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Expression of apolipoprotein A-I mRNA in liver and intestine of cecectomized rats fed beet fiber

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Sham-operated and cecectomized rats were fed a cholesterol-free diet with no added fiber (fiber-free) for 10 days, followed by the diet of 15% beet fiber for 10 days. The plasma cholesterol concentrations in rats fed the fiber-free diet were not significantly different between sham-operated and cecectomized groups. Plasma cholesterol concentrations in sham-operated rats were decreased by feeding the beet fiber diet, whereas those in cecectomized rats did not change. Final plasma total cholesterol concentrations in cecectomized rats were significantly higher than in sham-operated rats. This difference was due mainly to lower HDL cholesterol concentrations. The cecectomized rats also tended to have higher apolipoprotein A-I concentrations in plasma. Northern blot analysis revealed that the relative concentrations of ileal apolipoprotein A-I mRNA were the same in the two groups, while hepatic apolipoprotein A-I mRNA levels were significantly higher in cecectomized rats than in sham-operated rats. These data demonstrate that the cecectomy abolished the hepatic apolipoprotein A-I mRNA-lowering effect of dietary beet fiber, and it is suggested that the cecum plays an important role in the regulation of hepatic apolipoprotein A-I expression which seems to be responsible for the hypocholesterolemic effect of dietary beet fiber. (J. Nutr. Biochem. 6:380–384, 1995.)

Keywords: apolipoprotein A-I; mRNA; small intestine; liver; plasma cholesterol; beet fiber; rats

Introduction

It has been reported that plasma cholesterol concentrations were significantly lower in rats fed a cholesterol-free diet that contained beet fiber than in those fed the diet with no fiber.[1] The difference was mainly due to lower high density lipoprotein (HDL) cholesterol concentrations.[2] In rats, HDL is the main carrier of plasma cholesterol and the major protein component of circulating HDL is apolipoprotein A-I (apo A-I), which is synthesized in both the liver and small intestine.[3–5] Apo A-I synthesis is thought to be important in the regulation of plasma HDL concentrations.[6,7]

Recently we reported that cholestyramine (a bile acid sequestrant), which has no hypocholesterolemic effect, lowers the apo A-I mRNA concentration in the ileum but not the liver in rats fed cholesterol-free diet, whereas beet fiber, which has a hypocholesterolemic effect, lowers the apo A-I mRNA concentration in both the ileum and the liver.[8,9] Beet fiber increases in the bile acid excretion into the feces.[2] These observations suggest that the diminished absorption of bile acid in the intestine may alter ileal apo A-I gene expression but this does not necessarily cause any changes in plasma cholesterol concentrations. It is also suggested that the hypocholesterolemic effect of dietary beet fiber is associated with diminished expression of the hepatic apo A-I gene.

However, the mechanism by which dietary beet fiber lowers hepatic apo A-I mRNA concentration is unclear. Nishimura et al.[2] reported that cecectomy abolishes the plasma cholesterol-lowering effect of dietary beet fiber in

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rats fed a cholesterol-free diet. Thus the cecum may play a role in some changes in lipoprotein metabolism in rats fed beet fiber. So in this study, as a step to understanding the role of the cecum, we have determined the effect of cecectomy on apo A-I mRNA concentrations in the ileum and liver of rats fed beet fiber.

