Effect of Essential Amino Acid Deficiency on Lecithin - Cholesterol Acyltransferase in Rat Plasma : Dietary Factors Influencing Lecithin - Cholestetol Acyltransferase in Rat Plasma (Part 3)

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Effect of Essential Amino Acid Deficiency on Lecithin: Cholesterol Acyltransferase in Rat Plasma

Dietary Factors Influencing Lecithin : Cholestetol Acyltransferase in Rat Plasma (Part 3)

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The concentration of lipid components and lecithin : cholesterol acyltransferase (LCAT) activity in plasma of male rats fed on diets deficient in one of eight essential amino acids (EAA) were compared with those of the controls in two types of the experiments. Ingestion of the deficient diet for 14 days resulted in disorganized changes in the concentration of phospholipid and cholesterol with respect to EAA tested. The LCAT activity in general decreased by the feeding of EAA deficient diet, the decrease being significant in Met, Lys, Leu, Val or Trp deficiency. Changes in fatty acid specificity was different regarding each EAA deficiency.

In rats refed on diet deficient in EAA for 2 days after 2 day-fasting, the concentration of phospholipid and cholesterol tended generally to decrease. The LCAT activity tended to increase on refeeding the diet deficient in EAA and it was significant in Ileu or Thr deficiency. Relative formation of the monounsaturated fatty acid ester was decreased by refeeding EAA deficient diet.

Judging from fatty acid changes, decreased formation of monounsaturated ester of cholesterol was due mainly to that of the palmitoleate ester but not of the oleate ester.

No direct correlation could not be demonstrated between the changes in these two enzymatic characteristics and the nutritional status of the rats. The results indicate that each EAA has different effects on the lipid composition and the activity and fatty acid specificity of LCAT in rat plasma.

INTRODUCTION

Lecithin: Cholesterol acyltransferase (LCAT) in plasma plays an important role in formation of the bulk of cholesterol ester (Glomset, 1968 ; Chevallier et *al.*, 1971). It is also suggested that LCAT is closely related to the removal of triglyceride from blood stream (Schumaker and Adams, 1970). The activity of this enzyme appears to be influenced by the levels of the substrates, unesterified cholesterol and lecithin (Glomset, 1968 ; Monger and Nestel, 1967 ; Nichols and Gong, 1971 ; Raz, 1971). Fatty acid specificity of the enzyme is also presumably influenced by the fatty acid composition of lecithin (Swell and Law, 1968 ;

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Sugano *et al.*, 1969a ; Sugano, 1971). Thus, the change in the physiological status of animals appears to effect directly on the activity and fatty acid specificity of LCAT (Swell and Law, 1968 ; Sugano *et al.*, 1969a ; Sugano, 1971).

Only limited data are available pertaining to the effects of dietary nutrients on LCAT (Sugano and Portman, 1965 ; Well and Hogan, 1968 ; Hori *et al.*, 1973b). Nath and Singh (1970) reported that in rat the LCAT activity was influenced by the levels of dietary protein. However, according to our similar types of the experiments the activity was not influenced by the levels of dietary protein (Hori *et al.*, 1973a) and only the enzymatic specificity for relative formation of the each ester was altered by increasing the dietary protein levels. In addition to these dietary factors, the feeding habit of the animals also influences the activity and specificity of LCAT. When rats were refed for 2 days after fasting for 2 days, there were differences in the activity of LCAT among the rats refed each one of the diets containing 8, 20 or 32 % casein. Formation of choleterol arachidonate, however, enhanced by increasing levels of the protein (Hori *et al.* 1973a). Under the similar fasting-refeeding condition, the activity increased after refeeding the fat and carbohydrate free diet (93.5 % casein) compared to that after refeeding the fat and protein free diet (93.5% glucose) (Unpublished data). These observations also indicate that the activity and specificity are influenced by the levels of dietary protein.

On the basis of the effect of dietary protein on the concentration of plasma cholesterol components, it is obviously apparent that the quantity as well as the quality of this nutrient have a close relationship to the metabolism of cholesterol ester. The fact that proteins from different sources have different effects on the concentration of plasma cholesterol (Portman and Stare, 1959 ; McGregor, 1971) suggests that the differences in the amino acid composition influence differently on the metabolism of cholesterol. Feeding diets deficient in essential amino acid (EAA) or imbalanced in amino acid composition to rats frequently induced abnormalities in the metabolism of hepatic and plasma lipids (Yoshida and Harper,1960 ; Lyman *et al.*, 1964 ; Innami *et al.*, *1967* ; Yoshida *et al.*, 1967; Maeda *et al,, 1973).* Direct evidence to the cause of this alteration has not been clearly proved yet.

