Amount and quality of dietary protein regulate lecithin:retinol acyltransferase activity without change in cellular retinol-binding protein, type two in rat jejunum

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The effects of amount and quality of dietary protein on the cellular retinol-binding protein (type two) and two enzymes for retinol esterification, i.e., lecithin:retinol acyltransferase and acyl-CoA:retinol acyltransferase in rat jejunum were examined. Three groups of five rats were pair-fed defined diets for 14 days. The control group was fed a diet containing 20% casein; the two experimental groups received diets containing 5% casein or 20% gluten. Feeding the 5% casein diet or the 20% gluten diet decreased serum concentration of retinol and retinol-binding protein and increased liver total retinol and retinol-binding protein contents. The lecithin:retinol acyltransferase activity in jejunal microsomes, which was determined using retinol bound to cellular retinol-binding protein (type two), was significantly reduced in animals fed the 20% gluten diet (by 30%) as compared with animals fed the 20% casein diet. Feeding the 5% casein diet also led to a slight decrease in the segmental lecithin:retinol acyltransferase activity. The acyl-CoA:retinol acyltransferase activity was unaffected by feeding these diets. The jejunal cytosolic cellular retinol-binding protein (type two) content was also unchanged by feeding these diets. Feeding the 20% wheat gluten diet resulted in remarkable low levels of the unesterified retinol (by 64%), retinyl palmitate (by 32%), and protein (by 47%) in the whole jejunal mucosa, when compared with the corresponding values of the 20% casein group. These results suggest that the dietary protein malnutrition will suppress intestinal esterification of retinol absorbed by a decline of lecithin:retinol acyltransferase activity and without change in intestinal cellular retinol-binding protein (type two) level. The results also suggest that retinol uptake may decrease due to an impaired absorption process in protein malnutritional status. (J. Nutr. Biochem. 5:197–203, 1994.)

Keywords: cellular retinol-binding protein (type two); lecithin:retinol acyltransferase; acyl-CoA:retinol acyltransferase; protein malnutrition; intestinal jejunum; rat

Introduction

The esterification of retinol with long chain fatty acids occurs in the liver and other organs for storage of vitamin A. In the small intestine esterification of retinol is the final step in vitamin A absorption, which precedes incorporation into chylomicrons and the export of absorbed vitamin A into lymph.[1] The retinol absorbed in the enterocytes has been shown to bind to cellular retinol-binding protein, type two (CRBP(II)),[2] and this protein-ligand complex serves as substrate for microsomal lecithin:retinol acyltransferase (LRAT).[3] A second retinol esterification activity has been described, acyl-CoA:retinol acyltransferase,[4] which catalyzes esterification of free retinol, but not the retinol bound to CRBP(II).[5] Because CRBP(II) is localized in large quantity in small intestine,[6–9] it is likely that most of absorbed retinol can be bound to CRBP(II). The retinol bound to CRBP(II) is restricted to microsomal LRAT for esterification, suggesting that LRAT is the physiologic retinol esterifying enzyme in the small intestine.[10]

The protein deficiency has been documented to cause a decrease in serum retinol and retinol-binding protein (RBP) concentration, presumably due to reduced RBP biosynthesis

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in the liver.[11] In the small intestine the protein deficiency also decreases hydrolyzing activity for retinyl esters.[12] In addition, carotenoid utilization was shown to be particularly affected in severe protein energy malnutrition (PEM); not only is carotenoid absorption reduced by the decrease of micelle formation with bile acids,[13] but its cleavage to retinal by carotenoid dioxygenase in the intestinal mucosa cells is declined.[14] However, it is not known whether LRAT activity and/or CRBP(II) content are also altered in the small intestine of protein malnourished animals. Therefore, we considered it pertinent to examine whether the amount and/or the quality of dietary protein affect CRBP(II) and the activities of two retinol esterifying enzymes, i.e., LRAT and ARAT in rat jejunum. We report here that the activity of LRAT, but not ARAT, and the unesterified retinol content in jejunal mucosa were lowered by the dietary protein malnutrition, while the intestinal CRBP(II) level was unaffected by the diets.