Methods and materials

Animals and diets

Male Wistar rats (Japan SLC, Hamamatsu, Japan), 5-week-old at the start of the experiment, were housed in individual cages in a temperature-controlled (23 ± 2°C) room under a 12 hr light–dark cycle (light: 8:00 a.m.–8:00 p.m.). They were allowed free access to a purified 25% casein fiber-free diet (*Table 1*) and to water prior to the experiment. This diet is used as a standard rat diet in our laboratory since it yields maximal growth rates. After 5 days of consuming the fiber-free diet, rats weighing 138 ± 1 g (n = 12) were divided into two groups of 6 sham-operated and 6 cecectomized rats, and all rats were deprived of food for 24 hr before surgery. They were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, 35 mg/kg of body wt; Abbott Laboratories, North Chicago, IL USA). The cecum was isolated from the terminal ileum and ascending colon by ligation followed by resection. Only the abdominal cavity of the sham-operated rats were opened and exposed for just ~20 min, the same length of time as required for the cecectomy. All rats were not allowed food for the first 24 hr postoperatively, and then they were fed the fiber-free diet for 3 days for the recovery. Thereafter both groups were fed the fiber-free diet for 10 days (FF period), followed by the diet containing 15% beet fiber (*Table 1*) for 10 days (BF period). The composition of beet fiber (g/100 g) was as follows: moisture, 4.5; total dietary fiber, 81.1 (cellulose, 23.0; hemicellulose, 22.0; pectin, 19.0; lignin, 3.0; unidentified matter, 14.4); protein (N × 6.25), 9.0; lipid, 0.6; sucrose, 1.5; ash, 3.0.[1] Blood samples were collected from the tail vein on days 0, 3, 7, and 10 of the FF period and days 3, 7, and 10 of the BF period for determination of the total plasma cholesterol concentration.

Table 1 Composition of diets

Dietary component	Fiber-free (g/kg of diet)	Beet fiber (g/kg of diet)
Casein*	250	250
Sucrose	647	497
Corn oil†	50	50
Mineral mixture‡	40	40
Vitamin mixture§	10	10
Vitamin E‖	1	1
Choline chloride	2	2
Beet fiber	—	150

*Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand).
†Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg of diet) were added to corn oil.
‡The mineral mixture is identical with MM2 described by Ebihara et al.[20]
§The vitamin mixture was prepared in accordance with the AIN-76 mixture,[21] except that vitamin K as menadione and L-ascorbic acid were added to give 5.81 μmol/kg[22] and 284 μmol/kg[23] of diet, respectively.
‖Vitamin E (Granulated, Juvela, Eisai Co., Tokyo, Japan) supplied 423 μmol all-*rac*-α-tocopheryl acetate/kg of diet.

On the last day of the BF period, the animals were decapitated and blood was collected for determination of plasma cholesterol and apo A-I concentrations. The liver was excised and weighed. Liver samples were immediately plunged into liquid nitrogen and stored at −80°C for analysis of mRNA. The small intestine was excised and the luminal contents were washed out with 20 mL of ice-cold saline. The intestine was suspended vertically, and a 15 cm portion of the ileum (20 cm proximal from the ileocecal valve) was excised. The muscosa was scraped off with a glass slide and immediately plunged into liquid nitrogen and stored at −80°C for RNA extraction.

The study was approved by the Hokkaido University Animal Use Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Analyses of plasma cholesterol

Plasma was separated from blood sample by centrifugation at 4°C for 15 min at 1,500 g. Plasma total cholesterol and HDL cholesterol concentrations were measured by enzymatic methods using the Cholesterol C-Test and HDL-Cholesterol Test, respectively (Wako Pure Chemical Industries, Osaka, Japan).

Immunoblotting for plasma apo A-I quantitation

Whole plasma (0.5 μL) were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions,[10] and then electrophoretically transferred to nitrocellulose membrane (Hybond C extra, Amersham International plc., Amersham, UK) in a semidry electroblotting apparatus (Nippon Eido, Tokyo, Japan) at constant current of 170 mA for 1 hr. The blotting buffer contained 125 mM Tris, 960 mM glycine in 20% (vol/vol) methanol, pH 8.3. The membrane was then incubated in a blocking solution of 5% (wt/vol) skim milk in Tris-buffered saline (20 mM Tris-HCl, 150 mM NaCl, pH 7.4), followed by the incubation for 1 hr with a 1:1,000 dilution of the sheep antihuman apo A-I serum (Serotec Ltd., Oxford, UK) in the blocking solution containing 0.05% (wt/vol) of Tween 20. The membrane was then washed three times with Tris-buffered saline containing 0.05% (wt/vol) of Tween 20. Next, the membrane was incubated for 1 hr with a 1:2,000 dilution of a second antibody of rabbit antisheep IgG conjugated with horseradish peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA USA) in the same solution used in the incubation with the first antibody, followed by washing in the same way with the first antibody. Identification of the antigen–antibody complex was performed using Renaissance Western Blot Chemiluminescence Reagent (DuPont NEN Research Products, Boston, MA USA) as recommended by the manufacturer. The relative quantity of apo A-I was estimated by densitometry scanning (Dual-Wavelength Flying-Spot Scanner CS-9000, Shimadzu, Kyoto, Japan). By serial dilution analysis, 0.5 μL of whole plasma on SDS–PAGE provided a signal in the linear range of 0.2 to 1.4 μL of whole plasma for detection of apo A-I (data not shown).

