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Effect of Essential Amino Acid Deficiencies on the Liver Lipids of Rats

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The liver lipids of rats fed on the diets deficient in essential amino acid (EAA)* were compared with those of the corresponding pair-fed controls. The concentration and composition of hepatic lipids are modified specifically according to each EAA deficiency. In general, feeding the diets deficient in EAA resulted in the decrease in the concentration of hepatic phospholipid. This was most remarkable in Lys, Val or Leu (and perhaps Phe or Trp) deficiency. The decrease in the concentration of hepatic phosphatidylethanolamine (PE) was more predominant than that of phosphatidylcholine (PC). The concentration of triglyceride increased in rats fed on the diets devoid of each EAA except Val. The response of cholesterol concentration was scattering. In the liver and its microsomal PC percentage of linoleate increased in Ileu, Thr, Met or Leu (and Phe) deficiency and that of arachidonate decreased in Met, Leu or Val (Phe) deficiency, respectively. Consequently, the ratio of arachidonate to linoleate reduced in Thr, Ileu or Met deficiency. These observations, together with those demonstrated previously, indicate that the quantity and the quality of dietary protein significantly influence on the metabolism of fatty acids, particularly linoleate and arachidonate.

INTRODUCTION

Administration of a low protein diet to rat results in changes in the concentration as well as the composition of hepatic phospholipids. The concentration of phospholipids decreases and percentage of linoleate increases at the expense of arachidonate in PC. A close relationship between the extent of desaturation of linoleate and the dietary protein levels has been reported (Maeda *et al.*, 1973).

Obviously, protein nutrition should be understood not only at the quantitative but qualitative standpoints. Dietary deficiency (Hall *et al.*, 1952) and imbalance (Sidransky and Farber, 1958) of EAA produce accumulation of liver lipids. As to the mechanism of the fatty infiltration, Yoshida and Harper (1960) indicated that the increased hepatic lipogenesis appeared to be apparently responsible for the production of fatty liver due to amino acid imbalance. Lyman *et al.* (1950) and Wilfred and Sekhara (1969) obtained evidence supporting that damage to

* Following abbreviations are used in this paper. EAA: essential amino acid, PC: phosphatidylcholine, PE : phosphatidylethanolamine, SPH : sphingomyelin, LPC : lysophosphatidylcholine, TG : triglyceride, FFA : free fatty acid

lipoprotein synthesis in the liver, hence depression of release of the fatty components to blood stream, was not the important factor functioning to the accumulation of hepatic lipids.

By virtue of the tissue or cellular specificity in the fatty acid composition and of the molecular structure of the glycerides, each fatty acid appears to be metabolized very specifically (Hanahan and Blomstrand, 1956, Land, 1958). Thus, analyses of the fatty components in tissues of animals are of useful approach to the determination of these specificities. Only partial, but not systematic, information has been available on this problem (Sidransky and Virney, 1964).

This communication deals with the effect of EAA deficiencies on the concentration and composition of lipid components in rat liver and its subcellular fractions with special emphasis to those of phospholipids.

MATERIALS AND METHODS

Animals and diets

Male rats of a Wistar strain were housed in individual cage in an air-conditioned room at 22-24 °C. The composition of the experimental diet is shown in Table 1. The amino acid mixture according to Rama Rao *et al.* (1961) was used. One group of the animals was fed on the diets lacking one EAA and the other was pair-fed on the complete diet. Omitted EAA was replaced by the same amount of glutamic acid. The present paper was composed of three separate experiments.

Table 1. Composition of basal diets.

Components	%
Sucrose	70.85
Amino acid mixture ¹⁾	15.0
Corn oil ²⁾	5.0
Mineral mixture ³⁾	4.0
Vitamin mixture ³⁾⁴⁾	1.0
Choline chloride	0.15
Cellulose	4.0

1) Amino acid mixture according to Rama Rao (1961).

