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Dietary Fat Content Effects on Concentrations of Liver and Intestinal Fatty Acid Binding Proteins

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Summary. **Two fatty acid binding proteins, liver and intestinal, have been identified in the rat intestine. Both are thought to be closely related to the absorption and metabolism of fatty acids in the intestinal epithelium. However, the underlying mechanism is not clearly understood. The purpose of this study was therefore to investigate the roles of these two fatty acid binding proteins in the intestinal absorption of fatty acids.**

Rats were fed diets varying in fat content for two or four weeks. Liver and intestinal fatty acid binding proteins were extracted from the duodenum, jejunum and ileum, and their concentrations were measured using the single radial immunodiffusion method.

Rats fed with a high fat diet for two weeks had a significantly increased liver fatty acid binding protein concentration in the jejunum, and a significantly increased intestinal fatty acid binding protein concentration in both the jejunum and the ileum in comparison with the duodenum. Rats fed a high fat diet for four weeks also had a significantly increased liver fatty acid binding protein concentration in the jejunum, and a significantly increased intestinal fatty acid binding protein concentration in the ileum compared with that in the duodenum.

Since the increase in the concentrations of liver and intestinal fatty acid binding proteins varied depending on the amount of fat, we speculate that liver and intestinal fatty acid binding proteins possess different mechanisms in the absorption and metabolism of fatty acids.

Key words—fatty acid binding protein, liver fatty acid binding protein, intestinal fatty acid binding protein.

INTRODUCTION

Fatty acid binding proteins (FABPs) have been extracted from various organs in the rat. Although each FABP has a different physiochemical character, they have similar features. Each FABP has been named after the organ from which it was first extracted, including liver (L) , intestinal (I) and heart (H) FABP. While the liver contains only L-FABP and heart contains only H-FABP[1-4], both L-FABP and I-FABPs are found in the intestine[5]. Both L and I-FABPs are thought to be closely related to the abosrption and metabolism of fatty acids, including their intracellular transport, esterification, and the resynthesis of triglycerides in the intestinal epithelium[6-8].

L-FABP concentration was measured in the rat small intestine in 1974 by Ockner et al.[7]. I-FABP concentration was measured in the rat jejunum in 1985 by Bass et al.[9], and L-FABP concentration was measured in the human intestine in 1990 by Sakai et al.[10]. However, concentrations of L and I-FABPs in different parts of the intestine and their relation to diet have not been investigated. To understand further the roles of L and I-FABPs in the intestinal absorption of fatty acids, we measured the concentrations of L and I-FABPs in three portions of the intestine in rats fed with three different diets.

MATERIALS AND METHODS

Fifty male two-month-old Wistar rats were divided into the following five groups (each group n=10): 1) those fed freely with a normal diet for two weeks; 2) those fed with a high fat diet for two weeks ; 3)those

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fed with a high fat diet for four weeks; 4)those fed with a low fat diet for two weeks; and 5)those fed with a low fat diet for four weeks. The compositions of each diet are listed in Table 1. Further, fatty acid contents in the lard used in this study are listed in Table 2. After the designated period, rats were fasted overnight and sacrificed under ether anesthesia. There were no significant differences in body weight among the two-week groups, or between the four-week groups. Five cm segments were resected: from the duodenum, just distal to the pylorus ring; from the jejunum, just distal to Treitz's ligament; and from the ileum, just proximal to Bauhin's valve. The whole layer of each intestinal section was homogenized and then centrifuged for 40 min at 105,000 G. The resulting supernatant fraction was concentrated using a Centriprep (Amicon, Tokyo, Japan) ultra filter.

New Zealand white rabbits were immunized with the antigens L and I-FABPs (obtained from Dr. Ono[11])to produce rabbit anti-rat L and I-FABP antisera. The antisera were purified by ammonium sulfate precipitation and then concentrated using a Centriprep. The anti-L and I-FABP antibodies produced yielded single precipitation lines with L and I-FABP antigens, using the Ouchterlony method[12]. Furthermore, when anti-L and I-FABP antibodies were absorbed by L and I-FABP antigens, the anti-L and I-FABP antibodies did not yield a sedimentation line with the supernatant of rat intestinal homogenate. We therefore concluded that the anti-L and I-FABP antibodies produced were specific for L and I-FABP antigens.