RNA isolation and Northern blot analysis

Total RNA was isolated by the acid guanidium-phenol-chloroform method[11] from ileal mucosa and liver. Samples of total RNA (10 μg/lane) were electrophoresed on denaturing 2.2 M formaldehyde, 1% agarose gel[12] and transferred to nylon membrane (Hybond N^+, Amersham International). Blots were hybridized with an apo A-I probe of the 54 base oligonucleotide as previously described.[9] The probe was 3′ labeled using a nonradioisotopic system, DIG Oligonucleotide Tailing Kit (Boehringer Mannheim, Mannheim, Germany), and prehybridization, hybridization, and detection

were carried out with DIG-Luminescent Detection Kit (Boehringer Mannheim) as recommended by the manufacturer. The relative quantity of mRNA was estimated by densitometry scanning as for the immunoblots. By serial dilution analysis, 10 μg of total RNA on electrophoresis provided a signal in the linear range of 2 to 20 μg of total RNA for detection of apo A-I mRNA (data not shown).

Statistical analysis

Results were expressed as means ± SE and the statistical comparison of the mean were done by Student's *t*-test. Values in the text were means ± SE.

Results

Body weights on day 0 in the FF period were the same and averaged 152 ± 2 and 152 ± 2 g for sham-operated and cecectomized rats, respectively. Body weight gains per 10 days in the FF period were the same: 70 ± 3 and 67 ± 3 g for sham-operated and cecectomized rats, respectively. Similarly, body weight gains per 10 days in the BF period were the same: 71 ± 4 and 69 ± 4 g for sham-operated and cecectomized rats, respectively. Average daily food intakes in the FF period were 14.6 ± 0.5 and 14.3 ± 0.5 g and those in the BF period were 17.8 ± 0.6 and 17.7 ± 0.7 g for sham-operated and cecectomized rats, respectively. There was no significant difference in food intake between sham-operated and cecectomized rats in the two periods.

Figure 1 shows the time course of changes in plasma cholesterol concentration in sham-operated and cecectomized rats. In the FF period, there was no significant difference between the two groups. After 3 days of beet fiber feeding, plasma cholesterol concentrations were significantly lower in sham-operated rats than in cecectomized rats. The lower plasma cholesterol concentrations in sham-operated rats were maintained to day 10 in the BF period. Plasma cholesterol concentrations in sham-operated rats significantly decreased on day 10 in the BF period compared with those on day 10 in the FF period. In contrast, plasma cholesterol concentrations in cecectomized rats were not changed by feeding the beet fiber diet. The changes in plasma cholesterol concentration between each final day in FF and BF periods were significantly greater in sham-operated rats than in cecectomized rats (*Table 2*). Final plasma cholesterol concentrations were significantly lower