Methods and materials

Animals and diets

Five-week-old male Wistar rats (Japan SCL, Inc., Hamamatsu, Japan) were housed in individual wire cages. The animals received free access to a synthetic diet containing 20% casein *(Table 1)* for 9 days, and then they were divided into three groups of five rats. The animals were pair-fed for 14 days. The same amount of diet consumed by the 5% casein diet group, which was allowed to consume the diet ad libitum, was given to the other groups on the following day. Synthetic diets containing either 20% casein, 5% casein, or 20% gluten were prepared as shown in *Table 1*. At the end of the feeding period the animals were killed by decapitation and the small intestine, liver, and serum were collected. The experimental procedures used in the present study met the guidelines of the animal usage committee of the University of Shizuoka.

Preparation of intestinal samples

The entire small intestine was removed and the duodenum extending from the pylorus to the ligament of Treitz was discarded. The proximal half (jejunum) of jejunoileum was flushed with 10 mL of ice-cold 0.9% NaCl solution, cut along its length, and blotted with a wet paper. The mucosa from the jejunal segment was scraped using a glass microscope slide and was quickly frozen in liquid nitrogen and stored at −80° C. The jejunum mucosa was homogenized in 2 volumes (vol/wt) of 0.2 M potassium phosphate buffer (pH 7.2) containing 0.25 M sucrose using a Teflon-glass homogenizer. Small aliquots (1 mL) of the homogenates were kept at −80° C for assay of protein and retinoid contents. The rest of the homogenates were centrifuged at 10,000 *g* for 15 min (4° C), and the resulting supernatant was saved and the pellet was rehomogenized in 1 volume (vol/wt) of the same buffer, followed by recentrifugation at 10,000 *g* for 15 min. The supernatant liquids of the above two centrifugations were combined and centrifuged at 105,000 *g* for 1 hr (4° C). The resulting cytosol fractions were stored at −80° C for CRBP(II) assay. The microsomal pellets were suspended in 1 volume of 0.2 M potassium phosphate buffer (pH 7.2) containing 1 mM dithiothreitol, followed by recentrifugation at 105,000 *g* for 1 hr. The recovered pellets were suspended in 1 volume of the same buffer, and quickly frozen in liquid nitrogen before storage at −80° C. The microsome fractions were used for the assay of LRAT and ARAT activities. The protein content of the microsome preparation was determined by the method of Lowry et al.[15] with bovine serum albumin as standard.

Table 1 Composition of the diets

Ingredient	20% casein	5% casein	20% wheat gluten
Corn starch	15	30	15
Sucrose	50	50	50
Casein (vitamin-free)	20	5	—
Wheat gluten	—	—	20
Corn oil	5	5	5
Cellulose	5	5	5
DL-methionine	0.3	0.3	0.3
Mineral mixture*	3.5	3.5	3.5
Vitamin mixture*	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2

*AIN[76] mineral mixture and AIN[76] vitamin mixture (AIN 1977) purchased from Oriental Yeast, Co., Tokyo, Japan.

Assays of LRAT and ARAT activities

Microsomes were assayed for LRAT and ARAT activities using protein concentrations and incubation times that have been validated for initial rate determinations.

LRAT activity in the jejunal microsomes was determined according to the procedure described by Ong et al.,[5] using retinol bound to CRBP(II) as substrate. CRBP(II) was purified from rat small intestine as described previously.[16] The purified CRBP(II) (1.5 mg) was incubated with 1 μmole of retinol for 5 min at 4° C and unbound retinol was removed through a Sephadex G-25 column (PD-10, Pharmacia, Uppsala, Sweden) equilibrated with 0.2 M potassium phosphate buffer (pH 7.2). The CRBP(II)-retinol (3 μM) was incubated with microsomes (60 μg protein) in 0.2 M potassium phosphate buffer (pH 7.2) containing 2 mM dithiothreitol and 2.4 mg/mL bovine serum albumin (final volume, 250 μL) for 30 min at 37° C. The reaction was terminated by the addition of 1 mL of cold ethanol, and retinyl esters were extracted from the reaction mixtures into 4 mL hexane containing butylated hydroxytoluene (BHT, 200 μg/mL). A portion (3 mL) of the upper hexane phase was evaporated to dryness under nitrogen, and the residue was dissolved in 100 μL of methanol for high performance liquid chromatography (HPLC) analysis. The HPLC analysis was performed using a Shimadzu LC-6A system fitted with a μBondapak C18 column (10 nm particle, 3.9 mm × 25 cm; Waters Associates Inc. (Milford, MA, USA), with 100% methanol as a mobile phase at a flow rate of 2 mL/min. Absorbance of retinyl palmitate in the eluates was determined using a spectrophotometer (Shimadzu SPD-6AV) with a wavelength of 330 nm. An external standard of the purified retinyl palmitate was used for quantification of eluted retinyl palmitate peak.