Ouchterlony agar (1%) was made by adding 1.0 ml L-FABP or 0.5 ml I-FABP antiserum to 6 ml of the sample. The concentrations of L and I-FABP in each sample were measured by the single radial immunodiffusion (SRID)[13]method. The total protein concentration of each sample was measured by the Lowry method[14]. Values are expressed as the concentration of L and I-FABP per unit concentration of total protein (μg/mg soluble protein). Comparison among the five groups was made using a relative value of L and I-FABP concentration for each group to the mean concentration of L and I-FABP of the normal diet.

Values are expressed as mean$\pm$SD. Significant differences were determined by Fisher's PLSD. A p value<0.05 was considered significant.

RESULTS

Concentrations of L and I-FABP in the control group (Fig. 1). In the normal diet group, the concentrations of both L and I-FABP per soluble protein were the highest in the jejunum, followed by the ileum and duodenum, respectively. However, these differences were not significant.

Concentrations of L and I-FABP in each intestinal section under varying diet conditions (Figs. 2 and 3). In the groups fed with a high fat diet for 2 or 4 weeks, L-FABP concentration was significantly higher in the jejunum than other sections. I-FABP concentration, however, was significantly higher in the ileum than in

Table 1. Dietary contents (weight %)

		Normal diet (%)	High fat diet (%)	Low fat diet (%)
Carbohydrate	β-corn starch	37	8	47
	α-potato starch	10	10	10
	Granulated sugar	5	5	5
Protein	Casein	25	25	25
Fat	Lard	6	40	0
Others		17	12	13

Table 2. Fatty acid contents in lard (weight %)

Palmitic acid	$C_{16}H_{32}O_2$	29.8	Saturated fatty acid
Stearic acid	$C_{18}H_{36}O_2$	12.7	43.5
Others		1.0	
Oleic acid	$C_{18}H_{34}O_2$	47.8	Unsaturated fatty acid
Linoleic acid	$C_{18}H_{32}O_2$	3.1	56.5
Others		5.6	

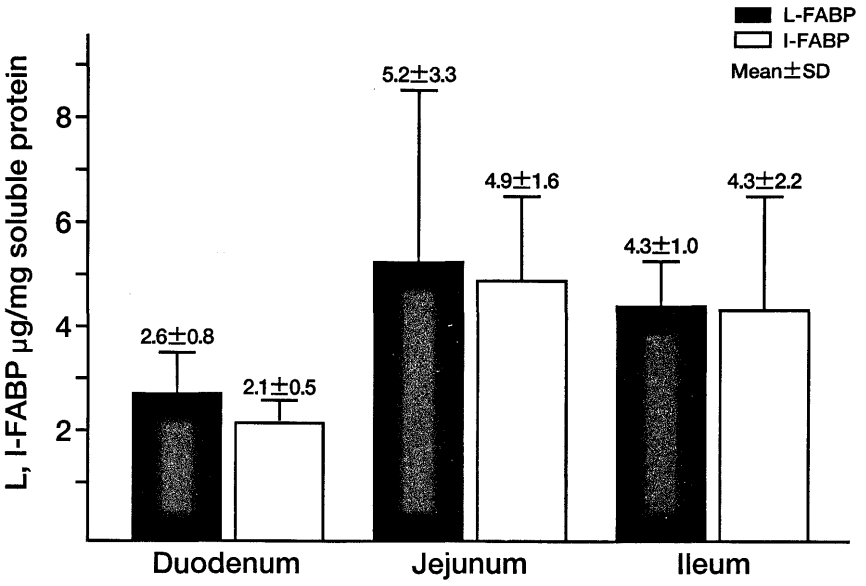

Fig. 1. Concentrations of L, I-FABP in the normal diet group.

