Dietary Protein-dependent Changes in Rat Intestinal 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Activity

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The effects of dietary protein on intestinal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase were studied in rats. When the rats' diet was changed from a commercial non-purified diet to a purified diet containing either soybean protein or casein as a protein source, the reductase activity in the jejunum increased progressively while that in the ileum decreased. The extent of the change in the jejunal activity was prominent in rats fed soybean protein. When rats were given a purified diet containing casein for 8 days then a purified diet containing soybean protein, the enzyme activity increased progressively both in the jejunum and ileum. The protein-dependent change became apparent within 1 to 3 days. Among the various animal (sardine and lactalbumin) and vegetable (soybean and wheat gluten) proteins tested, soybean protein was the one that had a specific effect on the intestinal reductase. The contents of mucosal cholesterol was essentially the same except for a slight higher level for the ileum from rats fed lactalbumin. The concentration of serum cholesterol were lower in rats fed vegetable proteins, while the hepatic cholesterol-lowering action of dietary protein was evident only with soybean protein.

Cholesterol metabolism in experimental animals and humans is profoundly influenced by the type of dietary protein. It is now well established that vegetable protein as compared with animal protein has a significant antihypercholesterolemic effect in experimental animals with alterations in many aspects of cholesterol metabolism.¹⁾

Recent studies have indicated that long-term feeding of soy protein in relation to casein causes a significant increase in the rate of cholesterogenesis and the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, a rate limiting enzyme in cholesterogenesis, both in the liver and small intestine,^{2~4)} the major organs which contribute endogenous cholesterol in animals. However, the details of this phenomenon and the mechanism responsible for these protein-dependent changes, especially in the small intestine are not yet clear. Here, we report some aspects

of protein-dependent alterations in the activities of HMG-CoA reductase in rat small intestine.

MATERIALS AND METHODS

Materials. DL-[3-¹⁴C]3-Hydroxy-3-methylglutaryl-CoA was synthesized chemically from DL-[3-¹⁴C]3-hydroxy-3-methylglutaric acid (Amersham International plc Buckinghamshire) and CoA (Oriental Yeast Co., Tokyo) as described previously.⁵⁾ Various proteins employed for dietary studies were obtained from the following sources; milk casein from Wako Pure Chemicals, Osaka, soy protein isolate from Fuji Oil Co., Osaka, wheat gluten and lactalbumin from Tokyo Kasei Co., Tokyo and sardine protein from Niigata Tekkosho Co., Tokyo. The protein contents of these preparations were 90, 84, 74, 77, and 89%, respectively. Other chemicals used were of reagent grade.

Animals and diets. Male Sprague-Dawley rats weighing 150~180 g purchased from Seiwa Experimental Animals, Fukuoka were used throughout. Animals were housed individually in an air-conditioned room in which the

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temperature was 22~24°C, the light was on from 08:00 to 20:00 hr, and experimental diets and water were freely available. The composition of the basal diet was, in weight per cent: protein 20; mineral mixture 4; vitamin mixture 1; choline chloride 0.15; cellulose powder 2; corn oil 5, and sucrose to 100. Mineral and vitamin mixtures as described by Harper⁶⁾ were purchased from Oriental Yeast Co., Tokyo. Detailed experimental protocols are given in the RESULTS section.

Assay of intestinal HMG-CoA reductase. The small intestine from the ligament of Treitz to the ileocecal valve was divided into four segments of equal length. Intestinal cells in the upper (jejunum) and lower (ileum) fourth were isolated.^{7,8)} The activity of HMG-CoA reductase was measured in the homogenate of the isolated intestinal cell as described in detail previously.⁸⁾ The protein content in the cell homogenate was measured by the method of Lowry *et al.*⁹⁾

Lipid analysis. Lipids in liver and blood serum were measured as described previously.⁵⁾ Mucosal cholesterol was measured in the nonsaponifiable lipid fraction using an assay kit (cholesterol B-test, Wako Pure Chemicals Co., Osaka).

Statistical analysis. Data were analyzed either by the one or two way analysis of variance where appropriate. Where the former analysis was adopted, differences between pairs of means were all inspected.¹⁰⁾

RESULTS

In the first trial, the commercial stock diet (Type NMF, Oriental Yeast Co., Tokyo) was replaced by the purified experimental diets containing either soybean protein or casein and changes in the activities of intestinal HMG-CoA reductase were measured for 5 days. No difference was found in the growth and food intake between the two groups of rats.