2) Contains the following percentages of fatty acids: 14:0 ; 0.2, 16:0 ; 9.6, 16:1 ; 0.6, 18:0 ; 1.8, 18:1 ; 25.6, 18:2 ; 61.6, 18:3 ; 0.6.

3) Obtained from the Tanabe Amino Acid Research Foundation.

4) Vitamin A 2400IU, vitamin D₂ 2001U and vitamin E 10mg per 100g diet were added to the mixture.

Lipid analysis

The rats were killed by decapitation 2 weeks after the feeding and the livers were quickly removed. Liver microsomes and mitochondria were fractionated according to Schneider and Hogeboom (1950) in an ice cold solution of 0.25 M sucrose containing 0.001 M EDTA. Lipids were extracted according to Folch *et al.* (1957). Total lipids, cholesterol and lipid phosphorus were determined by the method of Bragdon (1960), Sperry and Webb (1950) and Gomori

(1942), respectively. The distribution of the phospholipid components was determined after separating them by thin-layer chromatography (TLC) (Rouser *et al.*, 1966). Triglyceride content was calculated by subtracting phospholipid and cholesterol from total lipids. Fractionation of lipids by TLC and determination of fatty acid composition by gas-liquid chromatography were performed as described elsewhere (Sugano *et al.*, 1969).

RESULTS

Food intake and growth

Table 2 shows that daily food intake was the minimum (4.8 g) in Ileu deficiency, though the differences of initial body weights of rats used in each experiment should be taken into consideration. The animals in Lys or Trp deficiency ingested above 8 g daily and this may indicate that the requirement of these two EAA for growth diminishes accompanying maturity of rats. When compared with the pair-fed controls, the loss of body weight was remarkable in Thr or Ileu (and Phe or Trp) deficiency.

Table 2. Body weight and food intake.^{a)}

Expt. no.	Diets		Body weight (g)		Changes of body weight (g/2 weeks)	Average of food intake (g/day)
			Initial	Final		
	Thr deficient	(4)	144 ± 10	109 ± 5	-3.5	6.7
	Pair	(4)	142 ± 7	145 ± 8	+3	6.7
	Ileu deficient	(4)	141 ± 6	92 ± 9	-4.9	4.8
	Pair	(4)	141 ± 6	128 ± 5	-1.3	4.8
		(4)				
	Met deficient	(4)	165 ± 2	138 ± 1	-2.7	7.3
	Pair	(4)	161 ± 4	135 ± 3	-2.6	7.3
	Lys deficient	(4)	164 ± 4	139 ± 4	-2.5	8.2
Pair	(4)	159 ± 3	135 ± 3	-2.4	8.2	
3	Leu deficient	(3)	200 ± 20	165 ± 17	-3.5	7.3
	Pair	(3)	199 ± 21	164 ± 15	-3.5	7.3
	Val deficient	(3)	198 ± 23	154 ± 18	-4.4	6.4
	Pair	(3)	194 ± 22	146 ± 15	-4.8	6.4
	Phe deficient	(3)	192 ± 25	154 ± 16	-4.8	7.1
	Pair	(2)	183	150	-3.3	7.1
	Trp deficient	(3)	189 ± 27	157 ± 23	-3.2	8.7
	Pair	(2)	181	166	-1.5	8.7

a) Values are the mean ± SE. Figures in parentheses represent the number of rats.

Hepatic lipid components

As shown in Table 3, the relative liver weight was apparently similar between the deficient and control rats, except in Thr or Ileu deficiency where the weight considerably decreased.

