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Low protein diets posttranscriptionally repress apolipoprotein B expression in rat liver

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In an attempt to elucidate molecular mechanisms related to low protein diet-induced fatty livers, apolipoprotein (apo) mRNA and its expression in the liver were studied in rats. Rats were fed for 14 days a diet containing 5% and 20% casein, and whey protein equivalent in protein to these caseins. In the protein-deficient groups, liver triacylglycerols elevated to 1.5-2 fold higher than did the diet sufficient groups. The mRNAs for apo A-IV, apo B and apo E were 20 to 50% lower in the deficient groups than in the sufficient groups, but apo A-I mRNA was not reduced. The rate of transcription of apo A-IV, B, and E genes in hepatic nuclei isolated from those animals was not altered with different levels of dietary protein. Apo B concentration in the serum was lowered and correlated positively with apo B mRNA abundance but the concentrations of apo IV and apo E were elevated in the deficient groups. It thus appears that a low protein diet decreases apo B synthesis through a posttranscriptional mechanism and thereby depresses secretion of triacylglycerol-rich lipoproteins from the liver. (J. Nutr. Biochem. 7:381–385, 1996.)

Keywords: apoprotein mRNA; expression of apoprotein gene; low protein diet

Introduction

Most studies show that rats develop a fatty liver when fed a low-protein diet, the alteration of liver lipid transport that may result from an impaired synthesis of apolipoprotein (apo) in very low density lipoproteins (VLDL).[1–5] A depressed ability of hepatic cells to assemble and secrete triacylglycerol-rich lipoproteins appears to be regulated to a decreased synthesis of apo B, a major apoprotein in hepatic VLDL.[1–3] Davis et al. showed that a specific reduction in apo B synthesis was associated with a reduction in the capacity to secrete triacylglycerols from rat hepatocytes.[6]

Experiments with animals revealed that protein deficiency resulted in decreased mRNA of secretory proteins in the liver including albumin, transferrin, transthyretin, and β-fibrinogen.[7–9] A search of the literature revealed no documentation of the effect of a low protein diet on an apoprotein gene expression in the rat liver, except for one report on a reduction of apo E mRNA.[8] We report here that a low protein diet led to a decreased mRNA for apo E as well as apo A-IV and apo B, but no apo A-I, and reductions of these apo mRNAs could not be attributed to mechanisms of transcription.

Methods and materials

Animals and diets

Five-week-old male ExHC rats, bred in Animal Facilities of Kyushu University, were maintained on a commercial nonpurified diet (NMF, Oriental Yeast Co., Tokyo, Japan) for 1 week before feeding them the experimental diets. ExHC rats are established strains isolated from Sprague-Dawley rats exhibiting hypercholesterolemia upon feeding cholesterol-containing diets, but maintaining a normal cholesterol level when on a cholesterol-free diet.[10] The rats were divided into four groups of six, according to the

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dietary protein source (casein and whey protein) and a level (sufficient and low). Casein (a chemical grade), composed of approximately 86% protein, was purchased from Wako Pure Chemicals Co., Osaka, Japan and whey protein concentrate, approximately 74% protein, was from Snow Brand Milk Co., Kawagoe, Japan. Casein at the level of 5% and 20%, respectively, or whey protein isolate at the level of 5.8% and 23.2%, respectively, were added to the purified diet recommended by the American Institute of Nutrition[11] as a source of protein. Ingredients other than protein were as follows (by wt %): 10 soybean oil, 1.0 vitamin mixture (AIN^{TM76}), 3.5 mineral mixture (AIN^{TM76}), 0.2 choline bitartrate, 0.3 DL-methionine, 5.0 cellulose, 15 corn starch, and to 100 at the expense of sucrose. Rats, maintained freely on one of the four diets and water for 14 days, were anesthetized with diethyl ether and killed by withdrawing aortic blood, the livers were immediately isolated.