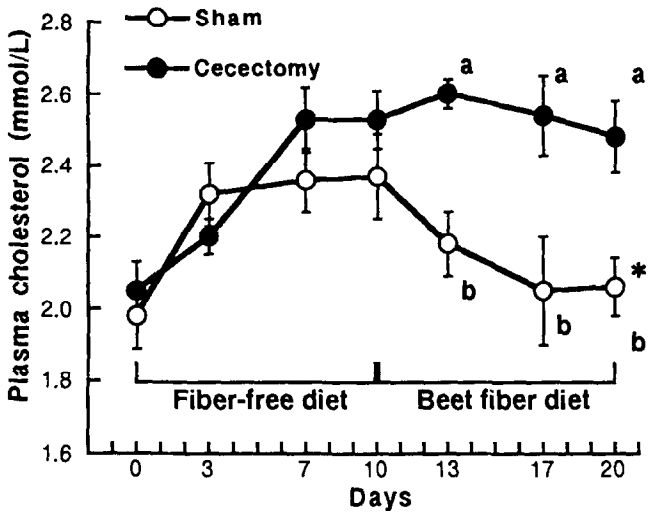

Figure 1 Changes in plasma cholesterol concentrations in sham-operated and cecectomized rats fed a fiber-free diet for 10 days followed by a beet fiber diet for 10 days. Each point is the mean ± SE of 6 rats. Values at each time point with different common letters are significantly different ($p < 0.05$). Values with an asterisk are significantly different ($p < 0.05$) from values at day 10 on fiber-free diet in each group.

in sham-operated rats than in cecectomized rats (*Table 2*). This difference was due mainly to lower HDL cholesterol concentrations. Relative liver weights were the same: 4.77 ± 0.11 and 4.87 ± 0.12 g/100 g of body wt for sham-operated and cecectomized rats, respectively.

Figure 2 shows the relative concentrations of plasma apo A-I in sham-operated and cecectomized rats as estimated by immunoblotting analysis. The value in cecectomized rats was expressed relative to the average value in sham-operated rats which was normalized to 100. Final plasma apo A-I concentrations in cecectomized rats tended to be higher than in sham-operated rats.

The relative quantities of apo A-I mRNA were determined by Northern blot analysis in the ileum and liver (*Figure 3*) The values of apo A-I mRNA on cecectomized rats were expressed relative to the average values in sham-operated rats which were normalized to 100 (*Figure 4*). In the ileum of rats fed beet fiber, the relative quantities of apo A-I mRNA were unaffected by cecectomy. In contrast, the

Table 2 Effect of cecectomy on changes in plasma total cholesterol concentration and final plasma cholesterol concentrations in rats fed the beet fiber diet for 10 days*

Treatments	Changes in plasma total cholesterol†	Final plasma cholesterol		
		Total (mM)	HDL (mM)	VLDL + IDL + LDL‡ (mM)
Sham	−0.31 ± 0.11	2.00 ± 0.10	1.44 ± 0.11	0.56 ± 0.06
Cecectomy	−0.04 ± 0.08	2.49 ± 0.09	1.77 ± 0.12	0.72 ± 0.07
P	<0.05	<0.01	<0.05	<0.1

*Values are means ± SE, $n = 6$ per group.
†Change in plasma total cholesterol concentration was calculated as the difference between the values on day 10 in the FF period and day 10 in the BF period.
‡The concentration of (VLDL + IDL + LDL) cholesterol was calculated as the difference between total plasma cholesterol and HDL cholesterol.

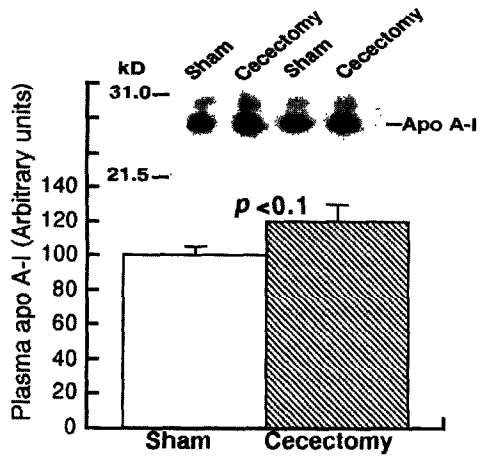

Figure 2 The effect of cecectomy on the relative quantity of plasma apo A-I in rats fed the beet fiber diet for 10 days. Results are the means ± SE of 6 rats. The values in cecectomized rats were expressed relative to the average value in sham-operated animals which was normalized to 100. Inset illustrates the representative immunoblots of whole plasma.

hepatic concentrations of apo A-I mRNA in cecectomized rats fed the beet fiber diet were significantly higher than in sham-operated rats fed the same diet (*Figure 4*).

