Upregulation of Apolipoprotein A-IV Gene Expression in Residual Ileum After Massive Small Bowel Resection Requires the Biliary Secretion in Rats (43971)

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Abstract. **Small bowel resection results in adaptation of residual intestine, but little is known about the postresectional response of apolipoproteins synthesized mainly in the small intestine. We have investigated the postresectional response of apolipoprotein A-I and A-IV gene expression in residual ileum and assessed the mechanism of response, particularly the role of biliary secretion. Time course of changes in apolipoprotein A-I and A-IV mRNA levels was examined by Northern blotting in the residual terminal ileum for 24 hr after 85% jejunoileal resection in fasted rats. Localization of these mRNAs was studied using *in situ* hybridization histochemistry. Effect of biliary diversion on the postresectional response of mRNAs and proteins was estimated by Northern blotting and immunoblotting, respectively. Apolipoprotein A-IV mRNA began increasing at 1 hr postresection, achieved a maximum by 12 hr (5-fold increase) and remained stable to 24 hr, while apolipoprotein A-I mRNA did not change. Apolipoprotein A-IV mRNA accumulated predominantly in the upper part of ileal villi and increased its intensity postresection, and apolipoprotein A-I mRNA was detected in the villus base to tip. The postresectional increase in apolipoprotein A-IV mRNA and protein was completely abolished by concurrent biliary diversion. The results suggest that the enterocytes in the ileal villi rapidly adapt to massive small bowel resection by increase in apolipoprotein A-IV gene expression which is mediated by biliary constituents but not luminal nutrients.** [P.S.E.B.M. 1996, Vol 211]

Apolipoproteins play a central role in lipid transport and metabolism and are closely associated with the pathogenesis of atherosclerosis (1). In rats, apolipoprotein A-I (apo A-I) and apo A-IV are major protein constituents of plasma high-density lipoproteins (HDL) (2). These apolipoproteins have been shown to stimulate cholesterol efflux from peripheral tissues (3–6) and to be the main activators of the enzyme lecithin:cholesterol acyltransferase (LCAT) (7–9). Thus, these proteins are key components of the reverse cholesterol transport process. In rats, the apo A-I and A-IV are synthesized mainly in the small intestine and to a lesser extent in liver (10).

The small intestine is capable of adaptive changes in its mucosal structure and function. For example, massive small bowel resection is an established animal model of the intestinal adaptation seen after disease or surgery (11). In response to the resection, mucosa in the residual small intestine becomes hyperplastic with tall villi, deep crypts, and enhanced absorption per unit length of intestine (12). Simultaneously, it seems that the enterocytes are immature after resection, as seen in a decrease in some of their metabolic functions. Buts *et al.* (13) demonstrated a drop in the

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amount of microvillous enzyme:sucrase per cell in entire villous-crypt unit in the residual intestine after the proximal bowel resection. The mucosal adaptation to small bowel resection is stimulated by several factors, including circulating hormones and luminal nutrients (14, 15). The phenomena of these adaptive changes have been well documented, and the mechanisms for these responses have been extensively studied. However, little is known about the postresectional response of apolipoproteins synthesized mainly in the small intestine. Unless the synthesis of apolipoproteins in the remaining intestine or the liver increased after the small bowel resection, the plasma apolipoprotein concentrations would decrease through the diminished delivery of apolipoproteins from intestine. To our knowledge, however, there is no report that shows the postresectional decrease in apolipoproteins in plasma of experimental animals and patients with short bowel syndrome. So it is theoretically possible that the residual ileum would show the compensatory increase in the synthesis of apolipoproteins after the small bowel resection.

