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EFFECT OF γ-LINOLENIC TRIGLYCERIDE ON THE LIPID METABOLISM IN RATS FAT-FREE DIET

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SUMMARY

To observe the effect of γ-linolenic triglyceride on the fatty acid composition of various tissues, rats maintained with a fat-free diet frr 3 months were administered a single dose of 0.4 ml. of safflower oil or γ-linolenic triglyceride in the first experiment, and transferred to diets containing 5% (w/w) safflower oil or γ-linolenic triglyceride in the second experiment. The fatty acid composition of total lipids and lipid fractions of the plasma, liver and intestinal mucosa were determined at intervals of 2 and 6 hours, and 2 and 14 days after feeding.

The livers from the animals fed a fat-free diet showed evidence of cellular degeneration and some fatty infiltration, but cellular regeneration and little fatty infiltration, but cellular regeneration and little fatty infiltration were found in the histological sections of rats fed γ-linolenic triglyceride.

An increased concentration of cholesterol in the liver and a reduced cholesterol content in the plasma of EFA-deficient rats were observed. After the supplemental feeding, there was a rapid recovery in cholesterol concentration. There was no differences among rations.

Lipids from the deficient animals were low in linoleic and arachidonic acids and high in palmitoleic, oleic, and eicosatrienoic acids. The supplemental feeding resulted in a rapid increase in linoleic and arachidonic acids and decrease in palmitoleic, oleic and eicosatrienoic acids in all three fraction, particularly in plasma phospholipids and in liver cholesterol esters.

It was seen that the degree of recovery in arachidonic acid levels of rats fed γ-linolenic triglyceride was larger than that fed safflower oil in plasm and liver phospolipids and in plasma cholesterol esters, and less in triglycerides. Therefore, it is concluded that a small amount (approximately 11 per cent) of γ-linolenic acid supplemented to linoleic acid is more effective than safflower oil for the recovery of tissue lipids in EFA-deficient rats.

INTRODUCTION

The essential fatty acid (EFA) deficiency syndrome was first recognized and described in young rats in 1929 by Burr and Burr[1]. In 1929 McAmis, Anderson, and Mendel[2] also studied the effect of a fat-free diet in weanling rats. The EFA-deficiency syndrome has been studied extensively ever since.

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The term "essential fatty acid" was introduced by Burr and Burr[3] in 1930 for linoleic acid (cis, cis-9, 12-octadecadienoic acid). They also suggested that linolenic acid (cis, cis, cis-9, 12, 15-octadecatrienoic acid) might have a similar effect. Linolenic acid was found effective in curing fat-deficient rats in a subsequent work, Burr, Burr and Miller[4]. In 1938 Turpeinen[5] found methyl arachidonate to be three times as potent as linoleate in promoting growth. Two years later a number of reports confirmed the essentiality of arachidonic acid in fat-deficiency[6)~8)]. Over the years EFA have been reviewed in great detail several times[9)10)]. A new component was added to the list of EFA in 1953 when Thomasson[11] found γ-linolenic acid (cis, cis, cis-5, 8, 11-octadecatrienoic acid) to be as active as linoleic acid for growth in EFA-deficiency.

Briefly, the scheme of formation of arachidonic acid from linoleic is as follows[12)~15)]:

$$\begin{array}{c} CH_3—(CH_2)_4—(CH{=}CH—CH_2)_2—(CH_2)_6—COOH \\ \downarrow \text{linoleic} \\ CH_3—(CH_2)_4—(CH{=}CH—CH_2)_3—(CH_2)_3—COOH \\ \downarrow \gamma\text{-linolenic} \\ CH_3—(CH_2)_4—(CH{=}CH—CH_2)_3—(CH_2)_5—COOH \\ \downarrow 8, 11, 14\text{-eicosatrienoic} \\ CH_3—(CH_2)_4—(CH{=}CH—CH_2)_4—(CH_2)_2—COOH \\ \text{arachidonic} \end{array}$$

This pathway was traced by a series of experiments involving the administration to rats of carbon-14-labeled acids followed by location of the C^{14} in arachidonic acid.

During the last 15 to 20 years the development of a number of new techniques and analytical methods for fat research has improved our understanding of the function and metabolism of the unsaturated fatty acids, however, there are many problems still unsolved.

In recent years the availability of gas-chromatographic methods has made possible the detailed study of the composition of complex fats. It has been possible to study the metabolism of each fatty acid upon the metabolism of others.

Clinically, a large dose of ethyl linoleate has been used for the treatment of the disturbances in lipid metabolism[16]. The purpose of the present study was to observe the effect of γ-linolenic acid supplemented to linoleic acid on the recovery of the disturbances in lipid metabolism.

MATERIALS AND METHODS

Newly weaned female Moriyamaso strain rats were maintained in raised wire-screen-bottom cages and fed a fat-free diet *ad libitum* for 3 months.

The composition of the basic fat-free diet* was as follows: (in per cent)

* The basic diet was obtained from Nutritional Biochemicals Corporation, Cleveland

Vitamin-test casein, 18.0; sucrose, 74.0; salt mixture, 4.0; α-cellulose, 4,0; and vitamin mixture. The vitamin mixture contained: (in mg/kg of diet) vitamin A, 4.00; thiamiue, 30.00; riboflavin, 30.00; pyridoxine, 8.00; vitamin B_{12}, 0.05; vitamin D_2, 4.00; vitamin E, 230.00; vitamin K_5, 2.00; DL-Ca pantothenate, 100.00; niacin, 100.00; *i*-inositol, 220.00; *p*-aminobenzoic acid, 75.00; biotin, 0.20; folic acid, 1.00; and choline chloride, 1000.00.
Salt mixture was followed the formula of Wessoa[17].