In the present study the effects of EAA deficiency on the activity and fatty acid specificity of plasma LCAT in rats were compared with those of the corresponding controls.

EXPERIMENTAL

Animals and diets

Male Wistar rats, which were obtained from the Experimental Animal Institute, Kyushu University School of Medicine and were maintained on a commercial pellet ration (Oriental Rat Chow, type NMF), were fed on the diets deficient in one of eight EAA for 14 days (Expts. l-3) or were fed on these diets for 2 days after 2 day fasting (Expt. 4). A basal diet consisted of the composition shown in Table 1. In the deficient diet, EAA omitted was replaced by the similar quantities of glutamic acid. Pair-feeding (Expts. l-3) and *ad libitum*-feeding (Expt. 4) rats were served as the controls. All rats were freely access-

ed water.

Table 1. Composition of diet.

Ingredients	%
Amino acid mixture"	15
Sucrose	70.85
Corn oil[2]	5
Mineral mixture[3]	a
Vitamin mixture[3]	1
Cellulose	4
Choline chloride	0.15

1) Products of Ajinomoto Co., Inc. Mixed acccording to Rama Rao *et al. (1961).*
2) Contained vitamin A, 2400 IU; vitamin D, 200 IU ; and vitamin E, 10 mg per 100g diet.
3) Purchased from Tanabe Amino Acid Research Foundation.

Assay of the activity and fatty acid specificity of LCAT

Rats were sacrificed by decapitation and blood was collected in tubes containing heparin. The methods adapted for the measurement of the activity and fatty acid specificity were those described in detail by Sugano (1971). In brief, 0.5 ml of fresh plasma was incubated with 0.05 μCi of cholesterol-4-^{14}C dissolved in 0.05 ml of 0.5 % Tween 20 in saline at 37°C for 3 hrs. Cholesterol-4-^{14}C (specific activity, 49.9 mCi/mM) was purchased from Daiichi Pure Chemicals Co., Ltd. By this method, it is capable of measuring the apparent initial rate of the activity. Individual cholesterol esters were separated by thin-layer chromatography (Sugano, 1971). LCAT activity was expressed as percentage of cholesterol -4-^{14}C which had been esterified during incubation. Fatty acid specificity was expressed as percentage of the radioactivity distribution in newly formed individual cholesterol esters.

Determinations of lipids

After extraction of plasma lipids with chloroform-methanol (2 :1, v/v), cholesterol and lipid-phosphorous were determined as described by the methods of Sperry and Webb (1950) and Gomori (1942), respectively. Gas-liquid chromatography of fatty acids were performed as previously described (Sugano et al., **1969b).**

RESULTS

Expts. l-3 : Feeding on EAA deficient diet for 14 days

Growth and food intake

Table 2 shows body weight change and food intake. Although direct comparisons of the data of each experiment are difficult because of the differences

Table 2. Body weight and average food intake.

Groups	Body weight[1] Initial g	Body weight[1] Final g	Body weight[1] Change g	Food intake g/day
Expt. 1				
Thr —[2] (4)[3]	144 ± 10	109 ± 5	-35	6.7
Thr +[2] (4)	142 ± 7	145 ± a	+ 3	6.7
Ileu — (4)	141 ± 6	92 ± 9	—49	4.8
Ileu + (4)	141 ± 6	128 ± 5	-13	4.8
Expt. 2				
Met — (4)	165 ± 2	140 ± 1		7.3
Met + (4)	161 ± 4	135 ± 3	-25 -26	7.3
Lys — (4)	164 ± 4	139 ± 4	-25	8.2
Lys + (4)	159 ± 3	135 ± 3	-24	a. 2
Expt. 3				
Leu — (3)	200 ± 20	165 ± 17	-35	7.3
Leu + (3)	199 ± 21	164 ± 15	-35	7.3
Val — (3)	198 ± 23	154 ± 18	-44	6.4
Val + (3)	194 ± 22	146 ± 15	-48	6.4
Phe — (3)	192 ± 25	154 ± 16	-38	7.1
Phe + (2)	183	150	-33	7.1
Trp — (3)	189 ± 16	157 ± 23	-32	8.7
Trp + (2)	181	166	-15	8.7

1) Mean ± SE.
2) —: Deficient, +: Supplemented, pair-feeding.
3) Numbers of rats in parentheses.