We recently observed an increase in apo A-I (1.2-fold) and A-IV (3.2-fold) mRNA levels in the residual ileum at 15 days after an 85% of jejunoileal resection, and a transient decrease in apo A-I and A-IV levels in plasma 2–5 days after the resection followed by the recovery to the control levels in rats (16). These observations suggest that the ileum adapts to small bowel resection by selective increases in apo A-I and A-IV gene expression at pretranslational stage, which would contribute to the recovery of apo A-I and A-IV in plasma. However, the mechanism for the upregulation of apo A-I and A-IV gene expression after the resection is unclear. There are a number of reports suggesting that the intestinal expression of apo A-IV is regulated by an event associated with the absorption of dietary lipids (10, 17, 18). We previously reported that the chronic biliary diversion lowered the ileal apo A-I and A-IV mRNA levels in rats (19), and the findings suggest that the biliary constituents play an important role in ileal apo A-I and A-IV gene expression. So it is possible that the upregulation of ileal apo A-I and A-IV gene expression after the small bowel resection would be modulated by the facilitated uptake of dietary lipids or biliary constituents which will be normally absorbed in the proximal intestine. In the present study, we have investigated the time course of changes in apo A-I and A-IV mRNA levels in the residual ileum for 24 hr after massive small bowel resection in fasted rats (Experiment 1). Localization of these apolipoprotein mRNAs was examined by using *in situ* hybridization histochemistry in the ileum. Furthermore, to test above hypothesis we have determined the steady-state level of apo A-I and A-IV mRNA in the residual ileum at 24 hr after the resection or the resection plus biliary diversion in fasted rats (Experiment 2).

Materials and Methods

Animals. Male Wistar rats, 5 weeks old at the start of the experiment, were housed in individual cages in a temperature-controlled (23° ± 2°C) room with the dark period from 2000 to 0800 hr. They were allowed free access to water and a purified diet containing (as w/w) 25% casein, 65% sucrose, 5% corn oil, 4% mineral mixture, 1% vitamin mixture (20). This diet is used as a standard rat diet in our laboratory because it yields maximal growth rates. After 5 days of acclimation in Experiment 1, rats weighing 137 ± 1 g (n = 24) were divided into eight groups of three rats, and all rats were fasted for 18 hr before surgery. They were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital, 35 mg/kg body wt; Abbott Laboratories, North Chicago, IL). The resection was done at 0, 1, 3, 6, 9, 12, 18, and 24 hr before sacrifice. The resected rats underwent 85% jejunoileal resection followed by end-to-end anastomosis. This operation resulted in the removal of all bowel from a point 1 cm distal to Treitz ligament to a point 6 cm proximal to the ileocecal valve. All rats were not allowed food and water before sacrifice, which was performed between 1700 and 1800 hr. They were anesthetized by intraperitoneal injection of Nembutal, and after laparotomy the 5 cm of intestinal segment just proximal to the ileocecal valve was excised and the luminal contents were washed out with 10 ml of ice-cold saline. A 1-cm segment was excised, embedded in O.C.T. compound (Miles Scientific, Elkhart, IN), frozen in liquid nitrogen, and stored at −80°C for *in situ* hybridization. The mucosa of remaining segment was scraped off with a slide glass and immediately plunged into liquid nitrogen, and stored at −80°C for RNA extraction. In Experiment 2, rats weighing 139 ± 2 g (n = 18) were divided into three groups of 6 resected, 6 resected plus biliary diverted, and 6 sham-operated rats; and all rats were fasted for 18 hr before surgery. The small bowel resection was performed as described above. The biliary diversion was performed as previously described (19). In short, a catheter was inserted into the bile duct at a point just distal to the bifurcation of the common hepatic ducts, and the distal end of cannula for returning the biliary secretion was placed through the fistula at a point 4 cm distal to the junction of cecum and colon. For the sham-operated rats, the abdominal cavity was simply opened and exposed for ~50 min, the same length of time as required for the resection plus biliary diversion. All rats were not allowed food and water for 24 hr postoperatively, and then they were anesthetized by intraperitoneal injection of Nembutal, and after laparotomy the sample of intestinal mucosa

was obtained as described above and stored at −80°C for RNA extraction and immunoblot analysis. The liver was excised and immediately plunged into liquid nitrogen and stored at −80°C for RNA extraction.

The study was approved by the Hokkaido University of Animal Use Committee, and animals were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Hokkaido University.