A control group seceived the rat cubes which contain approximately 5 per cent fat.

The present study consists of 2 experiments. In the first experiment, rats were administered a single dose of 0.4 ml. of either safflower oil or γ-linolenic triglyceride by oral intubation and sacrificed at 2 and 6 hours after the administration.

In the second experiment, rats were fed diets supplemented with either safflower oil or γ-linolenic triglyoeride at a level of 5% (w/w), or fed the rat cubes and sacrificed at 2 and 14 days after the supplemental feeding.

The major fatty acid composition of the safflower oil and γ-linolenic tryglyceride** is shown in Table 1 and similar to one another except for γ-linolenic acid.

TABLE 1. Fatty acid composition of safflower oil and γ-linolenic triglyceride

	Safflower oil	γ-Linolenic triglyceride
palmitic	9.9%	9.4%
stearic	3.8	1.4
oleic	14.9	6.6
linoleic	71.7	71.1
γ-linolenic	—	11.3

At the indicated time after feeding, blood was drown by cardiac puncture and centrifuged immediately. The small intestine was placed in cold 0.9% sodium chloride and opened longitudinally. The contents of the small intestine were washed out with 0.9% sodium chloride. The segments of intestine were blotted dry, and the mucosa was scraped off with a glass slide. The liver and intestinal mucosa were immediately homogenized in a Potter-Elvehjem type homogenizer.

The lipid analyses were carried out as follows:

Cholesterol : the method of Leffler[18]
Phospholipid : the method of Fiske and Subbarow[19]
Total lipid : the modified method of Bragdon[20]
Esterified fatty acid: the method of Stern and Shapiro[21]

** γ-Linolenic triglyceride used in this study was supplied from Ono Pharm. Co. Ltd. Osaka, Japan.

To analyse the fatty acid composition of total lipids and lipid fractions, lipids were extracted by the procedure of Folch, *et al.*[22] and separated by thin layer chromatography, for cholesteryl esters, triglycerides, free aftty acids, cholesterol, and polar lipids. The polar lipids consisted mostly of phospholipids and, henceforth, this fraction is designated as phospholipids. The steryl esters may also contain a mixture of compounds, but they consisted mostly of cholesteryl esters and are so designated. All the total lipids and lipid fractions separated were sealed in each glass ample with about 5 ml. of 10% dry H_2SO_4 in methanol and placed in a 50°C water bath for four hours. The resulting methyl esters were analyzed by gas-liquid chromatography, and the results are reported as area percentage.

Histological sections from the livers in each treatment at 14 days after the supplemental feeding were stained with hematoxylin-eosin and sudan III and examined histologically.

RESULTS

1) Histological changes of the liver

Histological sections of the livers in all groups showed general cellular degeneration, and there were no differences in the degree of degeneration due to rations. The liver sections from animals fed a fat-free diet and those supplemented with safflower oil contained small fat globules within the cells in large areas, and large globules of fat within the cells in portal areas of the animals which had received the rat cubes. However, little fatty infiltration was found in the histological sections of rats fed γ-linolenic triglyceride, and large areas of cellular regeneration in the histological sections of the safflower oil and γ-linolenic triglyceride treated groups.

2) Plasma and liver lipid fractions (Table 2)

After feeding the basal fat-free diet for 3 months, there was a definite decrease in plasma lipid fractions particularly in plasma cholesterol.

After the supplemental feeding, there was a rapid recovery in plasma lipid fractions; within 2 hours total lipids had superceded the concentration observed in the control animals, phospholipids and esterified fatty acids approached the control level, but the recovery of plasma cholesterols took longer. There was no differences among rations.

In the liver of the EFA-deficient rats, accumulation of cholesteryl esters was observed, but the other lipid fractions showed the least difference in comparison with the control levels.

After the supplemental feeding, there was a recovery of the cholesterol concentration, but no consistent change was observed in phospholipids and total lipids.

TABLE 2. Plasma and liver lipid fractions

Group	Supplement	Duration	Plasma* (mg/dl)					Liver* (mg/g)				
			TC	E	P	TL	EFA	TC	E	R	TL	EFA
Control			84**	0.72	69	965	181	4.5	0.66	27.6	61.6	28.5
Fat-free			53	0.69	49	734	148	6.9	0.74	26.7	64.4	30.8
Experiment 1												
1	safflower oil	2 hrs.	60	0.75	73	1370	182	5.6	0.71	30.1	58.7	30.2
2	γ-linolenic	2 hrs.	64	0.72	72	1250	182	5.4	0.67	31.3	64.3	31.0
3	safflower oil	6 hrs.	66	0.73	79	1520	182	5.6	0.62	32.3	60.3	30.8
4	γ-linolenic	6 hrs.	73	0.77	78	1800	184	5.0	0.72	27.0	68.0	28.3
Experiment 2												
5	rat cubes	2 days	55	0.84	80	1490	179	5.5	0.69	32.5	46.7	28.6
6	safflower oil	2 days	58	0.83	86	1606	178	5.4	0.70	24.1	46.5	28.6
7	γ-linolenic	2 days	60	0.77	86	1904	167	5.7	0.70	30.6	49.2	27.3
8	rat cubes	14 days	68	0.87	88	1490	170	5.4	0.74	25.4	69.4	28.1
9	safflower oil	14 days	85	0.87	87	1680	179	5.6	0.71	27.9	78.2	27.7
10	γ-linolenic	14 days	89	0.87	83	1645	170	4.8	0.72	26.5	73.2	28.6

* TC. total cholesterol: E. (total cholesterol-free cholesterol)/total cholesterol
P. phospholipid: TL. total lipid: EEA. esterified fatty acid.
** Mean of 3 animals.