The ARAT activity was assayed according to the procedure described by MacDonald and Ong.[3] The reaction was initiated by incubating jejunal microsomes (60 μg protein) with 30 μM solvent (dimethyl sulfoxide)-dispersed retinol and 80 μM lauroyl-CoA in 0.2 M potassium phosphate buffer (pH 7.2) containing 20 mM bovine serum albumin and 5 mM dithiothreitol for 20 min at 37° C. Total volume of the reaction mixture was 250 μL. The extraction of the reaction mixture and HPLC analysis of the extracts were conducted by the same procedure described above for the LRAT assay. The external standard of the purified retinyl palmitate was used for quantification of eluted retinyl laurate peak.

Assay of CRBP(II)

The amounts of CRPB(II) in cytosol of jejunal mucosa were measured by a sandwich-type enzyme-linked immunosorbent assay with a monospecific antiserum, as described previously.[16]

Measurement of retinol and retinyl palmitate contents in jejunal mucosa

The jejunal mucosa homogenates were extracted for determinations of retinol and retinyl palmitate contents. Aliquots (200 μL) of the homogenates were gently mixed in glass-capped brown glass tubes with 2 vol of ethanol containing a known amount of the purified retinyl acetate as internal standard. The samples were then extracted with 5 mL of redistilled n-hexane containing BHT (2 mg/100 mL). Four mL of hexane phase was removed and evaporated to dryness under nitrogen gas. The residue was dissolved in 50 μL of methanol.

The analysis of retinol and retinyl palmitate dissolved in methanol was performed by HPLC using a Shimadzu LC-6A system fitted with a μBondapak C18 column (10 nm particle, 3.9 mm × 25 cm; Waters Associates, Inc.). The retinyl palmitate in the extract was determined by the same procedure described above for the LRAT and ARAT assay. The retinol in the extract was analyzed with 95% methanol/5% water as a mobile phase at a flow rate of 1 mL/min. Fluorescence of the retinol in the eluates was determined using a spectrofluorescence photometer (Shimadzu RF-530) with an excitation wavelength of 325 nm and an emission of 475 nm. Internal standard preparation of retinyl acetate was purified from commercial compounds (Sigma Chemical Co., St. Louis, MO USA) by chromatography on columns of neutral aluminum oxide weakened with 5% water.[17]

Measurement of serum retinol and liver total retinol

Serum retinol and hepatic total retinol were determined by a reverse phase HPLC. Serum retinol (200 μL of serum) was extracted as described in the previous report[18] according to the method of Catignani and Bieri.[19] The total retinol in the liver was extracted from the 10% liver homogenate, which was prepared with 50 mM Tris-HCl buffer (pH 7.5), containing 0.25 M sucrose, 25 mM $MgCl_2$, 16 mM EDTA, and 40 mM L-ascorbic acid. An aliquot (500 μL) of the homogenate was saponified for 20 min at 55° C with 2 mL of 20% KOH in 91% ethanol in the presence of 1% pyrogallol. Retinol was then extracted into 5 mL of hexane containing BHT (20 mg/L) and 2 mL water. Four mL of hexane phase was removed and evaporated to dryness under nitrogen gas. The residue was dissolved in 100 μL of methanol. The analysis of retinol dissolved in methanol was performed by HPLC as described above for the mucosal retinol assay.

Assay of serum and liver RBP

RBP concentrations in serum and liver were determined by enzyme-linked immunosorbent assay using anti-rat RBP-rabbit serum, according to the procedure described in the previous report.[16] Briefly, for the liver RBP assay, rat livers were homogenized in phosphate buffered saline (pH 7.5) containing 0.5% Triton X-100, 2.5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride using a Teflon-glass homogenizer. The homogenate was sonicated at a power output control of 2.5 for 30 sec using Branson sonifier (model 250, Branson Sonic Power Co., Danbury, CT, USA). After centrifugation of the treated homogenate at 105,000 *g* for 1 hr at 4° C, the resulting supernatant fraction was stored at −80° C and used for the RBP assay.