RNA Isolation and Northern Blot Analysis. Total RNA was isolated by the acid guanidium-phenol-chloroform method (21) from ileal mucosa and liver. Samples of total RNA (10 and 20 μg/lane for ileum and liver, respectively) were electrophoresed on denaturing 2.2 *M* formaldehyde, 1% agarose gel (22), and transferred to nylon membrane (Biodyne B, Pall BioSupport, East Hills, NY). Blots were hybridized with an apo A-I probe of the 54-base oligonucleotide as previously described (20). 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. After the detection, each filter was then sequentially rehybridized with the DIG-labeled apo A-IV and apo E probes of the 54-base oligonucleotide (20) and the DIG-labeled rat β-actin cDNA, and the detection was carried out similarly. The relative quantity of mRNA was estimated by densitometry scanning (Dual-Wavelength Flying-Spot Scanner CS-9000; Shimadzu, Kyoto, Japan).

***In Situ* Hybridization.** Frozen sections (8 μm) were prepared with a cryostat (MINOTOME; International Equipment Co., Boston, MA), thawed onto glass slides pretreated with poly-L-lysine, immediately dried with hair dryer, and then stored at −80°C until used.

The sections were fixed with 4% (w/v) paraformaldehyde in 0.1 *M* phosphate buffer pH 7.4 (PB) for 15 min, followed by digestion with 5 μg/ml proteinase K (Wako Pure Chemical Industries, Osaka, Japan) in 10 m*M* Tris-HCl, pH 8.0, containing 1 m*M* EDTA at 37°C for 10 min. After the digestion, the sections were treated with 4% (w/v) paraformaldehyde-PB for 10 min. Then, they were treated with 0.2 *N* HCl for inactivation of internal alkaline phosphatase, and were acetylated with 0.25% (v/v) acetic anhydride in 0.1 *M* triethanolamine-HCl pH 8.0 for 10 min. They were dehydrated with ethanol series (70%, 80%, 90%, and 100% ethanol for 15 sec each) and air dried. Hybridizations were performed overnight at 37°C under parafilm coverslips in a moisture chamber. Each section was covered with 30 μl of hybridization buffer composed of 50% (v/v) formamide, 10 mM Tris-HCl (pH 7.6), 200 μg/ml of *Escherichia coli* tRNA (Boehringer Mannheim), 1× Denhardt's solution (from 50 × stock solution, Wako Pure Chemical Industries), 10% (w/v) dextran sulfate, 600 m*M* NaCl, 0.25% (w/v) SDS, 1 m*M* EDTA, and 10 p*M* DIG-labeled oligonucleotide probe for apo A-I or A-IV. Following the hybridization, the coverslips were removed in 5 × SSC pre-warmed to 37°C. The sections were then washed with the following schedule: 2 × SSC/50% formamide at 37°C (1 × 30 min), 10 m*M* Tris-HCl, pH 7.6, containing 500 m*M* NaCl and 1 m*M* EDTA at 37°C (1 × 10 min), 2 × SSC at 37°C (1 × 20 min), and 0.2 × SSC at 37°C (2 × 20 min).

The sections were pre-blocked with 1.5% (w/v) blocking reagent (in DIG-Luminescent Detection Kit, Boehringer Mannheim) in 100 m*M* maleic acid/NaOH, pH 7.5, containing 150 mM NaCl (buffer 1) for 60 min. They were then incubated for 2 hr at room temperature with a 1:500 dilution of alkaline phosphatase-conjugated anti-DIG Fab fragment (in DIG-Luminescent Detection Kit, Boehringer Mannheim) in buffer 1. The slides were washed twice with 0.3% (w/v) Tween 20 in buffer 1 for 15 min followed by one wash in 100 m*M* Tris-HCl, pH 9.5, containing 500 m*M* NaCl and 50 m*M* $MgCl_2$ (buffer 3) for 5 min. They were then incubated for 48 hr at room temperature in buffer 3 with 0.45 mg/ml nitroblue tetrazolium (Wako Pure Chemical Industries) and 0.18 mg/ml 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt (Amresco, Solon, OH). The color reaction was stopped with 10 m*M* Tris-HCl, pH 8.0, containing 1 m*M* EDTA followed by washing with water, and mounted using Gel/Mount (Biomeda, Foster City, CA).

The control experiment was performed by processing with the labeled sense probes.