As shown in Fig. 1, as has been demonstrated previously,¹¹⁾ the purified diet evoked a striking increase in the activity of jejunal HMG-CoA reductase in both groups. The increment was, however, more substantial in rats fed soybean protein. Two way analysis of variance showed significant ($p < 0.05$) protein- and time-dependent changes in the enzyme activities. In contrast, ileal activities in both groups of rats tended to decrease in the course of the feeding period. Although the difference

FIG. 1. Changes in the Activities of Intestinal HMG-CoA Reductase after Replacement of a Non-purified Diet with Purified Diets Containing either Soybean Protein or Casein.

Rats weighing approximately 140 g were fed on a commercial non-purified diet for 10 days then the purified diet was given. Rats were killed on the indicated days and jejunal and ileal HMG-CoA reductase activities were measured. Values represent means $\pm$ SE of 3 rats except for those of 6 rats on day 0. —○—, soybean protein (jejunum); —●—, soybean protein (ileum); —□—, casein (jejunum); —■—, casein (ileum).

FIG. 2. Changes in the Serum Cholesterol Levels after Replacement of a Non-purified Diet with Purified Diets Containing either Soybean Protein or Casein.

Experiments were the same as described in the legend to Fig. 1. —○—, soybean protein; —□—, casein.

was less prominent than that found in jejunal reductase, the contour of the change appeared to depend on the type of dietary protein.

Although there was a temporary reduction of serum cholesterol level one day after the replacement of the diet in both groups of rats (Fig. 2), it thereafter increased in the casein group and returned to the initial level in the

FIG. 3. Changes in the Activities of Intestinal HMG-CoA Reductase after Replacement of a Purified Diet Containing Casein with That Containing Soybean Protein.

Rats weighing approximately 150 g were fed casein for 8 days and then changed to a soybean protein. Rats were killed on the indicated days, and jejunal and ileal HMG-CoA reductase activities were measured. Rats fed casein throughout the experimental period were also killed at the end of the experiment. Values represent means $\pm$ SE of 3 to 4 rats. —○—, soybean (jejunum); —●—, soybean (ileum); □, casein (jejunum), and ■, casein (ileum). *Significantly different from the value at day 0 at $p < 0.05$. + Significantly different from the corresponding values for rats fed casein throughout at $p < 0.05$.

soy protein group. Statistical examination by two way analysis of variance revealed that protein- and time-dependent changes were significantly different ($p < 0.05$).

In the next trial, the effects of replacement of dietary casein by soybean protein on the activities of small intestinal reductase were examined. Rats were fed casein for 8 days and then fed soybean protein periodically. One group of rats ate a casein diet throughout. As shown in Fig. 3, both jejunal and ileal activities increased for 3 days and reached a plateau thereafter. The increase was clearly more prominent in the jejunum than ileum. Although the activities of rats eating a casein diet throughout the experimental period also increased considerably, the values were significantly lower than corresponding values in rats fed soy protein.

The concentrations of mucosal cholesterol were not influenced by the replacement of dietary proteins throughout the experimental period both in the jejunum and ileum although

FIG. 4. Changes in the Serum Lipid Levels after Replacement of a Purified Diet Containing Casein with That Containing Soybean Protein.

The experiment was the same as that described in the legend to Fig. 3. —○—, soybean (cholesterol); —□—, soybean (triglyceride); —△—, soybean (phospholipid); ●, casein (cholesterol); ■, casein (triglyceride) and ▲, casein (phospholipid). *Significantly different from the value at day 0 at $p < 0.05$. + Significantly different from the corresponding values in rats fed casein throughout at $p < 0.05$.

they were consistently higher in the latter. The initial and final cholesterol values were in $\mu\text{g}/\text{mg}$ protein 26.4 ± 1.1 and 27.3 ± 0.8 for the jejunum and 45.9 ± 2.2 and 39.7 ± 5.3 for the ileum, and the jejunal and ileal values for rats fed casein throughout were 28.0 ± 1.8 and 40.0 ± 3.6 , respectively.

The levels of serum triglyceride and cholesterol decreased soon after the replacement of the dietary protein (Fig. 4). A similar but less pronounced decline in the phospholipid level was also observed. Feeding casein for an additional 7 days did not influence the serum lipid levels.