Other assays

Protein content in the jejunal mucosa homogenate was determined by the Lowry's method.[15] DNA content in the mucosa homogenate was determined according to the procedure of Burton,[20] with calf thymus DNA as standard. Serum albumin concentration was determined using an assay kit (Albumin B-test, Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis

All results were subjected to one-way analysis of variance. Differences in mean values between groups were tested using Tukey's multiple range test.[21]

Results

Final body weight, liver weight, serum albumin level, and protein and DNA concentrations in jejunal mucosa

The body weight at the end of the experimental period was lower in both groups of rats fed the 5% casein and the 20% wheat gluten diets, as compared with that in rats fed the 20% casein diet *(Table 2)*. The weight gain of the animals fed the 5% casein diet or the 20% gluten diet was approximately 60% and 50%, respectively, of that of the rats fed 20% casein diet. The liver weight of the animals fed the 5% casein diet and the 20% wheat gluten diet were significantly lower than that in the control group *(Table 2)*. Serum albumin concentration was slightly but significantly lower ($P < 0.05$) in the groups fed the 5% casein diet or the 20% wheat gluten diet than in the 20% casein group *(Table 2)*. The jejunum weights were similar among the three groups (data not shown). Also, the jejunum mucosa weights were not different among the three groups. The DNA contents in jejunal mucosa were not changed by feeding 5% casein and 20% wheat gluten diets. However, the protein levels expressed as the values per mg mucosa DNA and per whole mucosa of jejunal segment were significantly lower in rat jejunum of the 20% wheat gluten group as compared with the other groups *(Table 2)*.

Effects of amount and quality of dietary protein on jejunal activities of LRAT and ARAT

To examine whether dietary protein affects retinol esterification activity in jejunum, microsomes were prepared from the rats fed either 20% casein, 5% casein, or 20% wheat gluten diet, and the LRAT and ARAT activities were determined. The specific activity (per mg of microsomal protein) of LRAT was reduced in the rats fed the 20% wheat gluten diet to 71% of the level shown in the rats fed the 20% casein diet. There was no significant difference in the specific activity of LRAT between the rats fed the 5% casein diet and the rats fed the 20% casein diet *(Figure 1)*. When jejunal LRAT activity was expressed per total jejunal segment, the 20% wheat gluten group showed a significantly lower LRAT activity than the 20% casein group *(Figure 1)*. The jejunal total LRAT activity of the 5% casein group was slightly lower than that in the 20% casein group *(Figure 1)*. In contrast to LRAT activity, neither specific activity or jejunal total activity of ARAT was affected by dietary manipulation of the amount and quality of protein. Both the 5% casein diet group and the 20% wheat gluten diet group showed similar levels of ARAT activities as compared to the 20% casein group *(Figure 2)*.

Effects of amount and quality of dietary protein on jejunal CRBP(II) level

The animals fed the 5% casein diet and the 20% wheat gluten diet showed similar levels of the jejunal CRBP(II),

Table 2 Effects of amount and quality of dietary protein on jejunal mucosa protein and DNA contents, liver weights, and serum albumin concentration

Group	20% casein	5% casein	20% wheat gluten
Final body weight (g)	170 ± 3[a]	145 ± 2[b]	139 ± 2[b]
Liver weight (g)	7.3 ± 0.4[a]	5.7 ± 0.1[b]	5.7 ± 0.4[b]
Serum albumin (g/dL)	3.62 ± 0.04[a]	3.31 ± 0.04[b]	3.42 ± 0.05[b]
Jejunal mucosa			
weight (g)	1.12 ± 0.07	1.01 ± 0.05	0.99 ± 0.05
protein (mg/g)	112 ± 3[a]	118 ± 3[a]	68 ± 17[b]
(mg/mucosa)	124 ± 9[a]	125 ± 6[a]	66 ± 16[b]
DNA (mg/g)	5.27 ± 0.46	5.24 ± 0.43	6.26 ± 0.31

Data represent the mean ± SEM for five rats per group. The protein contents in jejunal mucosa shown are values of mg protein per g mucosa and mg protein per whole jejunal mucosa. Values not sharing a common superscript letter are significantly different at $P < 0.05$ as assessed by Tukey's multiple range test.