Immunoblotting for Ileal apo A-I and A-IV Quantitation. In Experiment 2, the immunoblot analysis of mucosal proteins in the ileum was performed for the semiquantitation of apo A-I and A-IV. The mucosa was homogenized on ice in 1% (w/v) Triton X-100, 1 m*M* phenylmethylsulfonyl fluoride, 5 m*M* EDTA, 450 μ*M* aprotinin in PBS (pH 7.4). Samples (100 μg total protein) were subjected to 12% (apo A-I) or 7.5% (apo A-IV) SDS-PAGE under reducing condition (23). Electrophoresed proteins were electrophoretically transferred to nitrocellulose membrane (Hybond C extra, Amersham International plc., Buckinghamshire, United Kingdom) and immunostained with the rabbit anti-rat apo A-I serum (a gift from Dr. Fumihiko Horio, Nagoya University, Nagoya, Japan) or the anti-human apo A-IV monoclonal antibody (Boehringer Mannheim) as previously described (16). The relative quantities of apo A-I and apo A-IV were estimated by densitometry scanning.

Statistical Analysis. Results were expressed as means ± SEM. In Experiment 1, we did not perform the statistical analysis, since the number of animals

per group was small (n = 3). In Experiment 2, the statistical comparison of the mean was done by Duncan's multiple range test.

Results

Figure 1A shows the representative Northern blots of ileal RNA for 24 hr after massive small bowel resection (Experiment 1). Notably, the signal of apo A-IV transcript increased for the first 12 hr postresection. In contrast, apo A-I and β-actin transcripts were relatively constant throughout the experimental period. In Figure 1B, values of apo A-I and A-IV mRNA were normalized by the value of β-actin mRNA, and the value at each time point were expressed relative to the average values on 0 time (nonresection) which were normalized to 100. The apo A-IV mRNA tended to increase at 1 hr postresection, linearly increased to 12 hr. and remained stable thereafter. In marked contrast, there was no increase in the apo A-I mRNA levels.

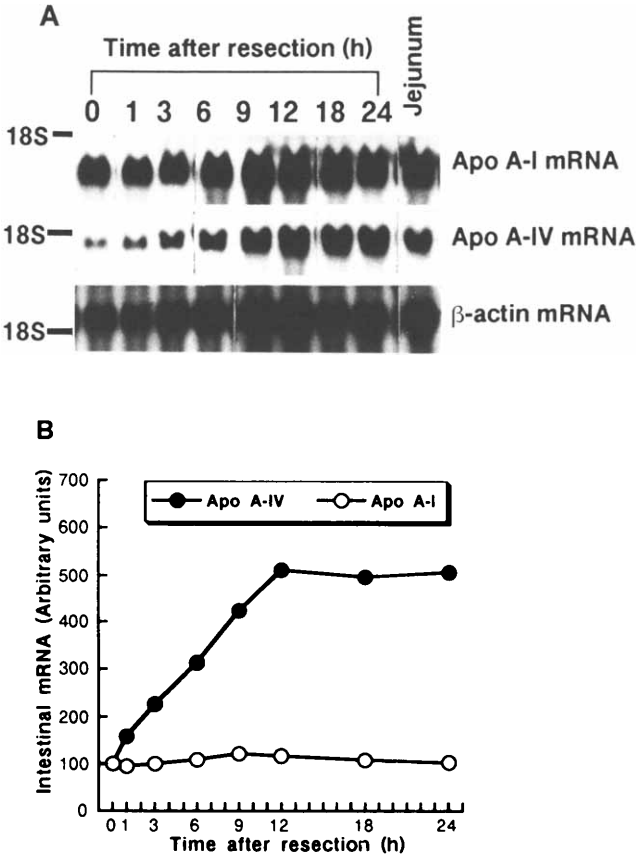

Figure 1. Time course of changes in apo A-I and A-IV mRNA levels in the residual ileum after massive small bowel resection (Experiment 1). (A) The representative Northern blots of ileal RNA postresection. The blots of jejunal RNA from rats of time 0 (nonresection) are also shown. Upper, middle, and lower charts show the transcripts of apo A-I, apo A-IV, and β-actin, respectively. (B) The position of 18S ribosomal RNA. Changes in the relative levels of apo A-I and A-IV mRNA. Results are the mean ± SEM of three rats. The abundance of apo A-I and A-IV mRNA was normalized by comparing with each value of β-actin mRNA, and the values on each time point were expressed relative to the mean values of 0 time which were taken as 100.