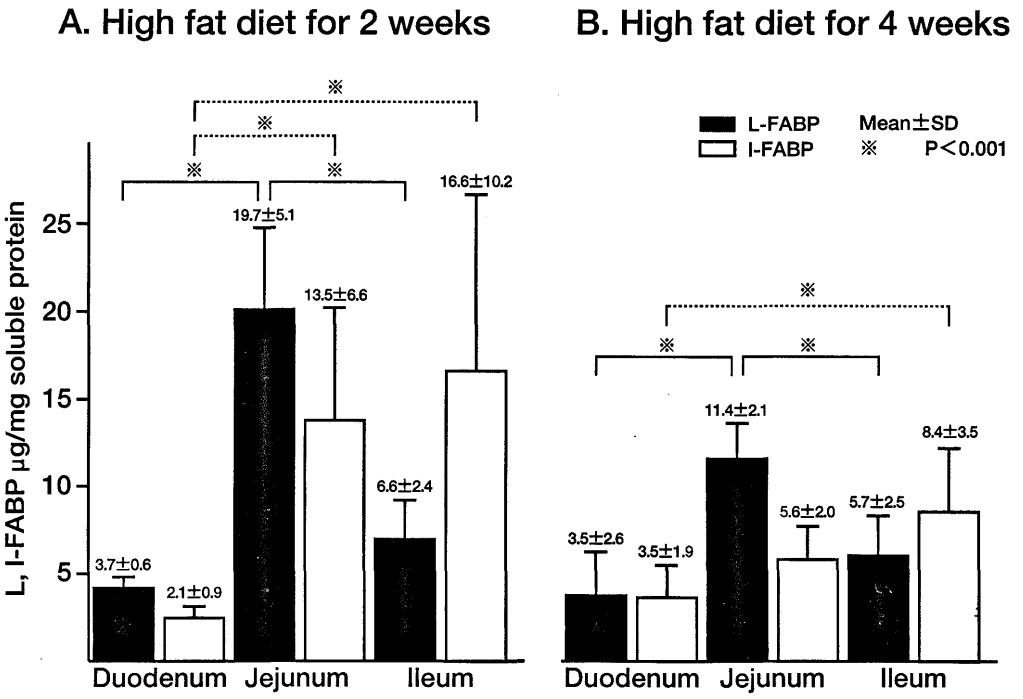

Fig. 2. Concentrations of L, I-FABP in the high diet 2(A) , 4(B) week groups.
A. The concentration of L-FABP was significantly higher in the jejunum than in the duodenum ($P<0.001$) and in the ileum ($P<0.001$). The concentration of I-FABP was significantly lower in the duodenum than in the jejunum ($P<0.001$) and in the ileum ($P<0.001$).
B. The concentration of L-FABP was significantly higher in the jejunum than in the duodenum ($P<0.001$) and in the ileum ($P<0.001$). The concentration of I-FABP was significantly higher in the ileum than in the duodenum ($P<0.001$).

the duodenum. In the group fed with a high fat diet for 2 weeks, I-FABP concentration was significantly lower in the duodenum than in the other sections. In the group fed with a low fat diet for 2 weeks, L-FABP concentration was significantly higher in the jejunum than in the duodenum. In the group fed with a low fat diet for 4 weeks, no significant difference was shown.

Relative concentrations of L and I-FABP in each dietary fat content (Table 3)

In the duodenum, the relative concentrations of L and I-FABP did not differ significantly from those in the normal diet group.

In the jejunum, the relative concentration of L-FABP in the groups fed with a high fat diet, for either 2 or 4 weeks was significantly higher than that in the normal diet group. The relative concentration of I-FABP in the groups fed with a high fat diet for 2 weeks was significantly higher than that in the normal diet group. Interestingly, those rats fed with a high fat diet for four weeks had significantly lower relative concentrations of the L and I-FABP than the rats fed with a high fat diet for two weeks. Also, the relative concentration of L and I-FABP in the low fat diet group did not differ significantly from that in the normal diet group.

In the ileum, the relative concentration of L-FABP in each diet group did not differ significantly from that in the normal diet group. The relative concentration of I-FABP, in the high fat diet for the 2 week group however, was significantly higher than that in the normal diet group. The relative concentration of I-FABP in the low fat diet group did not differ significantly from that in the normal diet group.

To summarize, in those groups fed with a high fat diet for 2 weeks, the concentration of I-FABP increased significantly in the jejunum and ileum, whereas the concentration of L-FABP significantly increased only in the jejunum compared with that in the normal diet group.