TABLE 3. Fatty acid composition of plasma lipids

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Control			17.9**	4.4	11.4	22.0	19.0	0.3	18.0	22.3	37.0	0.02
Fat-free			18.0	13.2	11.6	38.5	3.4	12.0	1.5	50.5	4.9	8.0
Experiment 1												
1	safflower oil	2 hrs.	24.9	10.2	7.8	34.6	13.8	5.5	3.2	40.1	17.0	1.7
2	γ-linolenic	2 hrs.	20.1	12.3	9.9	31.1	10.0	8.9	3.6	40.0	13.6	2.5
3	safflower oil	6 hrs.	24.6	7.4	12.6	21.1	20.2	5.2	6.2	26.3	26.4	0.9
4	γ-linolenic	6 hrs.	22.5	7.0	12.6	24.9	21.1	4.2	5.6	29.1	26.7	0.8
Experiment 2												
5	rat cubes	2 days	24.1	9.8	8.8	30.4	7.2	1.6	9.8	32.0	17.0	0.16
6	safflower oil	2 days	25.9	8.3	14.5	28.0	8.8	1.0	14.0	29.0	22.8	0.07
7	γ-linolenic	2 days	24.7	8.7	13.1	27.7	9.9	1.0	12.4	28.3	22.3	0.07
8	rat cubes	14 days	20.3	5.4	16.5	22.8	14.6	1.2	14.5	24.0	29.1	0.08
9	safflower oil	14 days	23.0	3.2	16.6	14.5	13.7	0.6	26.3	15.1	39.9	0.02
10	γ-linolenic	14 days	21.6	3.9	19.9	14.5	12.8	1.0	25.0	15.5	37.8	0.04

* Only the major fatty acids are represented; the number before the colon denotes number of carbon atoms, and the number after the colon, number of double bonds.
** Percentage of total fatty acids. Mean of 3 animals.

3) Fatty acids composition of total lipid

a) Plasma The concentrations of the major plasma fatty acids of rats are shown in Table 3. The combined concentration of linoleic and arachidonic

acids in the control group was 37% of the total fatty acids. In the deficient animals, the concentration of linoleic and arachidonic acids was greatly reduced, accounting for only about 5% of the total acids whereas palmitoleic, oleic and eicosatrienoic acids greatly increased.

Feeding various diets to the EFA-deficient animals resulted in a rapid recovery in the plasma fatty acid composition in 6 hours. However, the recovery of eicosatrienoic and arachidonic acids took a considerable period. The analysis indicated a slight peculiarity after 14 days of the supplemental feeding; stearic acids had increased and oleic acids had decreased over the control level; linoleic acid had also decreased from the value observed for 6 hours and arachidonic acid had superceded the concentration observed in the control animals. There was no differences in fatty acid levels between rats fed safflower oil and those fed γ-lenolenic triglyceride.

b) Liver In the EFA-deficiency, as observed in the plasma fatty acid composition, palmitoleic, oleic, and eicosatrienoic acids also increased and linoleic and arachidonic acids were greatly decreased.

TABLE 4. Fatty acid composition of liver lipids

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Control			23.9**	4.3	14.1	15.6	20.6	0.4	15.6	16.0	36.2	0.03
Fat-free			20.3	13.4	14.8	38.4	2.2	7.5	1.8	45.9	4.0	4.4
Experiment 1												
1	safflower oil	2 hrs.	21.1	5.8	28.8	29.9	4.4	5.8	4.8	35.7	9.2	1.2
2	γ-linolenic	2 hrs.	23.9	9.1	18.4	31.1	3.7	7.0	5.0	38.1	8.7	1.4
3	safflower oil	6 hrs.	22.6	4.6	24.2	23.5	11.5	4.7	7.6	28.2	19.1	0.6
4	γ-linolenic	6 hrs.	24.8	8.1	23.7	26.3	6.6	3.0	5.7	29.3	12.3	0.6
Experiment 2												
5	rat cubes	2 days	26.0	5.0	31.6	20.9	6.5	0.5	8.9	21.4	15.4	0.06
6	safflower oil	2 days	21.6	4.8	24.1	23.0	5.5	0.8	13.8	23.8	19.3	0.06
7	γ-linolenic	2 days	26.2	6.6	23.9	24.8	6.8	0.4	10.1	25.2	16.9	0.04
8	rat cubes	14 days	22.8	3.8	30.7	16.6	11.4	0.3	14.0	16.9	25.4	0.02
9	safflower oil	14 days	22.5	5.7	25.2	20.6	9.6	0.2	14.7	20.8	24.3	0.01
10	γ-linolenic	14 days	23.2	5.9	23.3	20.2	10.1	0.3	16.0	20.5	26.1	0.02

*, ** see Table 3

Feeding various diets to the deficient animals resulted in a slower rate of change of the same nature in the fatty acid comosition of liver lipids than plasma lipids. Oleic and linoleic acids did not approach the control levels after 14 days. However, the level of stearic acid maintained above the level in the control animals.

c) Intestinal mucosa The linoleic and archidonic acid contents of the intestinal mucosa of the deficient rats were very low. A striking feature of the deficiency was that the amount of eicosatrienoic acid was greater than that of linoleic and arachidonic acids.