Hepatic total lipids tended to increase in rats fed Thr or Ileu (and Phe) deficient diets and to decrease in Leu or Val deficiency. On feeding the diets

Table 3. Liver lipid composition.^{a)}

Expt. no.	Group	Liver weight (% of body weight)	Total lipids (mg/g liver)	Phospholipids (mg/g liver)	TG (mg/g liver)	Cholesterol (mg/g liver)		Ester %
						Total	Free	
1	Thr deficient (4)	4.4 ± 0.1**	40.8 ± 5.0	28.1 ± 1.6	10.1	2.56 ± 0.1	2.03 ± 0.1	20.5
	Pair (4)	6.8 ± 0.3	35.8 ± 3.2	30.1 ± 0.1	3.4	2.35 ± 0.1	1.96 ± 0.1	16.5
	Ileu deficient (4)	3.7 ± 0.6*	39.6 ± 4.2	26.9 ± 1.9	10.1	2.61 ± 0.2	2.26 ± 0.2	13.9
	Pair (4)	5.6 ± 0.3	33.1 ± 0.6	30.8 ± 0.4	trace	2.34 ± 0.1	1.88 ± 0.1	19.6
2	Met deficient (4)	4.1 ± 0.3	33.8 ± 3.1	26.7 ± 1.4	5.0	2.18 ± 0.1	1.81 ± 0.1	16.9
	Pair (4)	3.7 ± 0.4	35.6 ± 2.5	30.1 ± 1.9	3.1	2.42 ± 0.2	2.15 ± 0.2	11.3
	Lys deficient (4)	4.0 ± 0.2	37.8 ± 8.0	25.5 ± 0.6*	8.7	2.24 ± 0.1	1.86 ± 0.1	17.2
	Pair (4)	3.8 ± 0.3	39.8 ± 3.1	30.7 ± 1.5	6.6	2.47 ± 0.2	2.16 ± 0.1	12.7
3	Leu deficient (3)	3.9 ± 0.2	34.6 ± 0.9	27.9 ± 0.6*	4.4	2.37 ± 0.1**	2.09 ± 0.1*	11.7
	Pair (3)	3.3 ± 0.1	40.9 ± 2.1	35.5 ± 0.5	2.3	3.14 ± 0.1	2.67 ± 0.1	14.8
	Val deficient (3)	3.6 ± 0.1	30.9 ± 2.4	26.5 ± 1.2*	2.0	2.45 ± 0.1**	2.27 ± 0.1*	7.3
	Pair (3)	3.1 ± 0.1	43.1 ± 4.0	37.7 ± 1.9	4.4	3.29 ± 0.1	3.00 ± 0.2	8.8
	Phe deficient (3)	3.5 ± 0.2	49.7 ± 3.9	29.9 ± 0.3	17.2	2.62 ± 0.1	2.31 ± 0.1	11.9
	Pair (2)	3.3	42.8	37.3	2.2	3.38	2.99	11.8
	Trp deficient (3)	3.7 ± 0.2	40.8 ± 2.2	29.1 ± 1.8	9.0	2.71 ± 0.2	2.24 ± 0.2	17.4
	Pair (2)	3.2	44.6	36.2	5.1	3.29	2.74	17.0

a) Figures in parentheses represent the number of rats.

* P < 0.05. ** P < 0.01.

Table 4. The composition of liver phospholipids (%).

Fraction	Leu		Val		Phe		Trp	
	deficient (3)	Pair (3)	deficient (3)	Pair (3)	deficient (3)	Pair (2)	deficient (3)	Pair (2)
PE	27.4 ± 0.5	29.4 ± 1.0	25.4 ± 0.7	30.0 ± 0.9*	27.2 ± 0.4	30.2	27.5 ± 0.6	31.5
FC	50.0 ± 0.6	47.4 ± 0.7	49.9 ± 1.8	46.2 ± 1.2	48.9 ± 0.2	47.5	51.2 ± 0.9	45.3
SPH	6.3 ± 0.9	4.9 ± 0.6	5.4 ± 0.6	5.2 ± 0.1	4.7 ± 0.4	4.9	4.7 ± 0.2	5.2
LPC	10.7 ± 1.0	11.9 ± 0.1	11.6 ± 0.2	10.8 ± 0.5	12.3 ± 0.5	11.7	10.9 ± 0.2	11.2
Others	5.6 ± 0.8	6.4 ± 0.2	7.7 ± 3.0	7.8 ± 1.7	6.4 ± 1.3	5.8	5.7 ± 0.2	6.8

* P < 0.05.