DISCUSSION

FABP was first extracted from the rat liver and intestine by Ockner et al.[6] in 1972. FABP has a binding affinity with long chain fatty acids, its Co-A derivative, and dyes of amino-azo carcinogens. Furthermore, FABP is thought to be closely related to the intracellular transport and esterification of fatty acids and the resynthesis of triglycerides. Other studies suggest that FABP transfers fatty acids to microsomes where they activate fatty acyl-CoA, and to mitochondria where they are used in beta oxida-

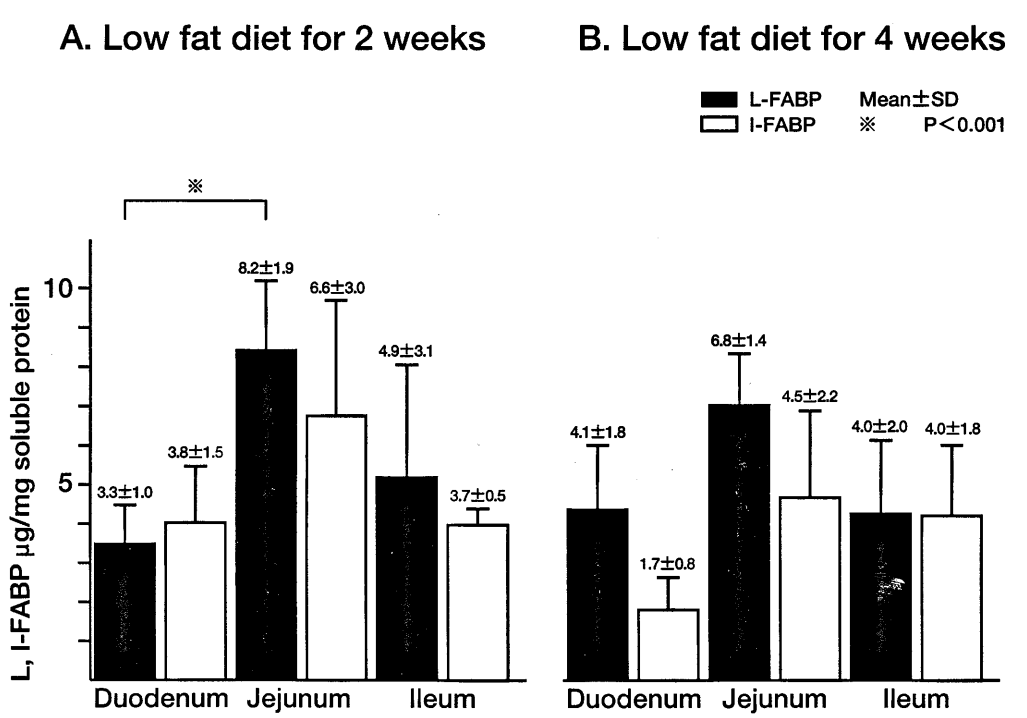

Fig. 3. Concentrations of L, I-FABP in the low fat diet 2(A), 4(B) week groups.
A. The concentration of L-FABP was significantly higher in the je-junum than in the duodenum ($P<0.001$).

Table 3. The relative concentrations of L, I-FABP in the duodenum (D), jejunum (J) and ileum (I) following diets of varying fat contents

		L-FABP			I-FABP	
D	N	1.0±0.3			1.0±0.2	
	H2	1.4±0.2			1.0±0.4	
	H4	1.3±1.0			1.7±0.9	
	L2	1.3±0.4			1.8±0.7	
	L4	1.6±0.7			0.7±0.4	
J	N	1.0±0.6	p<0.001	p<0.01	1.0±0.3	p<0.001
	H2	3.8±1.0	p<0.001		2.8±1.4	p<0.001
	H4	2.2±0.4			1.1±0.4	
	L2	1.6±0.4			1.4±0.6	
	L4	1.3±0.3			0.9±0.4	
I	N	1.0±0.2			1.0±0.5	p<0.001
	H2	1.5±0.6			3.9±2.4	
	H4	1.3±0.6			1.9±0.8	
	L2	1.1±0.7			0.9±0.1	
	L4	0.9±0.5			0.9±0.4	

N, normal diet group; H2, high fat diet for 2 weeks; H4, high fat diet for 4 weeks; L2, low fat diet for 2 weeks; L4, low fat diet for 4 weeks.

tion[15,16].