TABLE 5. Fatty acid composition of intestinal mucosa lipids

Group	Supplement	Duration	16:0 *	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Control			27.4	8.9	5.8	26.8	21.7	trace	4.7	26.8	26.4	0
Fat-free			23.8 **	16.7	5.2	45.8	1.7	3.7	0.4	49.5	2.1	9.9
Experiment 1												
1	safflower oil	2 hrs.	18.1	6.2	7.6	31.2	32.0	1.7	1.1	32.9	33.1	1.5
2	γ-linolenic	2 hrs.	21.3	7.4	5.6	31.2	26.0	2.3	1.4	33.5	27.4	1.6
3	safflower oil	6 hrs.	22.2	8.6	6.4	45.1	22.1	1.2	1.3	36.3	23.4	1.4
4	γ-linolenic	6 hrs.	25.2	8.9	5.4	45.2	19.9	0.9	0.7	36.1	20.6	1.5
Experiment 2												
5	rat cubes	2 days	33.3	12.4	6.3	38.2	3.8	trace	1.0	38.2	4.8	0
6	safflower oil	2 days	31.6	10.2	8.8	35.7	7.5	trace	2.7	35.7	10.2	0
7	γ-linolenic	2 days	34.7	10.9	7.9	37.5	5.0	trace	1.4	37.5	6.4	0
8	rat cubes	14 days	26.1	12.8	4.1	42.6	10.5	trace	0.5	42.6	11.0	0
9	safflower oil	14 days	31.3	10.7	3.7	39.6	10.8	trace	1.2	39.6	12.0	0
10	γ-linolenic	14 days	29.9	12.0	3.3	39.9	11.1	trace	1.0	39.9	12.1	0

*, ** see Table 3

When the rats previously receiving a diet depleted of EFA were supplemented, decrease in the levels of palmitoleic, oleic, and eicosatrienoic acids and increase in the levels of palmitic and linoleic acids were noticed. The amount of linoleic acid of the mucosa in 2 to 6 hours reflected that of the supplements, and the recovery of linoleic acid took a considerable period. There was no differences in fatty acid levels between rats fed safflower oil and those fed γ-linolenic triglyceride.

4) Fatty acid composition of lipid fractions

a) Plasma The principal effect of a fat deficient diet on the component fatty acids in plasma lipids is a marked decrease in linoleic and arachidonic acids and a marked increase in oleic and eicosatrienoic acids in all three categories, particularly in cholesterol esters except for a marked increase in eicosatrienoic acid in phospholipids.

The supplemental feeding resulted in a rapid recovery of the fatty acid composition in all three fractions; the most rapid in phospholipids, the second in cholesterol esters, and the last in triglycerides. It was seen that the degree of recovery in arachidonic acid levels of rats fed γ-linolenic triglyceride was larger than that fed safflower oil.

TABLE 6. Fatty acid composition of plasma cholesterol esters

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Control			24.5**	5.8	2.3	12.7	18.3	trace	35.4	12.7	53.7	0
Fat-free			12.1	27.7	2.5	38.4	5.5	9.9	0.7	48.3	6.2	17.6
Experiment 1												
1	safflower oil	2 hrs.	13.3	16.9	2.3	27.4	5.9	15.3	10.1	42.7	16.0	1.5
2	γ-linolenic	2 hrs.	11.0	26.4	2.2	28.6	5.5	11.0	8.8	39.6	14.3	1.3
3	safflower oil	6 hrs.	15.3	15.5	6.3	26.4	12.1	8.6	11.0	35.0	23.1	0.8
4	γ-linolenic	6 hrs.	12.6	18.8	3.2	20.9	12.4	8.1	12.5	29.0	24.9	0.8
Experiment 2												
5	rat cubes	2 days	23.2	10.1	7.5	22.6	14.9	1.2	13.3	23.8	28.2	0.09
6	safflower oil	2 days	19.4	11.7	5.9	23.2	10.6	1.2	27.9	24.4	38.5	0.04
7	γ-linolenic	2 days	15.7	8.9	3.9	23.6	9.3	1.1	32.1	24.7	41.4	0.03
8	rat cubes	14 days	19.0	10.0	6.0	18.7	17.0	1.4	22.7	20.1	39.7	0.06
9	safflower oil	14 days	17.1	7.3	6.8	16.2	11.1	0.5	38.2	16.7	49.3	0.02
10	γ-linolenic	14 days	11.5	3.9	5.0	14.0	11.8	0.4	50.5	14.4	62.3	0.01

*, ** see Table 3

TABLE 7. Fatty acid composition of plasma triglycerides

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Fat-free			21.2**	16.3	1.5	53.9	1.8	1.6	trace	55.5	1.8	∞
Experiment 1												
1	safflower oil	2 hrs.	25.0	8.7	3.4	41.1	18.2	0.9	trace	42.0	18.2	∞
2	γ-linolenic	2 hrs.	26.0	12.4	2.4	46.1	17.7	0.6	trace	46.6	17.7	∞
3	safflower oil	6 hrs.	24.4	8.9	4.3	29.5	25.8	1.4	0.7	30.9	26.6	2.2
4	γ-linolenic	6 hrs.	26.5	11.2	3.5	31.2	19.8	0.9	0.5	32.1	20.3	2.2
Experiment 2												
5	rat cubes	2 days	27.7	11.1	5.6	40.1	7.9	1.3	0.9	41.4	8.8	1.3
6	safflower oil	2 days	24.5	11.7	4.6	42.9	8.8	1.2	1.2	44.1	10.0	0.9
7	γ-linolenic	2 days	25.7	16.2	4.7	36.7	7.3	0.8	1.3	37.5	8.6	0.7
8	rat cubes	14 days	25.7	4.2	11.5	34.7	11.6	0.7	4.9	35.4	16.5	0.13
9	safflower oil	14 days	25.6	5.6	15.5	27.2	13.8	0.9	8.2	28.1	22.0	0.11
10	γ-linolenic	14 days	23.4	6.9	12.7	28.9	11.3	0.5	10.3	28.4	21.6	0.05

*, ** see Table 3

b) *Liver* The fatty acid composition of liver lipid fractions in the EFA-deficiency was similar to that of plasma lipid fractions; there was a marked decrease in palmitic, linoleic, and arachidonic acids and an increase in palmitoleic, oleic, and eicosatrienoic acids.