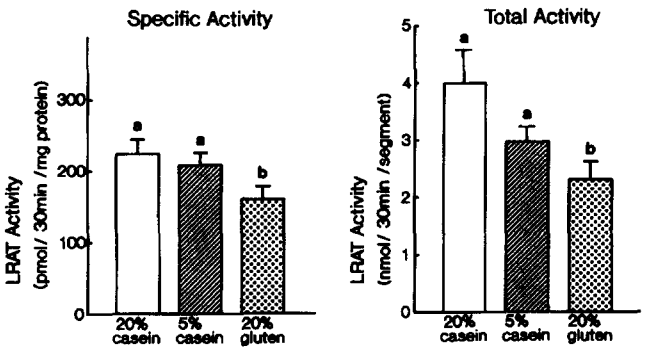

Figure 1 Effects of amount and quality of dietary protein on LRAT activity in jejunum. Either 20% casein or 20% gluten diet was pair fed to rats fed the 5% casein diet for 14 days. The specific activity of LRAT is expressed on the basis of mg microsomal protein (left panel), and the total activity of LRAT is expressed per total jejunal segment (right panel). Each bar represents the mean ± SEM for five rats. Values in each panel not sharing common superscripts are significantly different from one another at $P < 0.05$ assessed by Tukey's multiple range test.

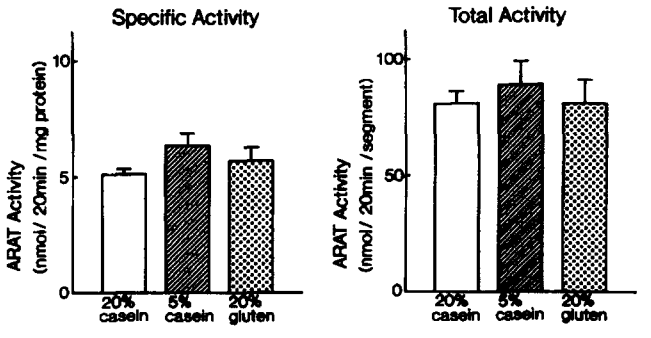

Figure 2 Effects of amount and quality of dietary protein on ARAT activity in jejunum. Either 20% casein or 20% gluten diet was pair fed to rats fed the 5% casein diet for 14 days. The specific activity of ARAT is expressed on the basis of mg microsomal protein (left panel), and the total activity of ARAT is expressed per total jejunal segment (right panel). Each bar represents the mean ± SEM for five rats.

which were expressed on the basis of mg DNA and whole mucosa, to the values of the 20% casein group *(Table 3)*.

Effects of amount and quality of dietary protein on unesterified retinol and retinyl palmitate contents in jejunal mucosa

To examine whether dietary protein affects retinol absorption and metabolism in jejunum, we determined unesterified retinol and retinyl palmitate levels in the jejunal mucosa homogenates. The unesterified retinol levels expressed on the basis of mg mucosa DNA and whole mucosa of jejunal segment were significantly low ($P < 0.05$) in rats fed the 20% wheat gluten diet, showing approximately 40% of the level shown in rats fed the 20% casein diet. Feeding the 5% casein diet tended to decrease the unesterified retinol content in the jejunal mucosa, although the level was not statistically lower than that of the control group. The retinyl palmitate content per mg DNA of jejunal mucosa in the 20% wheat gluten group was about 50% of that shown in the 20% casein group *(Table 3)*. The retinyl palmitate content in whole mucosa of jejunal segment was also significantly lower ($P < 0.05$) in rats fed the 20% wheat gluten diet, showing approximately 70% of that shown in rats fed the 20% casein diet *(Table 3)*. In the 5% casein group, the retinyl palmitate levels expressed on the basis of g tissue and whole mucosa were similar to the levels of the 20% casein group *(Table 3)*.