Table 3. Plasma lipid concentration[1].

Groups	Phospholipid μg/ml	Cholesterol Total μg/ml	Cholesterol Free μg/ml	Cholesterol Ester %
Expt. 1				
Thr — (4)	1535	823	275	66.6
Thr + (4)	1359	981	283	71.2
Ileu — (4)	1715	1019	366	64.1
Ileu + (4)	1503	855	298	65. 2
Expt. 2				
Met — (4)	1845	746	274	63.3
Met + (4)				
Lys — (4)	1350 1630	692 767	198 254	71.4 66.8
Lys + (4)	1295	539	190	64.9
Expt. 3				
Leu — (3)	1700	909	292	67.9
Leu + (3)	1537	854	264	69.0
Val — (3)	1490	876	282	67.8
Val + (3)	1367	864	279	67. 7
Phe — (3)	1792	851	287	66.3
Phe + (2)	1298	795	237	70.2
Trp — (3)	1213	633	203	67.9
Trp + (2)	1322	683	211	69.9

1) Values obtained from pooled plasma. See also Table 2.

in the initial body weights of the rats used, there was little difference in the body weight changes between the deficient and control groups except Thr, Ileu or Trp deficiency.

Lipid concentration

The concentration of phospholipid and cholesterol in plasma is shown in Table 3. The data were obtained from pooled plasma due to the limited quantity of the specimens available. Except Trp deficiency, the concentration of phospholipid tended to increase in all of the deficient groups and this was most predominant in Met., Lys or Phe deficiency. Changes in the concentration of cholesterol due to deficiency were scattering depending on EAA tested ; the decrease of cholesterol in Thr deficiency and the increase in Ileu or Lys deficiency, the changes being due mainly to those in the esterified form. Unesterified cholesterol remained unchanged in each EAA deficiency.

LCAT activity and specificity

Figure 1 illustrates the LCAT activity *in vitro*. The activity tended to decrease in Met, Lys, Leu, Val or Trp deficiency.

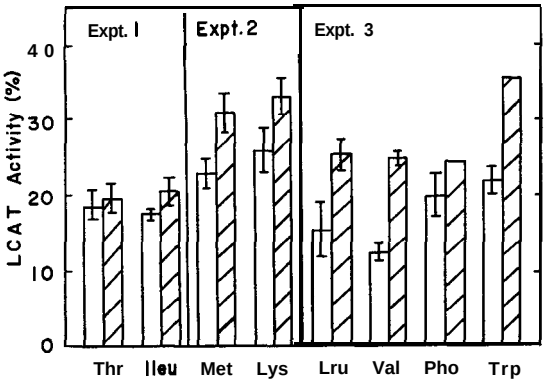

Fig. 1. Activity of plasma LCAT. The activity is expressed as percentage of cholesterol-4-W which was esterified during *incubation* at *37°C* for 3 hrs. ▭: Deficient, ▨: Supplemented. See also Table 2.

Table 4 represents the distribution of the radioactivity among the individual esters formed by LCAT (fatty acid specificity of LCAT). Except for Lys deficiency, there were considerable alterations in fatty acid specificity after feeding diet deficient in EAA. Remarkable changes were observed in Ileu deficiency in which formation of the oleate ester decreased and that of the linoleate increased. Thr deficiency decreased relative formation of cholesterol oleate. The change in fatty acid specificity in Met deficiency was shown as the increase in formation of cholesterol linoleate at the expense of the arachidonate. In Leu or Val (and perhaps in Trp) deficiency, the proportion of cholesterol palmitate and pentaenoate tended to increase. Formation of the arachidonate ester was depressed by Val deficiency.

Table 4. Fatty acid specificity of plasma LCAT[1].