The supplemental feeding resulted in a rapid recovery of the fatty acid composition in all three fractions. In the case of cholesterol ester, oleic and eicosatrienoic acids were most rapidly reduced. However, the levels of linoleic

TABLE 8. Fatty acid composition of plasma phospholipids

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3/20:4
Fat-free			18.5**	8.8	22.1	29.5	1.9	15.9	1.2	45.4	3.1	13.5
Experiment 1												
1	safflower oil	2 hrs.	26.2	4.3	28.7	24.1	6.1	5.5	2.6	29.6	8.7	2.2
2	γ-linolenic	2 hrs.	31.7	2.8	28.7	20.8	4.8	7.0	2.5	27.8	7.3	2.8
3	safflower oil	6 hrs.	31.5	4.5	26.0	16.9	10.7	5.2	3.8	22.1	14.5	1.4
4	γ-linolenic	6 hrs.	31.3	3.9	31.2	14.5	9.7	3.6	4.4	18.1	14.1	0.8
Experiment 2												
5	rat cubes	2 days	30.3	4.8	41.7	13.0	5.6	trace	4.8	13.0	10.4	0
6	safflower oil	2 days	29.3	2.6	37.9	12.5	7.7	1.2	7.3	13.7	15.0	0.16
7	γ-linolenic	2 days	24.0	3.1	34.1	13.5	7.5	1.3	10.2	14.8	17.7	0.16
8	rat cubes	14 days	28.8	4.2	30.2	14.0	10.8	trace	9.7	14.0	20.5	0
9	safflower oil	14 days	27.6	3.6	31.9	13.6	11.7	trace	8.5	13.6	20.2	0
10	γ-linolenic	14 days	28.0	3.7	31.0	13.0	10.2	trace	12.6	13.0	22.8	0

*, ** see Table 3

TABLE 9. Fatty acid composition of liver cholesterol esters

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3/20:4
Control			33.7**	9.9	9.7	18.8	8.8	trace	13.6	18.8	22.4	0
Fat-free			17.0	24.1	6.0	52.3	2.3	1.5	trace	53.8	2.3	∞
Experiment 1												
1	safflower oil	2 hrs.	26.1	14.8	7.5	43.5	2.6	1.3	1.3	44.8	3.9	1.0
2	γ-linolenic	2 hrs.	28.8	20.7	4.6	39.5	2.4	1.7	1.2	41.2	3.6	1.4
3	safflower oil	6 hrs.	27.4	15.2	4.7	38.3	6.9	1.3	1.4	39.6	8.3	1.0
4	γ-linolenic	6 hrs.	28.1	21.0	5.4	38.0	5.5	1.1	1.3	39.1	6.8	0.9
Experiment 2												
5	rat cubes	2 days	34.0	20.5	9.8	20.1	5.1	trace	2.3	20.1	7.4	0
6	safflower oil	2 days	34.4	16.4	13.4	23.3	6.7	trace	2.7	23.3	9.4	0
7	γ-linolenic	2 days	39.6	20.5	11.6	21.4	6.3	trace	4.5	21.4	10.8	0
8	rat cubes	14 days	32.6	10.1	26.8	19.6	5.5	trace	4.9	19.6	10.4	0
9	safflower oil	14 days	30.6	7.6	25.4	18.1	7.6	trace	7.1	18.1	14.7	0
10	γ-linolenic	14 days	30.6	12.2	23.0	20.6	6.1	trace	4.9	20.6	11.0	0

*, ** see Table 3

and arachidonic acids after 14 days remained below the levels in the control animals. By contrast, stearic acid, which in the deficient animals was not differed from that in the control animals, increased greatly after the supplemental feeding. There was no differences in fatty acid levels between rats fed safflower oil and those fed γ-linolenic triglyceride. In the case of triglyceride, the effect of the fat-deficient diet in the component fatty acids was not significant and a rate of the recovery was also slower. In the case of phos-

TABLE 10. Fatty acid composition of liver triglycerides

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Fat-free			26.4**	11.7	3.0	52.2	2.5	1.5	trace	53.7	2.5	∞
Experiment 1												
1	safflower oil	2 hrs.	33.2	11.9	3.3	43.9	4.6	0.6	trace	44.5	4.6	∞
2	γ-linolenic	2 hrs.	31.1	12.6	3.1	47.0	3.4	0.7	trace	47.7	3.4	∞
3	safflower oil	6 hrs.	29.1	7.3	2.3	45.7	10.6	0.6	0.6	46.3	11.2	1.0
4	γ-linolenic	6 hrs.	28.3	11.9	3.6	45.0	7.3	1.1	0.8	46.1	8.1	1.4
Experiment 2												
5	rat cubes	2 days	36.3	9.5	9.8	36.6	4.1	0.4	1.9	37.0	6.0	0.3
6	safflower oil	2 days	36.8	9.4	7.7	43.0	5.2	0.3	1.5	43.3	6.7	0.2
7	γ-linolenic	2 days	33.1	10.3	6.9	40.2	6.1	trace	1.1	40.2	7.2	0
8	rat cubes	14 days	30.2	6.7	12.9	31.9	14.5	trace	2.1	31.9	16.6	0
9	safflower oil	14 days	35.5	6.8	14.7	28.0	9.7	trace	4.0	28.0	13.7	0
10	γ-linolenic	14 days	31.1	8.2	14.1	29.7	11.2	trace	3.7	29.7	14.9	0