Serum concentration of retinol and RBP, and liver total retinol content

Serum retinol concentrations of rats fed the 5% casein diet and the 20% wheat gluten diet were decreased to approximately 80% of the concentration in the 20% casein group *(Table 4)*. The serum RBP concentrations were also significantly lower ($P < 0.05$) in these groups than those in the 20% casein group *(Table 4)*. The liver total retinol (retinol plus its esters) content per g tissue increased by 28% in the 5% casein group and by 22% in the 20% wheat gluten group, as compared with the 20% casein group *(Table 4)*. The total retinol level in whole liver was similar among the three groups. The liver RBP levels of rats fed the 5% casein diet and the 20% wheat gluten diet were 1.5 to 1.7 fold higher than that of rats fed the 20% casein diet. The RBP lev-

Table 3 Effects of amount and quality of dietary protein on CRBP(II) level and unesterified retinol and retinyl palmitate contents in jejunal mucosa

Group	20% casein	5% casein	20% wheat gluten
CRBP(II)			
μg/mg DNA	16.8 ± 3.1	15.8 ± 1.7	16.5 ± 1.7
μg/mucosa	82.7 ± 7.8	85.9 ± 6.4	102.4 ± 13.6
Unesterified retinol			
ng/mg DNA	48.8 ± 8.4[a]	34.9 ± 3.8[a]	17.5 ± 2.9[b]
ng/mucosa	300.2 ± 57.0[a]	198.6 ± 19.6[ab]	106.5 ± 15.8[b]
Retinyl palmitate			
ng/mg DNA	9.4 ± 1.1[a]	8.7 ± 1.4[a]	4.8 ± 0.4[b]
ng/mucosa	43.8 ± 2.6[a]	39.9 ± 5.6[ab]	29.7 ± 1.6[b]

Data represent the mean ± SEM for five rats per group. Values not sharing a common superscript letter are significantly different at $P < 0.05$ as assessed by Tukey's multiple range test.

Table 4 Effects of amount and quality of dietary protein on serum concentrations of retinol and RBP and liver contents of total retinol and RBP

Group	20% casein	5% casein	20% wheat gluten
Serum retinol (μg/dL)	57.3 ± 1.1[a]	46.9 ± 1.2[b]	43.0 ± 2.2[b]
Serum RBP (μg/mL)	41.4 ± 5.4[a]	31.2 ± 1.8[ab]	27.8 ± 1.4[b]
Liver total retinol			
(μg/g)	140.8 ± 2.3[a]	180.8 ± 7.7[b]	171.4 ± 6.5[b]
(μg/whole liver)	1006.7 ± 31.8	1032.1 ± 37.7	1024.3 ± 41.1
Liver RBP			
(μg/g)	43.4 ± 1.1[a]	70.3 ± 4.9[b]	66.1 ± 4.5[b]
(μg/whole liver)	324.3 ± 22.7	404.4 ± 32.8	379.2 ± 29.4

Data represent the mean ± SEM for five rats per group. Values not sharing a common superscript letter are significantly different at $P < 0.05$ as assessed by Tukey's multiple range test.

els in whole liver were similar among the three groups *(Table 4)*.

Discussion

As far as we know, this study is the first to explore whether dietary protein affects intestinal CRBP(II) content and LRAT activity. In this study, the dietary protein malnutrition was achieved by feeding the rats the diets containing either reduced level of protein (5% casein) or low quality protein (20% wheat gluten). Although rats fed the 5% casein diet and 20% wheat gluten diet received the identical energy by pair feeding, their body weight gains and liver weights were depressed due to protein malnutrition *(Table 2)*. These dietary manipulations also brought about decreases in the serum levels of retinol and RBP *(Table 4)*, as well as a decrease in serum albumin concentration *(Table 2)*, in agreement with the known consequences of protein malnutrition documented in the previous studies.[22–25] Notably, the jejunal mucosa protein content was remarkably low in animals fed the 20% wheat gluten diet *(Table 2)*.

The animals fed these diets exhibited the elevated contents of hepatic total retinol and RBP per g tissue *(Table 4)*. Interestingly, the hepatic RBP was not decreased, but increased, by feeding the 5% casein and 20% wheat gluten diets. This finding is in agreement with the recent report showing that the RBP mRNA level in rat liver was unchanged by feeding diets containing low amounts or low quality of protein.[26] Our results in liver RBP would suggest that the protein malnutrition decreases the serum retinol-RBP level, presumably due to an impaired secretion system rather than to a decrease in RBP biosynthesis in liver.