Groups	Fatty acid specificity (%)				
	Palmitate	Oleate	Linoleate	Arachidonate	Pentaenoate
Expt. 1					
Thr — (4)	10.7 10.2 ± 6.2 0.6	13.5 7. Y ± 0.2*	24.4 23.5 ± 0.9 1.3	53.2 50.5 ± 0.9 1.4	3.8 2.3 ± 0.7 0.1
Thr + (4)	10.4 ± 0.1	9.8 ± 1.1*	28.3 ± 0.0"	47.6 ± 1.0	3.7 ± 0.4
Ileu — (4)	9.7 ± 0.2	17.0 ± 0.7	21.6 ± 0.7	48.1 ± 1.0	3.6 ± 0.4
Ileu + (4)					
Expt. 2					
Met — (4)	10.8 9.0 ± 0.9 0.4	8.3 7.8 ± 0.9 0.4	29.7 21.7 ± 0.6 1.0*	49.7 60.1 ± 2.4*0.4	1.5 1.4 ± 0.1 0.3
Met + (4)	9.5 ± 0.6	7.9 ± 1.2	22.4 ± 2.6	58.1 ± 3.3	2.1 ± 0.8
Lys — (4)	9.3 ± 0.2	8.6 ± 1.1	21.2 ± 1.0	59.3 ± 1.7	1.6 ± 0.2
Lys + (4)					
Expt. 3					
Leu — (3)	10.4 ± 0.2*	8.3 ± 0.6	27.0 ± 2.3	51.1 ± l.Y	3.3 ± 0.2**
Leu + (3)	12.6 8.4 ±±0.8* 0.1	10.6 10.0± 0.8 1.5	22.9 21.8± 0.4 1.1	55.7 52.± 2.3 0.9**	2.4 3 4 ±+ 0.3 0.4**
Val — (3)	8.6 ± 0.1	10.8 ± 0.8	23.5 ± 0.4	55.0 ± 0.6	2: 1 ± 0. 1
Val + (3)	9.7 ± 0.3	9.7 ± 1.1	30.8 ± 0.6	47.2 ± 1 4	2.6 ± 0.2
Phe — (3)	8.9	10.4	24.0	54.1	2.6
Phe + (2)	10.9 ± 0.1	7.6 ± 0.8	22.2 ± 0.6	55.8 ± 0.2	3.5 ± 0.2
Trp — (3)	7.4	8.1	22.1	59.7	2.7
Trp + (2)					

1) Fatty acid specificity is expressed as percentage of the radioactivity recovered in individual cholesterol esters after incubation of plasma with cholesterol-4-^{14}C at 37°C for 3 hrs. See also Table 2.

* and **: Significantly different from the corresponding control values at $P < 0.01$ and $P < 0.05$, respectively.

Table 5. Fatty acid composition of plasma cholesterol ester".

Groups	Fatty acid						
	16: 0	16: 1	18: 0	18: 1	18: 2	18: 3	20: 4
Expt. 1			5.0	9.3		1.7	
Thr —	21.4	6.2	3.6	11.1	18.5	0.9	38.8
Thr +	16.9	6.2	4.0	9.5	19.3	2.1	42.0
Ileu —	16.6		4.5	12.0	20.8	2.2	40.8
Ileu +	17.6	8:0			18.7		37.0
Expt. 2		3.2	1.1				
Met —	9.5	3.1	0.6	7.6	23.5	1.5	51.6
Met +	13.1	5.3	1.6	8.3	20.2	1.7	57.6
Lys —	...	3.3	0.6	...	16.7	1.7	53.3
Lys +	10.7			8.1	22.2		53.3
Expt. 3							
Leu —	11.7	2.9	0.3	4.5	21.7	0.4	58.4
Leu +	9.9	5.1	0.3	7.5	20.4	0.4	56.3
Val —	10.6	2.9	0.2	4.0	18.7	0.6	63.0
Val +	13.5	5.5	0.6	8.1	19.6	0.9	51.8
Phe —	11.3	2.1	0.2	3.8	28.2	0.4	54.0
Phe +	14.9	8.8	0.9	9: 6	20.9	1.3	43.6
Trp —	13.8	1.5	0.6	4.9	16.0	tr.	63.2
Trp 4	10.8	5.5	0.3	6.7	17.8	0.4	58.5

1) Values are weight percentage from pooled plasma. See also Table 2.

Table 6. Fatty acid composition of plasma phospholipid[1].

Groups	Fatty acid						
	16: 0	16: 1	18: 0	18: 1	18: 2	18: 3	20: 4
Expt. 3							
Leu —	41.5	1.1	19.7	6.2	23.1	tr.	8.4
Leu +	37.1	1.5	16.8	9.4	22.2	tr.	13.0
Val —	40.5	1.5	16.6	7.4	21.5	tr.	12.5
Val +	39.1	1.3	16.5	8.9	20.7	tr.	13.4
Phe —		1.0					8.0
Phe +	38.0	1.5	15.3	7.8	19.6	tr.	10.2
Trp —	41.1	2.3	19.4	7.4	19.5	tr.	11: 1
Trp +	37.8		17.1	7.6	19.4	tr.	15.8

1) See Tables 2 and 5.