*, ** see Table 3

TABLE 11. Fatty acid composition of liver phospholipids

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Fat-free			18.4**	9.5	25.2	27.4	1.8	12.6	3.4	40.0	5.2	3.7
Experiment 1												
1	safflower oil	2 hrs.	24.9	5.9	34.4	23.8	2.9	5.6	2.2	29.4	5.1	2.6
2	γ-linolenic	2 hrs.	28.5	6.6	27.6	23.9	2.8	6.7	2.8	30.6	5.6	2.4
3	safflower oil	6 hrs.	25.3	5.6	30.9	18.7	9.8	4.1	4.7	22.8	14.5	0.9
4	γ-linolenic	6 hrs.	23.9	6.6	33.8	18.7	7.2	3.6	3.9	22.3	11.1	0.9
Experiment 2												
5	rat cubes	2 days	32.0	3.1	44.6	10.3	4.3	0.6	5.1	10.9	9.4	0.12
6	safflower oil	2 days	25.7	4.5	41.5	13.5	4.5	0.9	8.6	14.4	13.1	0.10
7	γ-linolenic	2 days	28.8	4.6	37.9	14.2	5.0	0.5	12.9	14.7	17.9	0.04
8	rat cubes	14 days	26.1	3.8	36.7	11.7	10.6	trace	10.3	11.7	20.9	0
9	safflower oil	14 days	31.4	4.5	34.0	13.7	6.4	trace	9.0	13.7	15.4	0
10	γ-linolenic	14 days	29.4	2.4	37.6	9.5	7.2	trace	13.2	9.5	20.4	0

*, ** see Table 3

pholipid, a rate of the recovery was intermediate, and γ-linolenic triglyceride was more effective than safflower oil for the recovery of arachidonic acid.

c) Intestinal mucosa The fatty acid profiles of lipid fractions of intestinal mucosa in the FEA-deficiency resembled those of plasma and liver lipid fractions.

The supplemental feeding resulted in a recovery of the fatty acid composition in all three fractions. However, the level of arachidonic acid in cholesterol esters after 14 days remained below the level in the control animals,

TABLE 12. Fatty acid composition of intestinal mucosa cholesterol esters

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3/20:4
Control			25.8**	9.4	14.8	26.6	7.8	trace	12.7	26.6	20.5	0
Fat-free			16.0	16.5	11.5	38.7	2.5	4.9	trace	43.6	2.5	∞
Experiment 1												
1	safflower oil	2 hrs.	23.5	10.0	15.8	26.7	16.4	2.6	1.3	29.3	17.7	2.0
2	γ-linolenic	2 hrs.	22.4	9.2	19.5	24.8	14.7	3.6	4.6	28.4	19.3	0.8
3	safflower oil	6 hrs.	29.5	10.5	13.7	24.5	19.5	1.0	0.5	25.5	20.0	2.0
4	γ-linolenic	6 hrs.	17.0	9.2	12.4	25.9	21.9	1.7	5.8	27.6	27.7	0.4
Experiment 2												
5	rat cubes	2 days	32.7	11.0	14.7	24.6	6.3	0.4	4.8	25.0	11.1	0.08
6	safflower oil	2 days	26.0	18.0	16.4	21.5	6.6	trace	4.8	21.5	11.4	0
7	γ-linolenic	2 days	22.4	16.0	19.4	21.9	6.9	trace	4.9	21.9	11.8	0
8	rat cubes	14 days	25.9	7.9	23.8	21.7	10.1	trace	5.8	21.7	15.9	0
9	safflower oil	14 days	27.5	13.6	12.4	23.6	11.9	trace	7.2	23.6	19.1	0
10	γ-linolenic	14 days	25.6	10.4	13.3	25.4	9.3	trace	5.7	25.4	15.0	0

*, ** see Table 3

TABLE 13. Fatty acid composition of intestinal mucosa triglycerides

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3/20:4
Fat-free			23.2**	17.5	2.8	51.1	1.8	1.1	trace	52.2	1.8	∞
Experiment 1												
1	safflower oil	2 hrs.	18.8	8.9	5.8	37.5	25.8	0.7	0.2	38.2	26.0	3.5
2	γ-linolenic	2 hrs.	21.9	9.2	5.5	41.7	16.4	1.4	0.3	43.1	16.7	4.7
3	safflower oil	6 hrs.	20.8	9.0	5.1	43.1	18.8	0.4	0.2	43.5	19.0	2.0
4	γ-linolenic	6 hrs.	27.6	8.1	4.8	45.1	11.4	0.3	0.2	45.4	11.6	1.5
Experiment 2												
5	rat cubes	2 days	25.8	13.2	5.5	47.6	4.3	0.2	1.2	47.8	5.5	0.1
6	safflower oil	2 days	27.8	12.8	8.4	41.9	5.5	trace	1.2	41.9	6.7	0
7	γ-linolenic	2 days	27.6	14.0	5.8	45.0	4.8	trace	0.9	45.0	5.7	0
8	rat cubes	14 days	27.0	14.9	3.0	43.0	8.9	trace	0.3	48.0	9.2	0
9	safflower oil	14 days	29.0	14.8	4.3	37.6	9.4	trace	1.5	37.6	10.9	0
10	γ-linolenic	14 days	28.5	13.7	2.1	42.7	10.0	tracs	0.4	42.7	10.4	0

*, ** see Table 3

and it is suggested that a rate of the recovery may be slow in the other lipid fractions. It was seen that the differences in arachidonic acid levels and the triene/tetraene ratio between rats fed safflower oil and those fed γ-linolenic triglyceride were present in cholesterol esters in 2 to 6 hours.