Determination of mRNA

The determination of mRNA for apo A-I, A-IV, B and E, and β-actin was carried out as described previously.[12,13] Total RNA of the liver was isolated by ultracentrifugation, according to the method of Chirgwin et al[14] and blotted onto a nitrocellulose filter using a dot blot apparatus (Bio Rad Japan, Tokyo, Japan). The cDNAs for apo A-IV, B and E from rats, β-actin cDNA from humans, and a 30-oligomer corresponding to apo A-I cDNA were labeled with [γ-^{32}P]ATP (Amersham Japan, Tokyo, Japan) by the 5′-terminal labeling system, according to the instruction of manufacturer (MEGALABEL, Takara Shuzo Co., Kyoto, Japan) and hybridized for 36 hr at 65°C. After washing filters in 2 × SSC (0.3 M NaCL, 0.1 M sodium citrate) –0.1% SDS at room temperature and then in 0.2 × SSC (0.03 M NaCL, 0.01 M sodium citrate) –0.1% SDS at 65°C, they were exposed to x-ray films and autoradiograms of the blots were analyzed by quantitative scanning densitometry. Results for apoprotein mRNA abundance were corrected for variations in the amount of RNA loaded on the blots by normalization to the signal for β-actin mRNA.

Determination of the synthetic rate of apoprotein mRNA

Nuclei were isolated from the fresh liver and used for determination of newly synthesized mRNA, as described previously.[12,13] The number of nuclei was determined spectrophotometrically[13] and the nuclei of 8×10^7 were incubated with [α-^{32}P]UTP (Amersham Japan) at 30°C for 30 min. Newly synthesized RNA (1×10^7 cpm) was hybridized onto Zeta Probe membranes (Bio Rad Japan) with denatured plasmids containing apo A-IV, B and E sequences for 48 hr at 42°C. Radioactivities of individual blots were counted in a liquid scintillation counter (Tri-curb 2250, Packard Japan, Tokyo). The relative rates of apo A-IV, B and E mRNA synthesis (expressed in parts per million) were calculated by subtracting the counts per minute of [^{32}P] bound to the filters containing nonrecombinant plasmids from the counts per minute of [^{32}P] bound to the filters with plasmids containing the apo A-IV, apo B or apo E inserts, and by dividing the counts per minute of [^{32}P] bound by the [^{32}P] RNA input.

Analyses of lipids and apoproteins

Sera were separated from blood samples, at room temperature, by brief centrifugation. The liver lipids were extracted according to the method of Folch et al.,[15] and cholesterol, triacylglycerols and phospholipids were determined by the methods of Sperry and Webb,[16] Fletcher,[17] and Rouser et al,[18] respectively. Serum triacylglycerols and cholesterol were measured, using enzyme assay kits (Wako Pure Chemicals Co.). Concentrations of serum apo A-I, A-IV, B and E were measured by the rocket immunoelectrophoresis, as described previously.[12,13]

Statistical analysis

Data were analyzed by one way analysis of variance (ANOVA), followed by assessment of differences by Duncan's multiple range test.[19] Effects of the level and the source of dietary proteins were evaluated by two-way ANOVA.[20]

Results

Growth parameters

As shown in *Table 1,* body weight gain and feed efficiency were lower in rats fed low protein diets than in those fed sufficient protein diets, whereas liver weight was greater in the low protein groups than in the sufficient groups. Whey protein diets as compared with the corresponding casein diet resulted in a greater body weight gain, feed efficiency, and liver weight.

Liver and serum lipids

Table 2 shows concentrations of liver and serum lipids. Low protein diets resulted in an increase of the liver triacylglycerols, as compared with sufficient diets and the protein effect was more prominent in the casein group. In contrast, the

Table 1 Effect of dietary proteins on body weight gain, food intake, feeding efficiency, and relative liver weight

Group	Body weight gain	Food intake	Feed efficiency	Relative liver weight
	(g/14 day)	(g/14 day)		(g/100 g body wt)
Casein sufficient	31.3 ± 3.0^a	255 ± 15^a	0.11 ± 0.03^a	3.18 ± 0.05^a
Casein low	4.0 ± 9.2^a	258 ± 9^a	0.01 ± 0.02^b	3.68 ± 0.05^b
Whey sufficient	43.3 ± 3.2^c	215 ± 4^b	0.25 ± 0.01^c	4.19 ± 0.10^b
Whey low	13.4 ± 3.3^d	243 ± 5^a	0.08 ± 0.01^a	4.47 ± 0.06^c
ANOVA				
Source	0.01	0.01	0.01	0.01
Level	0.01	NS	0.01	0.01
Interaction	NS	NS	NS	0.01

Values represent means ± SE for six rats.
NS: not significantly different.
^{abcd}Values without a common superscript letter are significantly different at $P < 0.05$.