At the end of this syudy, no significant difference in body weight was seen between the high fat diet group and the low fat diet group. As the rats were fed ad libitum, we believe that less of the high fat diet was ingested by the rats, possibly bacause it was not as palatable. Consequently, the difference in body weight was not significant, allowing us to compare rats of similar weights.

FABP exists mainly in the villi of the mucosa[7]. However, because it would be technically difficult to resect the mucosa from the thin rat intestine, FABP was extracted from the whole layer of the intestinal segment.

The concentrations of both L and I-FABP per soluble protein were the highest in the jejunum, followed by the ileum and duodenum, respectively. These results correspond to the study by Ockner et al. which showed that the concentration of L-FABP decreased from the proximal, distal portion of the jejuno-ileum to the duodenum. The fact that the concentrations of L and I-FABP were the highest in the jejunum where fat is mainly absorbed suggests the relevance of FABP levels to fat absorption.

Although Ockner et al. suggested that the concentration of rat FABP in the middle and distal thirds of the small intestine increased significantly when ingesting a high fat diet, and that the concentration of FABP in the proximal third of the intestine decreased significantly when ingesting a low fat diet[7], we hypothesized that FABP would be induced in each level of the intestine to absorb the extra fat ingested, and that it would decrease in low fat diets. In the low fat diet group, however, the concentrations of L and I-FABER were not significantly lower than the normal diet group in any portion of the intestine. This may be because, in the normal diet state, L and I-FABP are at basal levels and do not decrease further when less fat is consumed.

As expected, the concentration of L-FABP in the groups fed a high fat diet for 2 or 4 weeks was significantly higher in the jejunum than for those fed a normal diet. In the jejunum and the ileum, the concentration of I-FABP in the rats fed a high fat diet for 2 weeks was higher than the normal diet group. Interestingly, in the jejunum, the concentrations of both L and I-FABP in the rats fed a high fat diet for 4 weeks were lower than for those on the high fat diet for 2 weeks. Possibly the concentrations of FABP initially rise with an increase in fat intake, and after adapting to the high fat diet fatty acids, can be absorbed with lower levels of FABP. Another possibility may be that there is a negative feedback control of FABP, which is activated between 2 and 4 weeks after the initiation of a high fat diet. Since the half life of L-FABP is comparatively short—, 3.1 days[17], we think further studies at shorter time intervals are needed to fully understand this phenomenon.

While only L-FABP exists in the rat liver, both L and I-FABP exist in the rat intestine. Thus, it is possible that these two FABPs operate differently in the absorption and metabolism of fatty acids. Kuo-

Tung and Judith Storch have reported that the rate of anthroyloxy fatty acid transfer from I-FABP is dependent on the concentration of acceptor membrane vesicles, and that the characteristics of fatty acid transfer from L-FABP are consistent with an aqueous diffusion mediated process[18]. With respect to the affinity of L, I-FABP for saturated or unsaturated fatty acid, Ockner et al. have showed that L-FABP in rats has a stronger affinity for unsaturated fatty acids than saturated fatty acids[6]. Furthermore, Richieri et al. have necently reported that L-FABP has a 5-fold greater affinity than dose I-FABP for unsaturated fatty acid[19]. Another study showed that unsaturated fatty acids were absorbed almost entirely in the proximal small intestine[20]. In this study, the concentration of L-FABP increased significantly in the jejunum (proximal small intestine), whereas the concentration of I-FABP increased significantly both in the jejunum (proximal small intestine) and the ileum (distal small intestine) when rats were fed with a high fat diet. Lard, which contains an equal amount of saturated and unsaturated fatty acids, was used for this study. As a result, L-FABP concentration may have increased in the jejunum to aid in the absorption of the unsaturated fatty acids. A study using diets only containing saturated or unsaturated fatty acids would serve toward a further understanding of their mechanisms.

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