DISCUSSION

In the present study, newly weaned rats were fed a fat-free diet for 12

TABLE 14. Fatty acid composition of intestinal mucosa phospholipids

Group	Supplement	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4	18:1 + 20:3	18:2 + 20:4	20:3 / 20:4
Fat-free			22.2**	9.7	14.7	36.1	1.6	10.4	2.3	46.5	3.9	4.5
Experiment 1												
1	safflower oil	2 hrs.	27.0	5.6	19.4	22.4	18.9	2.0	0.8	24.4	19.7	2.5
2	γ-linolenic	2 hrs.	26.8	7.1	16.4	24.7	16.4	2.1	0.6	26.8	17.0	3.5
3	safflower oil	6 hrs.	24.0	7.5	21.1	22.7	15.2	1.1	0.8	23.8	16.0	1.4
4	γ-linolenic	6 hrs.	31.1	8.0	19.1	21.4	12.8	1.4	0.8	22.8	13.6	1.8
Experiment 2												
5	rat cubes	2 days	36.7	7.3	23.3	19.8	5.6	0.3	0.9	20.1	6.5	0.4
6	safflower oil	2 days	32.0	5.8	24.2	20.3	7.0	0.5	2.0	20.8	9.0	0.2
7	γ-linolenic	2 days	34.0	5.7	25.0	18.5	7.7	0.5	2.1	19.0	9.8	0.1
8	rat cubes	14 days	32.1	6.3	17.9	22.3	12.1	trace	5.3	22.3	17.4	0
9	safflower oil	14 days	32.4	7.3	14.0	24.6	11.5	trace	5.5	24.6	17.0	0
10	γ-linolenic	14 days	34.3	8.7	15.3	21.5	9.7	trace	5.3	21.5	15.0	0

*, ** see Table 3

weeks. The period of time required for the onset of the gross symtoms associated with an unsaturated fatty acid deficiency in the rat is generally considered to be of the order of 3~6 months[9)23)]. Klein and Johnson[24)] found that the unsaturated fatty acid contents of rat livers, however, were altered as early as 2 weeks after the animals were given the deficient diet. These changes developed rapidly during the first 6 weeks. Thereafter, there was a slower rate of change of the same nature which continued for 12~24 weeks.

In the present investigation, fatty acids were used in the form of triglycerides. Thomasson and Gottenbos[25)] reported that triglycerides were more efficiently utilized than their methyl or ethyl esters when administered simultaneously with linoleic acid to EFA-deficient rats. The histological changes observed in the liver and kidney of rats fed methyl esters of fatty acids indicated[26)] that preparations of methyl esters of fatty acids might be toxic when fed at high levels.

In the first experiment, rats were administered a single dose of 0.4 ml. of each supplement. Rahm and Holman[27)] indicated that 200 to 400 mg/day was a useful dietary range for a short-term feeding study using fat-deficient rats. The rat cubes contained approximately 5 per cent fat. Therefore, in the second experiment, rats were fed diets supplemented with either safflower oil or γ-linolenic triglyceride at a level of 5% (w/w).

After feeding the basal fat-free diet for 3 months, there was a definite decrease in plasma lipid fractions, particularly in plasma cholesterol and the accumulation of cholesteryl esters in the liver. Accumulation of cholesterol esters in the liver of EFA-deficient rats was observed by Beveridge and Lucas[28)]. Alfin-Slater, Aftergood, Wells and Deuel[29)] observed an increased concentration

of cholesterol in the liver and adrenal glands and a reduced cholesterol content in plasma of EFA-deficient male rats. Studies of the cholesterol biosynthesis from labeled acetate revealed[30] that there was a marked decrease in incorporation of radioactive acetate into cholesterol in the fat-deficient animals. In the animals supplemented with linoleate cholesterol synthesis was essentially normal. Therefore, it was suggested[31] that in the absence of polyunsaturated fatty acids, cholesterol was esterified with more saturated fatty acids which might not as easily enter a lipoprotein complex for the subsequent transport from the liver to plasma and to the other tissues.

Determinations of the fatty acid composition from various organs of EFA-deficient rats disclosed very pronounced changes in the fatty acid pattern in the deficient animals and the result was similar to those in other studies (Table 15). Lipids from the deficient animals were low in linoleic and arachidonic acids and high in palmitoleic, oleic and eicosatrienoic acids. Increased amounts of monoenoic fatty acids in the livers of fat-deficient rats have been reported by Mead[37]. A characteristic progressive decrease of dienoic acid has been demonstrated in rats[38]. However, the most striking change in the polyenoic acid pattern was the pronounced increase of the amount of trienoic acid in the tisse lipids. This was first demonstrated by Nunn and Smedley McLean[39] who isolated an eicosatrienoic acid as its hexabromide from the livers of fat-