Discussion

In this study we used the cecectomized rats to gather information about the role of the cecum in the hypocholesterolemic effect of dietary beet fiber. We observed that the plasma cholesterol concentrations in sham-operated rats were decreased by feeding the beet fiber diet, while those in cecectomized animals were unchanged. The final plasma total and HDL cholesterol concentrations in cecectomized rats fed the beet fiber diet were higher than in sham-operated rats fed the same diet. These observations agree with Nishimura et al.[2]

Previous studies showed that the decrease in the plasma cholesterol concentration in rats fed the beet fiber was mainly due to a lower HDL cholesterol concentration,[2,9] and that plasma apo A-I concentrations also tended to be lower in rats fed a beet fiber diet than in those fed fiber-free diets.[9] The apo A-I is synthesized in both the liver and small intestine.[3–5] Our previous report showed that beet fiber lowers the apo A-I mRNA concentration in both the ileum and liver, whereas cholestyramine, which has no hypocholesterolemic effect, lowers apo A-I mRNA in the ileum but not the liver; thus it was suggested that the hypocholesterolemic effect of dietary beet fiber is associated with diminished expression of the hepatic apo A-I gene. In the present study, the final plasma apo A-I concentration tended to be higher in cecectomized rats than in sham-operated rats. Furthermore, we observed that the hepatic apo A-I mRNA in cecectomized rats fed the beet fiber diet was higher than in sham-operated rats fed the same diet. The data demonstrate

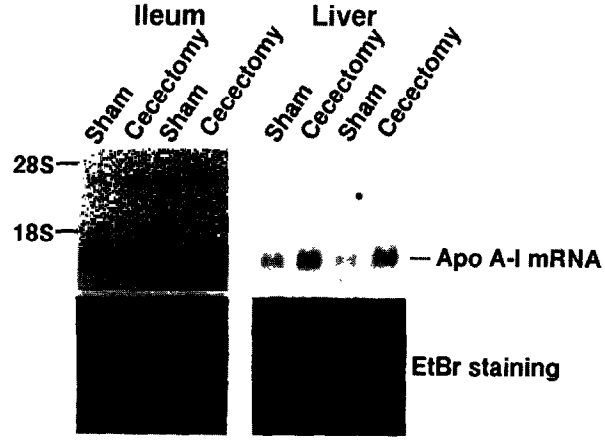

Figure 3 Representative Northern blots of ileal (left) and hepatic (right) RNA. The positions of the 18S and 28S ribosomal RNA are shown. Ethidium bromide staining of agarose gel are also shown (lower).