Table 2 Effect of dietary proteins on liver and serum lipids

Group	Liver (μmol/g)			Serum (mmol/L)	
	Triacylglycerols	Cholesterol	Phospholipids	Triacylglycerols	Cholesterol
Casein sufficient	15.5 ± 0.7[a]	15.8 ± 0.8[a]	32.9 ± 1.5[a]	1.35 ± 0.21[ab]	3.19 ± 0.24[a]
Casein low	31.2 ± 1.6[b]	14.0 ± 0.8[a]	21.3 ± 0.4[b]	1.76 ± 0.16[a]	2.54 ± 0.12[b]
Whey sufficient	13.2 ± 1.1[a]	15.3 ± 0.5[a]	31.9 ± 0.6[a]	1.56 ± 0.09[a]	2.29 ± 0.08[b]
Whey low	20.1 ± 0.6[c]	9.6 ± 0.5[b]	22.9 ± 0.6[b]	1.05 ± 0.08[b]	1.60 ± 0.04[c]
ANOVA					
Source	0.01	0.01	NS	NS	0.01
Level	0.01	0.01	0.01	NS	0.01
Interaction	0.01	0.01	NS	0.01	NS

Values represents mean ± SE for six rats.
NS: not significantly different.
[abc]Values without a common superscript letter are significantly different at $P < 0.05$.

concentrations of the phospholipids and cholesterol were lower in low protein group than in the corresponding sufficient group, and higher in the casein group than in the corresponding whey protein group. Although the level of dietary casein did not affect serum triacylglycerols, sufficient whey protein-fed rats had a higher concentration of triacylglycerols, as compared with low protein-fed rats.

Abundance and synthetic rate of apoproteins mRNAs

As shown in *Figure 1,* a low protein diet, as compared with the corresponding sufficient diet, resulted in a marked reduction of hepatic mRNAs for apo A-IV, B and E, but the apo A-I mRNA abundance was not influenced by the type and level of dietary protein. Although, according to the ANOVA as shown in the legend to *Figure 1,* the type of dietary protein had little influence on the abundance of these mRNAs, sufficient casein-fed rats had higher apo B mRNA than did the corresponding low protein-fed rats. The rates of the newly synthesized mRNAs for apo A-IV, B and E were not affected by the amount and type of the dietary proteins (in ppm): apo A-IV, from 3.2 to 4.4; apo B, from 3.3 to 4.4; apo E, from 4.2 to 5.6. The rate for apo A-I mRNA was not measured because the 30-oligomer corresponding to apo A-I cDNA was not large enough to hybridize the newly synthesized mRNA.

Serum apoproteins

As shown in *Figure 2,* the level of dietary protein influenced the serum apoprotein concentration, but in a different manner: sufficient protein diet as compared to the corresponding low protein diet resulted in a higher concentration of apo B than did the corresponding low protein diet whereas a low protein diet resulted in a higher concentration of apo A-IV and E than did the corresponding sufficient protein diet. The concentration of serum apo B was correlated positively to the apo B mRNA abundance in the liver ($r = 0.73$, $P < 0.001$); such a correlation was not seen for apo A-I, A-IV and E. Consumption of the diet with low casein also resulted in a higher apo A-I concentration than did the sufficient counterparts.

Discussion

Our study confirmed that low protein diets composed of either casein or whey protein elevated triacylglycerols in the liver.[1–5] Because triacylglycerols in the liver are secreted as triacylglycerol-rich lipoproteins in association with apoproteins and lesser hydrophobic lipids such as phospholipids and cholesterol, reduction in the synthesis of these lipoprotein-constituents results in a decreased secretion of triacylglycerols, and triacylglycerols accumulate in the liver.[6,21] Other studies showed a decreased incorporation of radioactive amino acids into VLDL-apoproteins in rats fed low protein diets,[2,3] thereby reflecting a decreased synthesis of VLDL-apoproteins. The present experiments provide evidence at the molecular level that a reduction in apoprotein synthesis in the liver of rats fed a low protein diet was attributed to a lowered abundance of the mRNAs for VLDL-apoproteins, especially apo B, which is a major constituent of nascent VLDL isolated from the endoplasmic reticulum and the Golgi apparatus in rat livers.[22,23] Although the synthetic rate of apo B was not directly measured in the present experiments, a positive correlation between serum apo B and the liver mRNA suggests that availability of the translatable apo B associated with triacylglycerolsin the liver was not sufficient to prevent steatosis in rats fed low protein diets. In contrast to the effect on the abundance of the liver apo B mRNA, low protein diets did not affect the rates of apo B mRNA; thus, the abundance of apo B mRNA in rats fed low protein diets may be regulated by a posttranscriptional mechanism such as stability of mRNA.