Table 5. Fatty acid composition of liver phosphatidylcholine.

Expt. no.	Group	Fatty acids (wt. %)						
		16:0	16:1	18:0	18:1	18:2	20:4	22:6
1	Thr deficient (4)	29.3 ± 0.9	2.1 ± 0.3	18.1 ± 1.0	10.1 ± 0.5	15.1 ± 0.6	19.5 ± 0.6	3.5 ± 0.1**
	Pair (4)	31.5 ± 0.9	2.9 ± 0.4	17.0 ± 1.2	10.7 ± 0.7	13.5 ± 0.5	20.2 ± 1.0	2.0 ± 0.3
	Ileu deficient (4)	26.7 ± 2.3	2.8 ± 0.5*	18.8 ± 1.5	8.9 ± 0.5**	17.3 ± 1.5*	18.7 ± 3.2	4.5 ± 0.7*
	Pair (4)	29.3 ± 1.0	4.6 ± 0.4	15.6 ± 1.0	13.7 ± 0.9	12.8 ± 0.7	18.7 ± 0.5	2.3 ± 0.3
2	Met deficient (4)	25.3 ± 2.4	2.6 ± 0.3	15.6 ± 1.0	10.9 ± 1.0	16.0 ± 0.5	23.2 ± 1.6*	4.0 ± 0.5
	Pair (4)	22.6 ± 2.1	2.2 ± 0.2	16.1 ± 0.3	8.6 ± 0.3	14.6 ± 0.7	28.2 ± 0.6	6.2 ± 1.0
	Lys deficient (4)	24.2 ± 1.5	2.1 ± 0.3	18.6 ± 1.1	8.3 ± 0.8	11.9 ± 1.6	27.8 ± 2.0	5.3 ± 0.5
	Pair (4)	24.1 ± 0.3	2.1 ± 0.1	17.5 ± 0.7	8.7 ± 0.7	12.6 ± 0.6	27.0 ± 1.0	6.1 ± 0.5
3	Leu deficient (3)	23.1 ± 0.4	2.1 ± 0.3	19.8 ± 0.6	9.5 ± 0.3	14.9 ± 0.3	24.4 ± 1.0*	4.6 ± 0.3
	Pair (3)	22.8 ± 1.3	2.4 ± 0.4	16.4 ± 1.1	11.1 ± 0.6	13.9 ± 0.5	28.7 ± 1.1	3.4 ± 1.2
	Val deficient (3)	26.1 ± 0.4**	2.4 ± 0.4	18.0 ± 1.2	9.2 ± 0.3	12.7 ± 1.0	25.5 ± 0.5**	4.7 ± 0.3*
	Pair (3)	22.9 ± 0.3	2.5 ± 0.2	17.2 ± 0.3	11.1 ± 0.4	13.3 ± 0.8	28.5 ± 0.1	3.1 ± 0.3
	Phe deficient (3)	24.0 ± 0.6	2.2 ± 0.4	19.6 ± 0.7	10.7 ± 3.4	16.6 ± 0.8	22.0 ± 0.6	3.3 ± 0.1
	Pair (2)	23.2	2.5	16.9	10.4	13.6	28.7	3.5
	Trp deficient (3)	24.0 ± 1.0	1.7 ± 0.3	21.9 ± 0.9	9.3 ± 1.0	10.1 ± 0.2	27.0 ± 1.2	4.8 ± 0.2
	Pair (2)	23.4	2.4	19.8	8.7	12.6	30.0	2.0

* P < 0.05.

** P < 0.01.

deficient in EAA resulted in general in the decrease of phospholipids, this being particularly remarkable in Lys, Leu or Val (and perhaps Phe or Trp) deficiency. In these groups of rats the decrease in the proportion of PE and thus the increase in that of PC was generally observed (Table 4). However, the net amounts of PC in whole liver decreased in these deficient groups.