To characterize the cellular basis of the pattern of gene expression, the same oligonucleotide probes were used for *in situ* hybridization. Figure 2 shows the expression of apo A-I and A-IV mRNA in the ileum at 0 time (nonresection) and 24 hr postresection. The hybridization signal of the apo A-I oligonucleotide probe was clearly seen in the mucosal epithelium from the villus base to tip at either 0 time (nonresection) and 24 hr postresection (Fig. 2, A and B, respectively), and the signal intensities were slighly less in the former. The hybridization signal of the apo A-IV probe was also detected in the ileal epithelium (Fig. 2, C and D), and the area limited to the upper part of villus. At 24 hr postresection (Fig. 2D), the apo A-IV mRNA dramatically increased its intensity but did not extend the positive area in comparison with the nonresected control (Fig. 2C). Neither apo A-I and A-IV mRNA were detected in the crypt region. Negative controls processed with labeled sense probes yielded no signal (Fig. 2, E and F, for apo A-I and A-IV, respectively).

Figure 3, A and B, shows the representative Northern blot of total RNA in the ileal mucosa and the relative quantities of apo A-I and A-IV mRNA in Experiment 2. The abundance of apo A-I and A-IV mRNA was normalized by comparing each value with that of β-actin mRNA, and the values on resected and resected plus biliary diverted rats were expressed relative to the mean value of sham-operated rats, which was taken as 100. The apo A-IV mRNA levels were significantly higher in the resected rats than in sham-operated and resected plus biliary diverted animals at 24 hr after the operations. There was no significant difference in the apo A-IV mRNA between sham-operated and resected plus biliary diverted rats. The apo A-I mRNA remained unchanged at 24 hr after the operations. Similarly, the immunoblot analysis of the mucosal protein in the ileum revealed that the concentrations of apo A-IV protein were significantly higher in the resected rats than the other groups while the apo A-I protein levels were the same (Fig. 4, A and B).

In the liver, the other principal tissue for apo A-I and A-IV expression, the abundance of apo A-I and A-IV mRNA was not changed by the small bowel resection or biliary diversion in Experiment 2 (Fig. 5). Similarly, hepatic apo E mRNA levels were the same in all the groups.

Discussion

Recently, we observed an increase in apo A-I and A-IV mRNA levels in the residual ileum at 15 days after massive small bowel resection in fed rats (16). However, the mechanism for the upregulation of apo A-I and A-IV gene expression has remained unclear. The most likely explanation is that the gene expression