However, intestinal cytosolic CRBP(II) levels were not affected by the protein malnutrition state, regardless of whether the diet contains a low level (5%) of protein or consists of a low quality of protein (wheat gluten) *(Table 3)*. The feeding period used in this study was 14 days, and it may be long enough to detect any effect of protein malnutrition on intestinal mucosa, considering that intestinal villus cells of adult rats turn over within 2 days.[27] Thus, although a detailed half life of intestinal CRBP(II) is unknown at present, it seems likely that the amount and the quality of dietary protein will not influence the CRBP(II) content. However, the nutritional status, such as vitamin A deficiency, also modulates CRBP(II) level.[28] The modulation of CRBP(II) level by the nutritional status appears to be dependent on the animal life stage and the types of nutrients.

This study provides the first evidence that the manipulation of dietary protein may affect the esterification of absorbed retinol in the small intestine. The specific and total activities of jejunal LRAT were significantly decreased in the rats fed the 20% wheat gluten diet, and feeding rats the 5% casein diet also led to a slight decrease in the total LRAT activity *(Figure 1)*. The difference in the magnitude of the decrease in jejunal LRAT activity caused by these two dietary manipulations may be ascribed to the fact that the 20% wheat gluten diet contains fewer essential amino acids, e.g., lysine, than the 5% casein diet. By contrast, the ARAT activities in jejunum were apparently unaffected by the protein malnutrition state used in the present study *(Figure 2)*.

Therefore, we concluded that intestinal LRAT activity, but not intestinal ARAT activity and CRBP(II), is readily modulated by the amount and/or the quality of dietary protein. As regards the difference in the sensitivity to dietary protein manipulations between LRAT and CRBP(II), we speculate that LRAT might be richer in essential amino acids than CRBP(II).

We have previously found that intestinal LRAT activity was decreased by the dietary depletion of vitamin A in chicks at early ages after birth.[28] Ong et al.[29] reported that at early ages (before weaning) the LRAT activity in rats was very high, followed by a gradual decline of the activity to the adult level, which was lower than that of suckling rats. Randolph and Ross[30] reported that the intestinal LRAT activity in vitamin A-depleted rats did not differ from that of vitamin A-adequate rats, suggesting that in adult animals the LRAT activity might be independent of vitamin A state. In contrast to the vitamin A effect, alteration of dietary protein level seems to modulate the LRAT activity in adult animals.

Feeding the 20% wheat gluten diet caused a remarkable decrease in the unesterified retinol and retinyl palmitate contents expressed per mg of mucosa DNA or per mucosa mass of the rat jejunal segment. The extent of these decreases was greater in the unesterified retinol content (by 64%) than in the retinyl palmitate content (by 32% to 49%) *(Table 3)*. The finding that the unesterified retinol and total protein contents in the jejunal mucosa were very low *(Tables 2, 3)*, was not consistent with the fact that the CRBP(II) level unchanged in animals fed diet containing the low quality protein. This result would suggest that retinol uptake was lowered due to an impaired carrier protein, which may be involved in retinol uptake into enterocytes. The reduced retinyl palmitate level may relate to the lowered LRAT activity, which was caused by the protein malnutritional status *(Figure 1)*.

Previous studies have suggested the existence of a membrane-associated carrier system for retinol absorption.[31,32] Their findings indicated that retinol uptake was saturable and its saturation was presumably the result of interaction of retinol with a carrier protein. The assumption that CRBP(II) works as the carrier protein was excluded by the study conducted by Said et al.[32] They showed that the excess cold retinol inhibited the labeled retinol uptake in jejunal everted sacs, but the excess cold retinal did not inhibit it, and suggested that the carrier protein involved in retinol uptake was not CRBP(II) because retinal competes with retinol for binding to CRBP(II).[2] Our results demonstrated that jejunal CRBP(II) level was unchanged, while the unesterified retinol level was lowered with the concomitant decrease of protein content in jejunal mucosa of rats fed the protein malnutritional diet, suggesting the existence of a carrier protein for retinol uptake, which might be modulated by the protein nutrition status.

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