TABLE 15. Fatty acid composition from various organs of EFA-deficient rats

	Reporter	Duration	16:0*	16:1	18:0	18:1	18:2	20:3	20:4
Total lipid									
Plasma	Walker, B. L.*** (32)	25 weeks	22.6**	9.1	9.4	33.9	2.4	15.6	2.0
	Sasaki, T.	3 months	18.0	13.2	11.6	38.5	3.4	12.0	1.5
Liver	Mohrhauer, H. *et al.* (33)	100 days	27.2	11.5	9.6	40.3	1.1	6.5	1.8
	Peluffo, R. O. *et al.* (34)	86 days	31.2	14.4	16.6	27.0	0.6	3.8	1.3
	〃	86 days	23.3	17.4	10.8	37.7	2.1	3.4	0.8
	Rahm. J. J. *et al.* (35)	90 days	27.7	12.1	11.7	30.9	1.4	9.9	3.6
	Walker, B. L.*** (32)	25 weeks	25.3	8.3	10.3	39.2	1.3	10.3	2.3
	Sasaki, T.	3 months	20.3	13.4	14.9	38.4	2.2	7.5	1.8
Triglyceride									
Plasma	Privett, O. S. *et al.* (36)	6 months	28.3	15.8	3.1	49.9	1.3	—	—
	Sasaki, T.	3 months	21.2	16.3	1.5	53.9	1.8	1.6	trace
Liver	Privett, O. S. *et al.* (38)	6 months	29.8	10.4	4.2	53.5	1.1	—	—
	Sasaki, T.	3 months	26.4	11.7	3.0	52.2	2.5	1.5	trace
Phospolipid									
Plasma	Privett, O. S. *et al.* (36)	6 months	23.9	7.6	16.7	28.3	1.5	19.8	1.7
	Sasaki, T.	3 months	18.5	8.8	22.1	29.5	1.9	15.9	1.2
Liver	Privett, O. S. *et al.* (36)	6 months	24.2	8.3	17.4	25.0	1.0	21.2	2.5
	Sasaki, T.	3 months	18.4	9.5	25.2	27.4	1.8	12.6	3.4

* see Table 3. ** Percentage of total fatty acids.
*** Fat-free diet+10% Hydrogenated coconut oil.

deficient rats. Eicosatrienoic acid from EFA-deficient rats mainly consist of eicosa-5, 8, 11-trienoic acid[33] which has been shown by Fulco and Mead[40] to be derived from oleic acid, and small amount of eicosa-7, 10, 13-trienoic acid[33] which has been suggested by Fulco and Mead[40][41] to may be derived from palmitoleic acid.

It was shown by Holman[42] that the ratio of trienoic to tetraenoic acids could be used to indicate the degree of EFA-deficiency in rats. The triene/tetraene ratio, or the ratio of 20 : 3 to 20 : 4, proved to be a useful parameter for describing linoleate metabolism. Above a triene/tetraene ratio of 0.4, normal conversion of linoleate to arachidonate did not appear to take place at a sufficient rate, and the 20 : 3 synthesis from oleate and palmitoleate became predominant[29]. In this study, the trine/tetraene ratio was above 0.4 in the EFA-deficiency and in 2 to 6 hours after the supplemental feeding, but in 2 to 14 days below 0.4 except for plasma triglyceride.

Regarding the enzymes involved in these *in vivo* interconversions, Mead[41] suggested that three enzyme systems were necessary to explain this phase of the metabolism of the unsaturated fatty acids. A polydehydrogenase, which adds double bonds toward the carboxy gronp of unsaturated fatty acids; an acyltransferase accomplishing the chin-lengthening process; and finally, the normal fatty acid degradation system operates on these unsaturated acids. Mohrhauer and Holman[43] observed that the order of affinity for the enzyme sites apparently depended on the degree of unsaturation of the acid (the order of affiinity was linolenate>linoleate>oleate). The appearance of C_{20} trienes in the animal in the absence of dietary linoleic acid could stem from the fact that with lower concentrations of the preferred substrate, linoleic acid, oleic acid might now complete favorably for the polydehydrogenase and be converted to the acid in question.

The supplemental feeding resulted in a rapid increase in linoleic and arachidonic acids and decrease in palmitoleic, oleic, and eicosatrienoic acids in all three fractions, particularly in plasma phospholipids and in liver cholesterol esters. The differences in arachidonic acid levels between rats fed safflower oil and those fed γ-linolenic triglyceride were present in plasma cholestrol esters and phospholipids and in liver phospholipids in 2 to 14 days, and in cholesterol esters of the intestinal mucosa in 2 to 6 hours. Dittmer and Hanahan[44] showed a greater incorporation of the absorbed C^{14} linoleic acid into phosphelidid than into the other lipid fractions. Studies with carboxyl-labeled linoleate by Mead and Fillerup[45] showed the more than half of the ingested linoleate appeared in the blood plasma as phospholipids half an hour later. This amount decreased only slightly later on. At the same time, a decrease in initial triglycerides and increase in sterol esters was observed. This rapid conversion of linoleate to phospholipids, and at a somewhat slower rate, to cholesterol esters, supposedly take place in the liver. Eicosatrienoic acid

formed from oleic acid in EFA-deficiency resembled arachidonic acid in its distribution in tissue lipids, which were found mainly in phospholipids and steryl esters[46)47)].

In reference to the histological sections of the livers, the liver sections from animals supplemented with safflower oil contained small fat globules. However, little fatty infiltration was found in the histological sections of rats fed γ-linolenic triglyceride.

The major fatty acid composition of γ-linolenic triglyceride was similar to that of the safflower oil except for γ-linolenic acid, and the former was more effective than the latter for the recovery of tissue lipids in the EFFA-deficient rats. Therefore, a smaller amount of pure γ-linolenic acid may be more effective and there remains a need for further investigation.

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