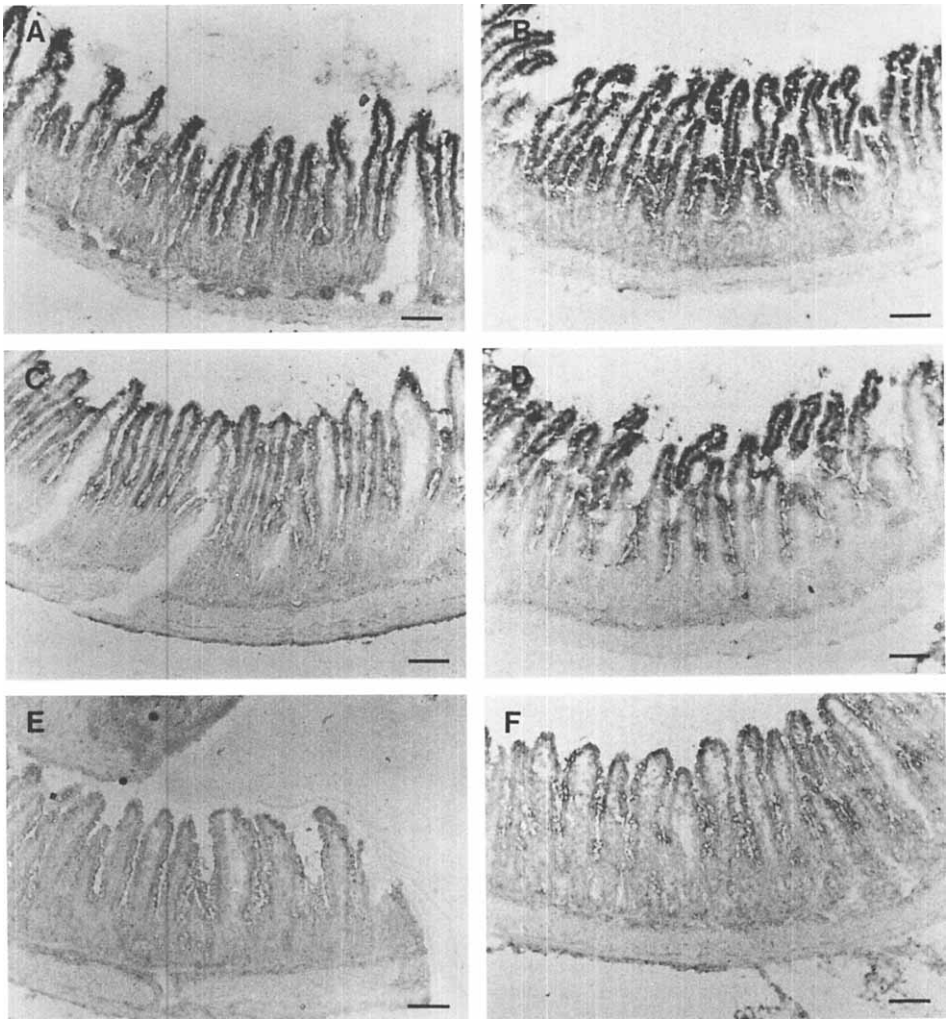

Figure 2. Expression of apolipoprotein A-I and A-IV mRNA in the residual ileum of rats at 0 (nonresection) and 24 hr after massive small bowel resection (bar, 100 μm; Experiment 1). *In situ* hybridization was performed using oligonucleotide probe, 3′ tailing labeled with DIG. (A) Antisense probe for apo A-I mRNA at 0 time. (B) Antisense probe for apo A-I mRNA at 24 hr postresection. (C) Antisense probe for apo A-IV mRNA at 0 time. (D) Antisense probe for apo A-IV mRNA at 24 hr postresection. (E) Negative control processed with labeled sense probe for apo A-I mRNA. (F) Negative control processed with labeled sense probe for apo A-IV mRNA.

of these apolipoproteins in the residual ileum might be stimulated by the facilitated absorption of dietary lipids that would normally be absorbed in the proximal intestine. There are a number of reports suggesting that the intestinal expression of apo A-IV is regulated by an event associated with the absorption of dietary lipids. Hayashi *et al.* (18) reported that apo A-IV and chylomicron output in mesenteric lymph increased dramatically after ingestion of dietary lipid and this was associated with an increased synthesis and secretion of apo A-IV by the small intestine. Elshourbagy *et al.* (10) and Apfelbaum *et al.* (17) also showed that apo A-IV synthesis by rat small intestine increased in response to dietary triacylglycerol and might be under pretranslational control. On the other hand, we have previously reported that the apo A-I and A-IV mRNA levels in the ileum were diminished by chronic (7 days) biliary diversion in rats (19). The findings suggest that some components in the biliary secretion play an important role in ileal apo A-I and A-IV gene expression. Thus, another explanation for the upregulation of apo A-I and A-IV gene expression in the residual ileum after the small bowel resection is that the expression of these apolipoproteins might be mediated by the biliary constituents. So in this study, as a step toward clarifying whether the upregulation of apo A-I and A-IV gene expression after the small bowel resection is associated with the dietary lipids or biliary secretion, we have investigated the effect of small bowel resection on ileal apo A-I and A-IV mRNA levels in fasted animals in which the contribution of dietary lipids could be negligible, and also examined the effect of concurrent biliary diversion on the postresectional response of apo A-I and A-IV mRNAs.