Fatty acid composition

The fatty acid compositions of cholesterol ester and phospholipid from pooled plasma are shown in Tables 5 and 6, respectively. The fatty acid composition of the latter was determined only in Expt. 3. Though modification of the fatty acid patterns of these lipid fractions was specific according to each EAA tested herein, it was much remarkable in the cholesterol ester fraction.

Expt. 4: Refeeding of EAA deficient diet for 2 days after 2 day-fasting

Body weight and food intake

Table 7 shows body weight change and food intake. Despite of 2-day-refeeding, ingestion of diet deficient in Ileu or Arg resulted in further loss of body weight, no weight gain being observed during refeeding period. Other groups of rats gained their- weights, though they could not restored their weights to

Table 7. Body weight and average food intake" (Expt. 4).

Groups		Body weight			Food intake
		Fasting	Refeeding	Final	g/day
Complete	(3)	265 ± 5	231 ± 3	250 ± 4	35
Ileu —	(3)	263 ± 8	232 ± 5	226 ± 6	19
Thr —	(3)	264 ± 6	233 ± 6	235 ± 5	17
Complete	(3)	264 ± 2	231 ± 3	250 ± 3	37
Met —	(3)	267 ± 5	231 ± 4	236 ± 3	24
Lys —	(3)	266 ± 5	232 ± 5	242 ± 5	31
Complete	(3)	251 ± 11	219 ± 6	241 ± 9	36
Arg —	(3)	252 ± 11	217 ± 12	206 ± 4	24
His —	(3)	250 ± 10	219 ± 5	228 ± 3	25
Phe —	(3)	251 ± 11	215 ± 6	220 ± 6	25
Trp —	(3)	248 ± 12	207 ± 11	211 ± 11	29
Leu —	(3)	249 ± 11	218 ± 13	224 ± 15	24
Val —	(3)	253 ± 13	219 ± 12	222 ± 14	21

1) Rats refed diet deficient in EAA for 2 days after 2 day-fasting. See also Table 2.

the levels at the initiation of fasting. The food intake in Lys deficiency was comparably high.

Table 8. Plasma lipids concentration[1] (Expt. 4).

Groups	Phospholipid μg/ml	Cholesterol		
		Total μg/ml	Free μg/ml	Ester %
Complete	1783 ± 136	636 ± 70	260 ± 29	58.3 ± 5.2
Ileu —	1096 ± 110**	572 ± 98	181 ± 14	67.4 ± 3.3
Thr —	1124 ± 166**	425	198	53.4
Complete	1933 ± 73	589 ± 16	248 ± 4	57.8 ± 1.1
Met —	1418 ± 28*	458 ± 33**	201 ± 10**	56.0 ± 1.3
Lys —	1320 ± 103*	446 ± 31**	184 ± 11**	58.7 ± 0.6
Complete	1810 ± 175	494 ± 44	180 ± 13	63.4 0.8
Arg —	1533 ± 220	508 ± 94	181 ± 21	63.4 ± 2.7
His —	1565 ± 108	453 ± 32	158 ± 14	65.2 ± 1.8
Phe —	1570 ± 155	473 ± 44	165 ± 15	65.0 ± 1.0
Trp —	1593 ± 35	464 ± 10	164 ± 2	64.6 ± 0.9
Leu —	1605 ± 138	438 ± 38	153 ± 15	65.2 ± 0.7
Val —	1253 ± 68*	368 ± 36	128 ± 15	65.3 ± 0.7

1) See Tables 4 and 7.

Lipid concentration

The concentration of phospholipid and cholesterol in plasma is shown in Table 8. The concentration of the former decreased in Ileu, Thr, Met, Lys or Val deficiency, and it tended to decrease in deficiency of other EAA. The concentration of cholesterol decreased only in Met or Lys deficiency. The ratio of esterified to total cholesterol remained unchanged in each EAA deficiency.