Similar contours of changes were also observed in the concentrations of hepatic lipids. However, the decrease in the phospholipid was regarded as representing a normal developmental process as the value after 7 days of feeding of soybean protein was indistinguishable from that observed in rats fed casein throughout. The initial and final values and those for rats fed casein throughout were, in mg/g tissue 3.33 ± 0.12 , 1.89 ± 0.15 , and 2.50 ± 0.50 for cholesterol, 31.7 ± 1.3 , 25.2 ± 0.4 , and 25.8 ± 1.40 for phospholipid,

TABLE I. ACTIVITIES OF INTESTINAL HMG-CoA REDUCTASE IN RATS FED DIFFERENT PROTEINS

Dietary protein	HMG-CoA reductase activities			
	Specific activity (pmol/min/mg protein)		Total activity (nmol/hr/segment)	
	jejunum	ileum	jejunum	ileum
Soybean protein	426 ± 48 ^a	254 ± 34 ^a	872 ± 101 ^a	112 ± 15 ^a
Wheat gluten	347 ± 21 ^{ab}	201 ± 29 ^{ab}	578 ± 48 ^b	70 ± 9 ^b
Lactalbumin	356 ± 26 ^{ab}	138 ± 22 ^b	627 ± 56 ^b	29 ± 4 ^c
Sardine protein	296 ± 37 ^b	186 ± 5 ^{ab}	459 ± 45 ^b	76 ± 21 ^b

Values represent means ± SE of 6 rats. Rats weighing approximately 200 g were given purified diets containing different protein for 7 days. Values in a column not sharing a common superscript letter are significantly different at $p < 0.05$.

TABLE II. SERUM AND HEPATIC LIPID LEVELS IN RATS FED DIFFERENT PROTEIN

Dietary protein	Serum lipids (mg/dl)			Hepatic lipids (mg/g)		
	Chol	TG	PL	Chol	TG	PL
Soybean protein	71.2 ± 3.7 ^a	160 ± 35 ^a	203 ± 16 ^{ab}	1.92 ± 0.11 ^a	13.9 ± 2.2 ^a	28.7 ± 0.7 ^a
Wheat gluten	69.3 ± 3.3 ^a	203 ± 21 ^a	202 ± 5 ^a	2.36 ± 0.11 ^b	25.4 ± 3.7 ^a	22.4 ± 0.8 ^b
Lactalbumin	73.0 ± 2.8 ^a	253 ± 28 ^a	206 ± 6 ^a	2.37 ± 0.16 ^b	27.5 ± 6.6 ^a	28.0 ± 0.3 ^a
Sardine protein	103.3 ± 4.8 ^b	328 ± 57 ^b	282 ± 8 ^b	2.65 ± 0.15 ^b	56.5 ± 8.4 ^b	27.8 ± 0.5 ^a

Values represent means ± SE of 6 rats. The experiment was the same as that described in the footnote of Table I. Values in a column not sharing a common superscript letter are significantly different at $p < 0.05$. TG, triglyceride; Chol, cholesterol; PL, phospholipid.

and 24.9 ± 5.1 , 12.0 ± 0.2 , and 29.5 ± 7.2 for triglyceride, respectively.

These observations clearly demonstrated that the intestinal HMG-CoA reductase and cholesterol metabolism were rapidly and profoundly modulated by the difference in the type of dietary protein. Based on these observations, the activities of intestinal HMG-CoA reductase in rats fed various vegetable and animal proteins were examined in the next experiment. Rats weighing approximately 200 g were fed purified diets containing either soybean protein, wheat gluten, lactalbumin, or sardine protein for one week. Food intake in rats fed wheat gluten (19 ± 0 g/day) was slightly but significantly lower than that in rats fed other proteins (22 ± 1 , 21 ± 1 , and 23 ± 1 g/day for soybean protein, lactalbumin and sardine protein, respectively). Rats fed animal protein grew more than those fed vegetable

protein and the growth of rats fed wheat gluten was extremely low (35 ± 3 , 9 ± 2 , 48 ± 4 , and 57 ± 2 g/7 days for soybean protein, wheat gluten, lactalbumin, and sardine protein, respectively).

As Table I shows, soybean protein tended to elevate the reductase activity both in the jejunum and ileum as compared with other protein sources. When the activities were expressed in terms of total activity (nmol/hr/segment), the differences were further exaggerated.