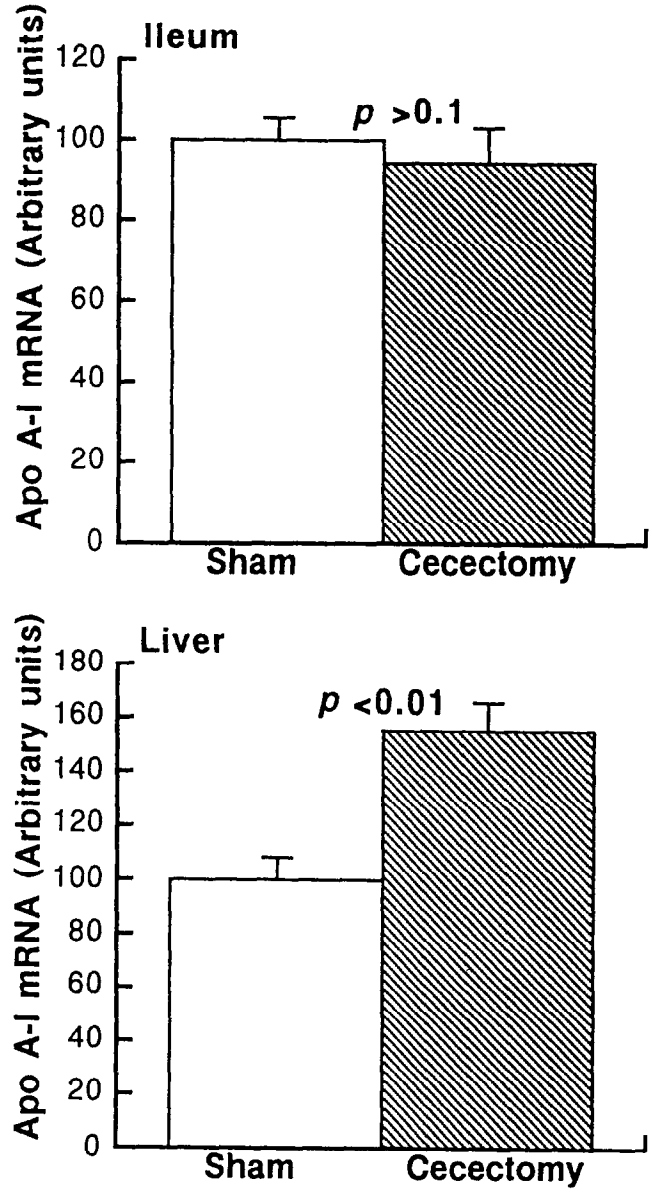

Figure 4 Effect of cecectomy on the ileal (upper) and hepatic (lower) apo A-I mRNA concentrations in rats fed a beet fiber diet for 10 days. Results are the mean ± SE of 6 rats. The values in cecectomized rats were expressed relative to the average value in sham-operated animals which was normalized to 100.

that the cecectomy abolishes the plasma apo A-I- and hepatic apo A-I mRNA-lowering effects of dietary beet fiber. Thus the cecum may be necessary for the decrease in hepatic apo A-I mRNA which seems to be involved in the hypocholesterolemic effect of dietary beet fiber.

It is debatable whether the cecectomy influences the hepatic apo A-I gene expression in rats fed a fiber-free diet. We observed that the plasma cholesterol concentrations in cecectomized rats were somewhat higher than in sham-operated rats on day 7 and 10 in the FF period (*Figure 1*). One explanation for this observation may be that the cecectomy increased the plasma cholesterol concentrations through the increased expression of hepatic apo A-I in rats fed the fiber-free diet. If this was so, it would be possible that the unchanged cholesterol concentrations in the plasma of cecectomized rats fed beet fiber resulted from a carry-over effect which was unrelated to the dietary beet fiber. In our preliminary experiment, however, the decrease in plasma cholesterol concentrations by switching from the casein diet to a soy protein diet (a known hypocholesterolemic diet) which contains no fiber in cecectomized rats was similar to that in sham-operated rats (unpublished data). These data suggest that the hypocholesterolemic action of soy protein does not require the cecum and that the cecectomy does not influence the plasma cholesterol concentration in rats fed the fiber-free diet.

It is still unclear how the cecum in rats fed the beet fiber influences the hepatic apo A-I gene expression. It has been suggested that the cholesterol-lowering effects of some dietary fibers may be related to short-chain fatty acid production in the colon including the cecum.[13–16] Nishimura et al.[2] also reported that the cecal short-chain fatty acids correlate negatively with the plasma total cholesterol concentration in rats fed a fiber-free diet or a diet containing 10% beet fiber. However, Arjmandi et al.[17] suggested that the plasma cholesterol-lowering effect of pectin in rats was not related to the concentration of propionate in portal venous blood. Beaulieu and McBurney[18] reported that the plasma cholesterol concentration was not reduced by cecal infusion of propionate in pigs. Thus it is disputable whether the availability of short-chain fatty acids are responsible for the hypocholesterolemic effect of dietary fiber. Furthermore, few studies have shown the effect of short-chain fatty acids on the hepatic apolipoprotein gene expression. Kaptein et al.[19] reported that 2 mM of butyrate increased the apo A-I mRNA in the human hepatoma cell line Hep G2. It is doubtful, however, whether butyrate that was fermented from dietary fiber in the lower digestive tract could reach the liver at enough of a concentration to alter the apo A-I mRNA concentration in vivo. Therefore further investigations are necessary to determine the cecum-derived factor(s) influenced on the apo A-I gene expression in the liver of rats fed dietary fibers.

In conclusion, we propose that the cecum may play an important role in the regulation of hepatic apo A-I gene expression, which seems to be responsible for the hypocholesterolemic effect of dietary beet fiber in rats.

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