The amount of TG obtained by calculation increased in each EAA deficiency except for Val and the extent of accumulation was remarkable in the rats deficient in Thr or Ileu (and perhaps Phe or Trp). An unusual low concentration of TG in the controls can be ascribed to the limited quantity of food ingested. The concentration of cholesterol decreased in Leu or Val (and Phe) deficiency and this was mainly due to the decrease of the free form.

Fatty acid composition

Hepatic PC : The fatty acid composition was altered specifically according to each deficient EAA and was much predominant with the polyunsaturated acids (Table 5). Met, Leu or Val (and Phe) deficiency caused the decreases in percentage of arachidonate. In some deficient groups docosahexaenoate increased. In addition to these changes oleate decreased and linoleate increased in Ileu deficiency, and palmitate increased in Val deficiency.

Microsomal PC : The fatty acid composition of microsomal PC was similarly modified as was in the whole liver PC. The relative ratio of major fatty acids is summarized in Table 6. The ratio of palmitoleate to palmitate decreased in Thr or Ileu deficiency and that of oleate to stearate decreased only in Ileu deficiency. The ratio of arachidonate to linoleate decreased in Thr, Ileu or Met deficiency, this being mainly due to the increase in linoleate with concomitant decrease in arachidonate. Although similar changes in the ratio of the polyunsaturated acid were observed in rats fed on diets devoid of Leu or Phe, there was limitation in evaluating the results because these were determined on the pooled samples.

The ratio of arachidonate to linoleate also decreased, but rather to the lesser degree compared with that of microsomal PC, in the mitochondria from Ileu, Met or Leu deficiency (Table 7). On the contrary, the ratio tended to increase in mitochondrial PC of Lys (and Trp) deficiency and in microsomal PC of Trp deficiency. These results may be related to the amounts of food intake (cf. Table 2).

DISCUSSION

There has been considerable information about the morphological and compositional changes in the liver of rats fed on diets deficient or imbalanced in EAA. Accumulation of hepatic lipids in rats fed on diets devoid of EAA has been reported (Sidransky and Farber, 1958; Lyman *et al.*, 1964). Imbalance in dietary amino acids causes enhancement of lipogenesis (Yoshida and Harper, 1960) and the increase in hepatic TG (Ogura *et al.*, 1965). These observations support the view that the effect of dietary protein on the hepatic lipids should be examined at the quantitative as well as qualitative standpoints.

Table 6. The ratio of fatty acids of liver microsomal phosphatidylcholine.^{a)}

Expt no.	Group		16:1/16:0	18:1/18:0	20:4/18:2
1	Thr deficient	(4)	0.065 ± 0.001*	0.613 ± 0.033	0.630 ± 0.100*
	Pair	(4)	0.080 ± 0.001	0.601 ± 0.090	1.110 ± 0.095
	Ileu deficient	(4)	0.074 ± 0.014*	0.464 ± 0.051*	0.650 ± 0.100"
	Pair	(4)	0.129 ± 0.006	0.843 ± 0.017	1.126 ± 0.130
2	Met deficient	(4)	0.117 ± 0.028	1.036 ± 0.179	0.726 ± 0.115"
	Pair	(4)	0.076 ± 0.004	0.638 ± 0.027	1.220 ± 0.089
	Lys deficient	(4)	0.115 ± 0.006*	0.610 ± 0.062	1.458 ± 0.147
	Pair	(4)	0.070 ± 0.008	0.593 ± 0.200	1.470 ± 0.079
3	Leu deficient	(3)	0.097	0.466	1.68
	Pair	(3)	0.103	0.597	2.13
	Val deficient	(3)	0.102	0.485	2.12
	Pair	(3)	0.099	0.6'6	2.01
	Phe deficient	(3)	0.093	0.483	1.51
	Pair	(2)	0.113	0.615	2.01
	Trp deficient	(3)	0.075	0.410	2.71
	Pair	(2)	0.083	0.439	2.25

a) Data in experiments 1 and 2 are the mean ± SE and in experiment 3 are the means of pooled samples.