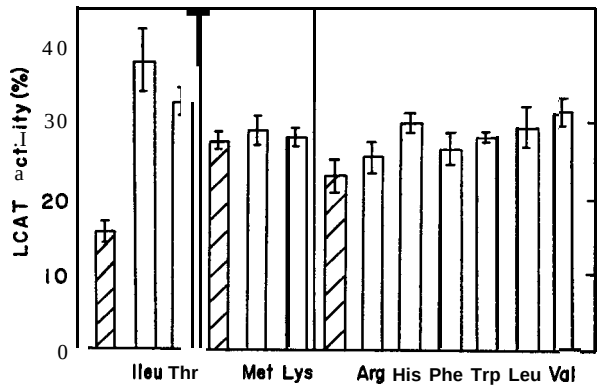

Fig. 2 Activity of plasma LCAT (Expt. 4). See also Table 7 and Figure 1.

LCAT activity and specificity

The activity and fatty acid specificity of LCAT in plasma are shown in Figure 2 and Table 9, respectively. Refeeding of Ileu or Thr deficient diet significantly

Table 9. Activity and fatty acid specificity of plasma LCAT[1] (Expt. 4).

Groups	Fatty acid specificity			
	Saturated	Monoenoic	Dienoic	Tetra and pentaenoic
Complete	6.6 ± 0.3	27.1 ± 2.5	19.8 ± 1.3	46.5 ± 2.4
Ileu —	6.9 ± 0.9	7.9 ± 1.4*	24.9 ± 1.5	60.3 ± 1.0'
Thr —	7.7 ± 0.1	18.8 ± 2.8	25.7 ± 1.2**	47.9 ± 1.6
Complete	7.2 ± 0.6	29.5 ± 2.3	22.1 ± 0.9	41.1 ± 2.0
Met —	8.3 ± 0.4	17.3 ± 1.5**	28.9 ± 1.5**	45.2 ± 1.9
Lys —	7.7 ± 0.2	15.5 ± 1.3*	27.5 ± 0.4*	49.3 ± 1.4**
Complete	7.3 ± 0.4	25.3 ± 0.6	20.4 ± 1.0	47.0 ± 0.4
Arg —	9.7 ± 0.7**	14.3 ± 4.2	30.3 ± 1.5*	45.5 ± 6.4
His —	6.9 ± 0.8	11.9 ± 1.7*	24.6 ± 2.7	56.6 ± 2.0**
Phe —	8.1. ± 0.6	12.7 ± 1.4*	25.5 ± 1.8	53.7 ± 1.8**
Trp —	8.0 ± 0.8	12.3 ± 1.8'	26.2 ± 2.3	53.1 ± 2.5
Leu —	8.0 ± 0.1	15.1 ± 2.8**	26.4 ± 1.8**	50.5 ± 1.8
Val —	9.3 ± 0.6	13.6 ± 1.9*	23.3 ± 2.3	53.7 ± 1.6**

1) See Tables 4 and 7.

increased the activity. Refeeding of His, Leu or Val deficient diet tended to increase the activity. Fatty acid specificity was remarkably changed by refeeding of EAA deficient diet. In general, relative formation of the monounsaturated ester was significantly decreased. Relative formation of the diunsaturated ester was increased by refeeding of diet deficient in Thr, Met, Arg or Leu and that of the tetra and pentaunsaturated ester fraction was increased by refeeding of Ileu, His, Phe or Val deficient diet. Refeeding of Lys deficient diet increased relative formation of the di and tetra and pentaunsaturated esters.

Fatty acid composition

The fatty acid composition of cholesterol ester from pooled plasma is shown in Table 10. A specific change in percentage of monounsaturated fatty acid was

Table 10. Fatty acid composition of plasma cholesterol ester[1] (Expt. 4).

Gronps	Fatty acid						
	16: 0	16: 1	18: 0	18: 1	18: 2	18: 3	20: 4
Complete	35.3	20.2	6.7	15.6	9.7 11.5	0.3	12.2
Ileu —	33.5	10.9	8.3	15.5		0.3	18.2
Thr —	26.6	10.8	7.3	19.1	16.5	tr.	18.6
Complete	11.0	25.3	0.5	11.2	18.2	tr.	33.7
Met —	13.4	8.0	0.4	11.5	28.6	tr.	33.7
Lys —	13.7	13.8	0.9	13.0	25.3	tr.	33.2
Arg —		6.4	0.9	...7.7	21.9	1.8	37.8
His —	12.6	16.7	0.6	7.87.2	27.6	0.7	44.2
Phe —	12.5	6.9	0.7	7.9	23.4 25.7	0.3 0.5	49.8 46.0
Trp —	13.8	8.4	0.8	6.7	25.3	0.7	44.6
Leu —	12.8	9.6	1.2	8.3	22.6	...	44.9
Val —	13.8	6.4	0.7	9.2	22.3	0.6	47.6