Low protein diets[9] or protein-deficient diets[8] were reported to decrease mRNAs for albumin, fetoproteins, ferritin, and apo E in the rat liver. In the present experiments, in addition to apo B and E mRNAs, the reduction of apo A-IV mRNA abundance in the liver was observed in rats fed low-protein diets. In analogy to apo B mRNA, the abundance of apo A-IV and E mRNAs also appeared to be regulated by a posttranscriptional mechanism because the rate of these mRNAs was not altered in rats fed low-protein diets. In contrast to apo A-IV, B and E mRNAs, apo A-I mRNA abundance was not lowered in rats fed a low protein diet. Since it has been reported that the level of high density lipoproteins (HDL), of which apo A-I is a major protein component, is normal in animals fed a low-protein diet[4,5] or in humans with untreated Kwashiorkor,[24,25] the resistance of HDL to low or deficiency protein diet can be attributed to the lack of response of apo A-I mRNA abundance to low protein diets. Why a low protein diet disproportionately

affected the abundance of apo A-I mRNA and the mRNAs is not clear, but compartmentalization of newly synthesized mRNAs for apoproteins may differ, as discussed by other workers.[26,27]

Previous experiments showed a decreased concentration of apo B_{48} and B_{100}, and apo A-IV and E in the serum VLDL and HDL fractions, respectively, in rats fed low-protein diets.[4,5] In contrast to those experiments, the present experiments revealed that low protein diets did not lower the concentrations of the serum apo A-IV and E, rather there was an elevation of the concentrations. The most probable explanation for the discrepancy between the present and previous experiments is differences in the methods used for determining serum apoproteins: whole serum was subjected to immunological determination of apoproteins in the present study, whereas lipoprotein fractions separated by ultracentrifugation were used for the apoprotein determination in the previous experiments, under which condition it was found that apo A-IV and E were easily dissociated from VLDL and HDL during their isolation by ultracentrifugation.[28] It is possible that catabolism of these apoproteins in the serum in rats fed a low-protein diet was not so rapid as in rats fed a sufficient protein diet, thus, whether or not low protein diets led to a reduction of translational activities of apo E and apo A-IV mRNAs would need to be determined.

Verkade et al found that inhibition of phosphatidylcholine synthesis resulted in impairment of the secretion of triacylglycerol-rich lipoproteins from primary cultured rat hepatocytes.[21] In the present experiments, a low-protein diet resulted in a reduction of liver phospholipids. Thus, in addition to a lowered availability of apo B, a limited association of phospholipids with triacylglycerol-rich lipoprotein particles in the liver may also be involved in the accumulation of liver triacylglycerols in case of protein deficiency.

Although the proteins used in our experiment are nutritionally adequate for adult rats,[11] a whey protein diet as compared with the corresponding casein diet improved feeding efficiency and increased the relative liver weight. These apparent differences in growth parameters, however, appeared to have no significant correlation with the abundance of hepatic apoprotein mRNAs and their rates of transcription. In addition, a low whey diet as compared with the