* P < 0.05.

Table 7. The ratio of fatty acids of liver mitochondrial phosphatidylcholine.^{a)}

Expt. no.	Group		16:1/16:0	18:1/18:0	20:4/18:2
1	Thr deficient	(4)	0.069 ± 0.009	0.850 ± 0.044	0.340 ± 0.064
	Pair	(4)	0.092 ± 0.014	0.946 ± 0.168	0.380 ± 0.104
	Ileu deficient	(4)	0.114 ± 0.021	0.758 ± 0.079*	0.578 ± 0.480
	Pair	(4)	0.166 ± 0.011	1.060 ± 0.037	0.803 ± 0.158
2	Met deficient	(4)	0.105 ± 0.014	0.809 ± 0.115	1.186 ± 0.089
	Pair	(4)	0.101 ± 0.014	0.624 ± 0.027	1.370 ± 0.095
	Lys deficient	(4)	0.109 ± 0.010	0.542 ± 0.058	1.808 ± 0.158
	Pair	(4)	0.095 ± 0.010	0.721 ± 0.066	1.345 ± 0.169
3	Leu deficient	(3)	0.095	0.511	1.72
	Pair	(3)	0.115	0.800	2.09
	Val deficient	(3)	0.094	0.566	2.04
	Pair	(3)	0.098	0.628	2.17
	Phe deficient	(3)	0.093	0.601	1.37
	Pair	(2)	0.119	0.704	2.02
	Trp deficient	(3)	0.048	0.337	2.67
	Pair	(2)	0.088	0.544	2.31

a) See table 6.

* P < 0.05.

The present study showed that the concentration of liver phospholipid decreased in each EAA deficiency, this being remarkable with PE (see Table 4), which in general contains polyunsaturated acids in the higher percentage compared with PC. Met, Leu or Val (and Phe) deficiency caused the decreases in percentage of arachidonate in liver PC. Viviani *et al.* (1964) have reported the

decreases in linoleate, arachidonate and docosahexaenoate in carcass phospholipid of rats fed on the diets deficient in Lys or Thr. In our experiments docosahexaenoate in liver PC increased in rats fed on diets deprived of Thr, Ileu or Val (and Trp). Arachidonate decreased in Met, Leu or Val (and Phe) deficiency and linoleate increased in Ileu deficiency. The discrepancies may partly be attributable to the difference among the specimens analyzed and partly to that in the pattern and quantity of dietary amino acids.

Innami *et al.* (1967) have demonstrated that arachidonate, in the form of the esterified fatty acids, in serum from rats deficient in Lys, Met, Trp, His, Phe or Val increased. This was not consistent with the present data on hepatic PC. Moreover, the unpublished data on the phospholipid and cholesterol ester fractions in plasma obtained from rats in this experiment does not necessarily agreed with those of the above authors.

The reduction of the concentration of arachidonate and that of the ratio of arachidonate to linoleate in hepatic PC may indicate that 6-desaturation reaction of the acyl moieties is suppressed and the pool size of arachidonate is significantly modified by feeding on the EAA deficient diets. In facts, Inkpen *et al.* (1969) and Peluffo *et al.* (1969, 1971) have shown that the levels of the dietary protein significantly influence the extent of the desaturation of linoleate. However, the mechanism by which desaturation is influenced by the level as well as the quality of the dietary protein remained obscure. Some of the dietary amino acids directly effect the production and secretion of several hormones (Munro *et al.*, 1963). Administration of estradiol to the rats reduces the quantity of the diene fraction and increases stearate and arachidonate in hepatic PC (Lyman *et al.*, 1963). It is, therefore, presumable that the modification of hormonal balance due to dietary protein malnutrition is partly responsible for the changes in the desaturation reaction. More detailed studies are required for further understanding of this problem.

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