1) See also Tables 5 and 7.

observed. Refeeding of EAA deficient diet decreased percentage of palmitoleic acid but did not that of oleic acid. The percentage of arachidonic acid was increased by refeeding of diet deficient in EAA except Met or Lys. However, refeeding of Met or Lys deficient diet increased percentage of linoleic acid. Refeeding of Thr or Arg deficient diet also increased the percentage of linoleic acid.

Table 11. Fatty acid composition of plasma phospholipid[1] (Expt. 4).

Groups	Fatty acid							
	16: 0	16: 1	18: 0	18: 1	18: 2	18: 3	20: 4	22: 6
Complete	25.4	4.0 12.0	20.0	16.0	20.9	0.6	10.8	1.4
Ileu —	33.5	[illegible]	15.4	16.9	13.6	0.4	7.3	tr.
Thr —	29.6	6.6 7.1	15.6	15.0	20.9	0.2	10.1	1.5
Complete	33.0	3.6	23.5	21.0	13.8	tr.	1.2	tr.
Met —	43.9	[illegible]	23.3	16.5	12.3	tr.	0.5	tr.
Lys —	27.3	6.2 7.3	22.5	16.9	23.0	tr.	4.2	tr.
Complete	40.9	3.2	22.4	16.1	12.3	tr.	0.5 [illegible]	tr.
Arg —	35.5 40.5	3.9	18.9 23.1	10.7	27.1	tr.	4.6 1.9	tr.
His —		4.6		10.9	*19.7*	tr.		tr.
Phe —	44.1	4.7	27. 2	14.2	9.6	tr.	0. 2	tr.
Trp —	44.4	5.2	20.1	15.4	11.2	tr.	0. 3	tr.
Leu —	41.9	4.3	27.2	15.3	10.4	tr.	tr.	tr.
Val —	43.5		25.2	14.6	12.0	tr.	0. 4	tr.

1) See also Tables 5 and 7.

As shown in Table 11, the changes in the fatty acid composition of phospholipid were much less remarkable than those of cholesterol ester and only small changes were seen after refeeding of Ileu, Met, Lys, Arg or His deficient diet.

DISCUSSION

Because of the effects of the quantity and quality of dietary proteins on the metabolism of plasma cholesterol ester and the possible role of plasma LCAT on the maintenance of the levels and compositions of cholesterol ester *in situ,* it seems to be of interest to investigate the influence of EAA deficiency on LCAT. In the present experiments with feeding and refeeding of EAA deficient diet, the effects of EAA tested on LCAT activity were not always similar. The activity tended to decrease in Met, Lys, Leu, Val or Trp deficiency in the feeding experiment and to increase in Ileu, Thr, His, Leu or Val deficiency in the refeeding experiment. In the previous paper we have shown (Hori *et al.,* 1973b) that the differences in the feeding habit on a sucrose diet result in different responses of the LCAT activity.

Generally, the increase of the dietary protein levels appears to elevate the concentration of esterified cholesterol in plasma (Nath and Singh, 1970 ; Hori *et al.,* 1973a). The present study indicated that the changes. in the concentration of cholesterol observed in Thr, Ileu or Lys deficiency in the feeding ex-

periment were attributable essentially to those in the esterified form. The decrease in cholesterol in refeeding of Met or Lys deficient diet was also remakable in the esterified form. However, the LCAT activity did not always parallel with the changes in the concentration of esterified cholesterol. Also, the activity did not always parallel with the concentration of phospholipid. In both experiments ERA deficincies tended to decrease the concentration of phospolipid.