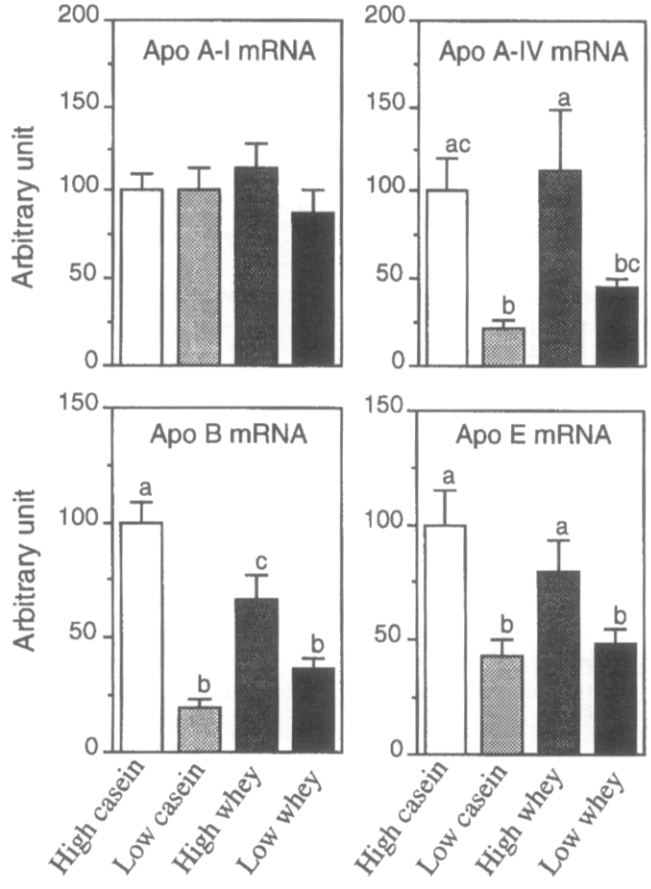

Figure 1 Effects of dietary proteins on the abundance of apoprotein mRNA in liver. Values are presented as means expressed as a percentage of the mean value for the group fed sufficient casein for each blot; hence, each column and vertical bar represent mean ± SE for six rats. [abc]Values without a common letter are significantly different at $P < 0.05$. ANOVA with significant difference was as follows: $P < 0.01$ for level and interaction in apo A-IV mRNA; $P < 0.01$ for level and interaction in apo B mRNA; $P < 0.01$ for level and $P < 0.05$ for interaction in apo E mRNA.

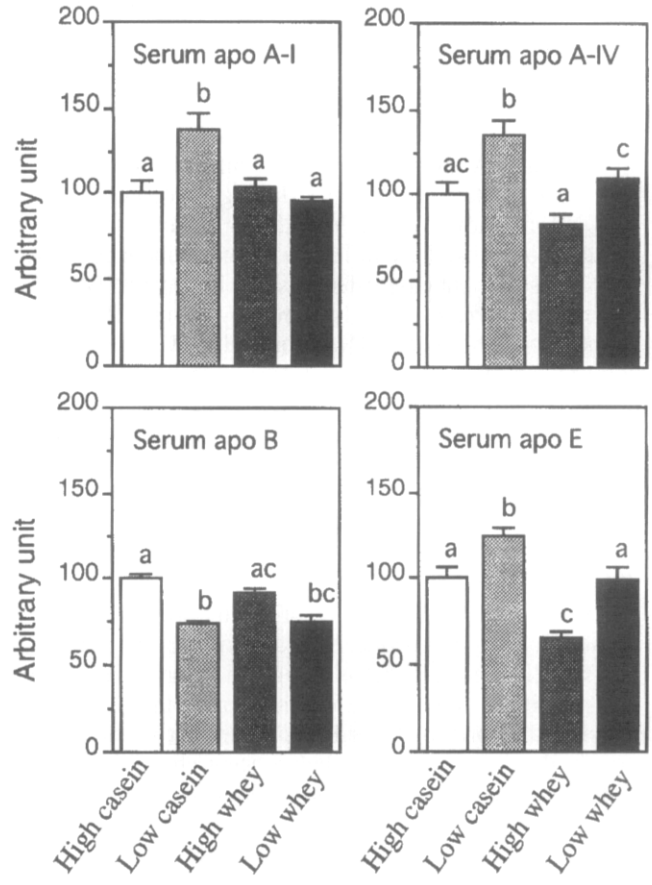

Figure 2 Effects of dietary proteins on the concentration of serum apoproteins. Values are presented as means expressed as a percentage of the mean value for the group fed sufficient casein for each rocket height; hence, each column and vertical bar represent mean ± SE for six rats. [abc]Values without a common letter are significantly different at $P < 0.05$. ANOVA with significant differences was as follows: $P < 0.01$ for source, $P < 0.05$ for level and $P < 0.01$ for interaction in apo A-I; $P < 0.01$ for source and level in apo A-IV; $P < 0.01$ for level in apo B; $P < 0.01$ for source and level in apo E.

corresponding casein diet led a decreased accumulation of liver triacylglycerols, suggesting that quality of dietary protein may ameliorate liver lipid accumulation.[29]

In the present study, whey protein diets resulted in a lower concentration of cholesterol in the liver and serum, and apoproteins other than apo B in the serum than did casein diets. Inconsistent results are, however, reported on the effects of whey protein diets and casein diets on the concentration of serum cholesterol in rats.[30,31] These discrepancies may attribute to the composition of diets as well as a strain of rats because ExHC rats, used in the present experiment, are a sensitive strain to dietary cholesterol.[10] In any events, a mechanism for hypocholesterolemic activity of whey protein remains to be solved.

We tentatively conclude that low protein diets posttranscriptionally suppress synthesis of VLDL-apoproteins, particularly apo B, and thereby the secretion of triacylglycerol-rich lipoproteins from the liver is impaired.

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