-deficient rats (22).

Animal studies have shown, however, that when linoleic acid is not present as 1% or more of total calories

5,8,11-eicosatrienoic acid appears (2, 8). This is the established biochemical indication of a metabolic deficiency state which has been repeatedly found characteristic of EFA deficiency (2, 8, 12-15). Under normal circumstances, the body converts linoleic acid (which cannot be biosynthesized) to arachidonic acid. When linoleic acid is not present in adequate amounts, the same enzyme system elongates and desaturates oleic acid (C18:1), a fatty acid which can be biosynthesized by the body, and produces 5, 8, 11-eicosatrienoic acid rather than arachidonic acid. This latter reaction is competitively inhibited by linoleic acid (23).

The increases in linoleic acid during the periods of total fasting and the intermittent feeding in Study II and the decrease in eicosatrienoic acid during the intermittent feeding would seem to indicate that repletion was occurring. The source of this linoleic acid was presumably adipose tissue triglyceride which contained 12% linoleic acid and which was mobilized during the periods of fast. These results suggest a theoretical basis for a therapeutic measure to prevent EFA deficiency during the clinical situation of hyperalimentation feeding. Clearly, the fasting of malnourished patients would not be appropriate, but the incorporation of a fat source containing linoleate into the hyperalimentation diet, as suggested by MacFayden et al., might well prevent EFA deficiency (24). While they did not study the EFA status of their patients, these investigators infused a 10% soybean oil emulsion (Intralipid®, Cutter Laboratories, Inc., Berkeley, Calif.) along with a formula containing 25% dextrose, 4.2% amino acids, vitamins, and electrolytes for a period of 4 h.

One point of interest which may be noted from examining the data during Periods A and B of our study is the source of the fatty acids in the free fatty acid fraction. It has been assumed that these are usually derived solely from adipose tissue. During Period A the relative fatty acid composition of the free fatty acid fraction was changing dramatically (Table III), yet the subject was not losing weight and presumably not mobilizing adipose tissue because of the continuous glucose infusion. Biopsies done at the beginning and end of the study showed similar linoleic acid contents of the adipose tissue, about 12% of the total fatty acids. In addition, the free fatty acid composition after the 24-h fast of Period B was similar to that at base line indicating that no gross changes in adipose tissue had occurred during Period A. The changes in triglyceride fatty acid composition during Period A were quite similar to those in the free fatty acid fraction. Since it is well established that the liver is the major site of triglyceride production, the free fatty acids might be derived via intravascular hydrolysis of triglycerides or, conceivably, via direct hepatic secretion of free fatty acids under the

circumstances of this study with a shutdown of free fatty acid outflow from the adipose tissue depots.

Complete correction of EFA deficiency occurred in 2 wk in Study II with reinstatement of dietary linoleic acid at 2.6% of total calories. This is in agreement with the usual recommendation of 1-2% of total calories as linoleic acid, but somewhat less than Collins and co-workers' recommendation of 4% of calories (15). They found that when linoleic acid intake decreased from 6.4 to 2.2% of calories, phospholipid eicosatrienoic rose from 2.4 to 3.6%. However, they were dealing with a severely depleted patient who, in addition, had short bowel syndrome and could not adequately absorb linoleic acid.

Collins et al. observed that arachidonic acid increased or decreased in direct response to dietary linoleic acid in their subject. Because of the biochemical pathway of linoleate to arachidonate, this seems logical. This correlation, however, was seen only in the phospholipid fraction during Study I in our studies. Perhaps similar changes would have developed in other fractions if the length of the dietary period was increased. The turnover time for arachidonic acid is somewhat slower than that for linoleic acid (25, 26).

In 1954 and 1958, Hanson, Wiese, and coworkers thoroughly investigated EFA deficiency in children (11, 27-30). They definitely established that a state of EFA deficiency can occur in growing humans. Their results were somewhat limited by their methodology which did not include gas-liquid chromatography, and results were expressed in terms of di-, tri-, and tetraenoic acid levels. Recently, in two studies, EFA deficiency occurred in infants receiving fat-free hyperalimentation diets similar to the ones used in the studies reported here (12, 14).

Since the work of Dudrick, Wilmore, Varn, and Rhoads (31) in 1968, the use of hyperalimentation diets which include glucose and amino acids, but no fat, have been extensively used for the extended nourishment of severely ill patients. Collins et al. (15) have reported the occurrence of EFA deficiency in a patient with a bowel resection who was maintained by fat-free intravenous alimentation for 100 days. He had no clinical evidence of malnutrition except a dermatitis, but his serum phospholipids contained 10% 5,8,11-eicosatrienoic acid. A fat infusion containing 6.6% of total calories as linoleate was then administered daily for 12 days. This caused a sharp decline in the 5,8,11-eicosatrienoic acid from 9.9 to 1.2% of total phospholipid fatty acids.

Patients receiving fat-free parenteral hyperalimentation diets are often ill and nutritionally depleted. They seem unusually susceptible to infection (32). Perhaps one contributing cause of the infection might be the associated EFA deficiency (3, 8). In view of the studies

we have reported, it would now seem advisable to supplement parenteral calories with linoleic acid. The addition of small amounts of linoleic acid to the diet, perhaps as little as 1-2% of total calories, would avoid EFA deficiency.

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