The LCAT activity may have a relationship to relative formation of cholesterol esters newly formed during incubation. LCAT in rat plasma has a specificity for fatty acids combined at the 2-position of lecithin in order of arachidonic acid > linoleic acid > oleic acid > palmitic acid (Sugano, 1971; Sgoutas, 1972). EAA deficiency produced the specific changes in fatty acid specificity of LCAT as shown in Tables 4 and 9. In feeding of Met, Val or Trp deficient diet, the activity appeared to decrease and relative formation of the palmitate increased and/or that of the arachidonate decreased (Leu deficiency also produced similar change). LCAT activity may relate to the changes in the rate of formation of the arachidonate or palmitate in these rats. In rats fed on Met or Phe deficient diet, however, formation of cholesterol arachidonate was decreased, whereas the activity tended to decrease in the former and remained undistinguishable in the latter. In rats refed on EAA deficient diet, a tendency to increase the activity was accompanied by the increase in relative formation of the polyunsaturated esters. On the other hand, refeeding of Met or Lys deficient diet increased relative formation of these esters but the activity remained unchanged. Thus, it is difficult to correlate simply the change in the activity to that in the specificity. However, increased activity seems to be accompanied by increased relative formation in the linoleate and arachidonate, though the same is not always true *vice versa.* Althongh relative formation of cholesterol arachidonate is enhanced by increasing the dietary protein levels, the steady effects of EAA deficiency on formation of this ester were obscure.

Refeeding after fasting increased relative formation of the monounsaturated ester by LCAT (Hori *et al.*, 1973a). Refeeding of EAA deficient diet, however, significatly decreased relative formation of the monounsaturated ester. However, it seems likely from the data in Table 10 that refeeding of the deficient diet depresses enzymatic formation of cholesterol palmitoleate. Formation of cholesterol oleate by LCAT appeared to be uninfluenced. Palmitoleic acid in phospholipid did not appreciably decreased, though that in cholesterol ester significantly decreased by refeeding of EAA deficient diet. These observations suggest that refeeding of EAA deficient diet induces the decrease not only in the synthesis of palmitoleic acid but also in the specificity of the enzyme for this acid.

Refeeding after fasting causes abrupt and considerable alterations in the metabolism of lipids in the hepato-plasmic system (Allman et al., 1965 ; Park *et al.*, 1972) and significantly decreases the concentration of linoleic and arachidonic acids in liver. Relative formation of cholesterol linoleate and arachidonate by LCAT is, however, maintained comparatively high (Hori *et al.*, 1973a and 1973b) Thus, formation of polyunsaturated cholesterol esters by LCAT becomes particularly important at the physiological standpoints. In rats refed after fasting on diets containing 18% casein with different levels of linoleic acid, relative

formation of cholesterol linoleate by LCAT increases proportionally with increasing levels of this essential fatty acid (Unpublished data). In the present experiment, food intake was not necessarily proportional to the percentages of linoleic and arachidonic acids in phospholipid and cholesterol ester. Also, *in vitro* relative formation of cholesterol linoleate and arachidonate was not essentially proportional to the amount of the diet ingested. Although relative formation of cholesterol arachidonate is also enhanced by increasing the dietary protein levels in the condition of refeeding as well as feeding, the responses of the rate of cholesterol arachidonate formation to dietary deficiecy of EAA were not always similar with respect to each EAA tested. The proportion of cholesterol oleate formed by LCAT was high in the pair-feeding control groups of Thr or Ileu deficiency. This may relate to relatively small change in body weight of these rats during experiments.

In patients with cholestasis and LCAT deficiency or guinea-pigs fed cholesterol, the decrease of LCAT activity is accompanied by the abnormalities of lipoproteins in plasma (Glomset and Norum, 1973). Maeda et al. (1973) indicated that the EAA deficiency changed the metabolism of hepatic lipids. Therefore, EAA deficiency seems to influence the concentration and pattern of lipoproteins in plasma. In the present experiments, the patterns of plasma lipoproteins on paper electrophoresis and disk electrophoresis on polyacrylamide gel were examined in rats fed and refed on Thr, Ileu, Met or Lys deficient diet. In rats fed on Thr or Ileu deficient diet percentage of high density lipoprotein was increased but in rats fed on Met or Lys deficient diet the pattern of lipoproteins remained unchanged. Refeeding of Thr, Ileu or Lys deficient diet increased percentage of high density lipoprotein, but that of Met deficient diet did not change the pattern of lipoproteins. The LCAT activity *in vitro* is controlled by the concentration of proteins in lipoproteins as well as by that of lipid components (Fielding *et al.*, 1972). EAA deficient diets may produce the changes in these parameters in plasma lipoproteins which in turn activate or inhibit the activity of LCAT.

Though interrelationship of EAA deficiency and the activity of LCAT was not fully clear at present, the present study indicated that each EAA had different effects on the metabolism of cholesterol as well as the activity and fatty acid specificity of LCAT in rat.

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