Cholesterol concentrations in jejunal mucosa were apparently the same among the various groups (31.6 ± 1.8 , 28.5 ± 1.0 , 33.7 ± 1.4 , and 31.3 ± 1.4 μ g/mg protein for soybean protein, wheat gluten, lactalbumin, and sardine protein, respectively). On the while, concentrations of ileal cholesterol in rats fed lactalbumin (64.6 ± 1.5 μ g/mg protein) were

distinctly higher than those in other groups (48.7 ± 2.5 , 46.4 ± 2.0 , and $51.6 \pm 1.8 \mu\text{g}/\text{mg}$ protein for soybean protein, wheat gluten, and sardine protein, respectively). The values in the ileum were again considerably higher than those in jejunum.

Serum lipid concentrations in rats fed fish protein appeared to be higher than in those fed vegetable protein (Table II). Concentrations of hepatic cholesterol and triglyceride in rats fed soy protein were the lowest among the various groups. Rats fed gluten showed the lowest hepatic phospholipid content among the groups of animals fed different protein.

DISCUSSION

Recent observations indicated that the type of dietary protein affected hepatic and intestinal cholesterologenesis in rats. Reiser *et al.*²⁾ found dietary soy protein as compared with casein enhanced HMG-CoA reductase in the liver. Nagata *et al.*³⁾ confirmed this observation and also found a stimulation by soybean protein of the incorporation of intraperitoneally administered [$1\text{-}^{14}\text{C}$] acetate into cholesterol in the small intestine. The latter observation was further sustained by the finding that soybean protein increased the activities of intestinal HMG-CoA reductase.⁴⁾ The enhancement by soybean protein of intestinal HMG-CoA reductase was again confirmed in this study. Moreover, protein-dependent alterations in the activities of HMG-CoA reductase as well as the lipid contents in serum and liver were established in a relatively short period, within 3 days. The rapid response to dietary protein implies the participation of a short-term regulation mechanism including phosphorylation-dephosphorylation of the enzyme molecule.^{8,12)} We had demonstrated that this type of mechanism is functioning in the diet-induced changes in intestinal HMG-CoA reductase activities.¹³⁾

In general, vegetable protein gave lower serum cholesterol levels than animal protein in rats.^{1,3,4,14)} Among the various proteins tested here, soybean protein specifically stimulated

the reductase activity, thus the enhancement of intestinal HMA-CoA reductase did not appear to be a general feature for vegetable protein. However, an apparent untoward effect of the vegetable protein on the growth of animals should be taken into consideration in evaluation of our data. As the response to the different proteins was more prominent in the active site for nutrient absorption, the jejunum rather than the ileum, dietary protein presumably exert its biological effect after being digested and absorbed by the epithelial cells.

Alteration in the tissue content of cholesterol is presumed to be a factor in regulating HMG-CoA reductase.^{15,16)} In this study no apparent change due to the type of dietary protein in the mucosal cholesterol content were seen thus the feedback mechanism may not account for the observed changes in the reductase activity. On the other hand luminal bile acids has been shown to inhibit the reductase activity in the small intestine.¹⁷⁾ However, this mechanism also unlikely to be responsible for the protein-dependent changes in the reductase activity as soy protein enhances the excretion of bile acids.^{1,3,14)}

The active synthesis of cholesterol in the small intestine may be connected with chylomicron formation and rapid cell renewal. Thus, the modulation of one of these processes would affect intestinal cholesterologenesis and hence the activities of HMG-CoA reductase. Indeed, the dietary fat-dependent increase in formation of chylomicrons^{18,19)} and hypertrophy of intestinal mucosa induced by diabetes^{20,21)} are accompanied by an enhanced activity of HMG-CoA reductase. It is not clear which of these mechanisms is responsible for the protein-dependent changes of the enzyme activity. As the soy protein as compared with casein rather depressed chylomicron formation and apo A-I synthesis in the small intestine,⁴⁾ alterations of lipoprotein formation, if any, may not be responsible for the observed changes. The decrease rather than the increase in the concentration of serum cholesterol in rats fed soybean protein favors this assumption. Although information with

regard to the dietary factors which affect cell proliferation in the small intestine is very scanty, it is certain that the dietary manipulation also affects this process.^{22~24)} However, the possibility that dietary protein affects the HMG-CoA reductase through the change in the rate of cell renewal still remains obscure.

In conclusion, the type of dietary protein caused profound alterations in the activities of intestinal HMG-CoA reductase. Modification of the activities due to the dietary manipulations occurred within a fairly short period, suggesting a participation of a short-term regulation mechanism. Among the various proteins tested, soybean protein appeared to have a specific biological action in this respect.

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