There are three important findings to note in the present study. The first is that the residual ileum of fasted rats showed the rapid increase in apo A-IV mRNA after massive small bowel resection. This re-

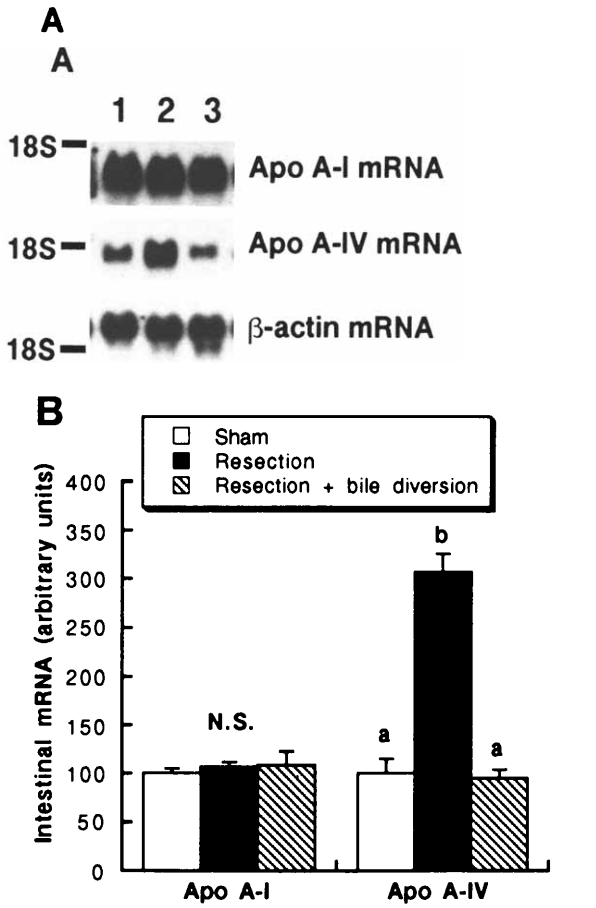

Figure 3. Effect of massive small bowel resection and biliary diversion on the relative quantities of ileal apo A-I and A-IV mRNA (Experiment 2). (A) The representative Northern blots of ileal RNA in sham-operated (Lane 1), resected (Lane 2), and resected plus biliary diverted (Lane 3) rats. Upper, middle, and lower charts show the transcripts of apo A-I, apo A-IV, and β-actin, respectively. The position of 18S ribosomal RNA is shown. (B) The relative concentrations of apo A-I and A-IV mRNA in the ileum. Results are the mean ± SEM of six rats. The abundance of apo A-I and A-IV mRNA was normalized by comparing with each value of β-actin mRNA, and the values on resected and resected plus biliary diverted rats were expressed relative to the mean value of sham-operated rats, which was taken as 100. Results not sharing a common letter are significantly different ($P < 0.05$). N.S., not significant.

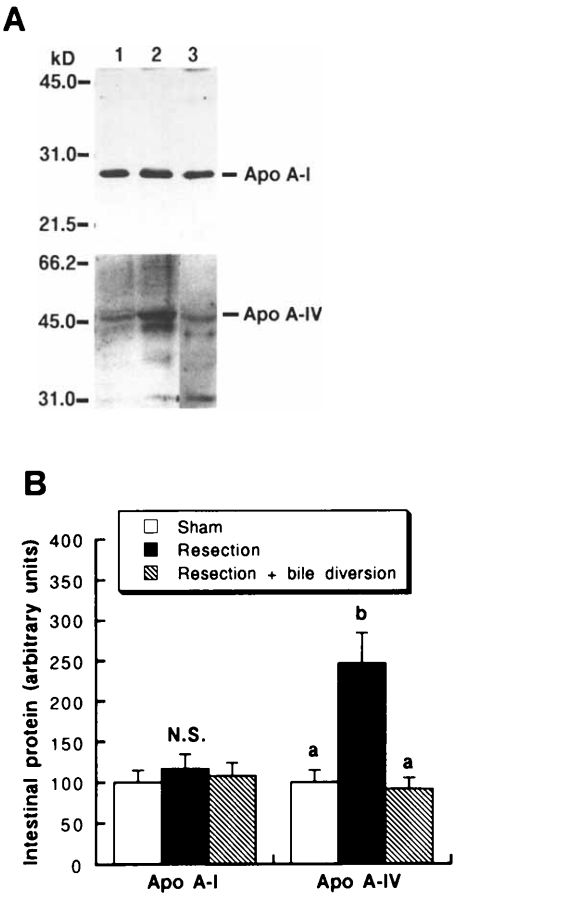

Figure 4. Effect of massive small bowel resection and biliary diversion on the relative quantities of ileal apo A-I and A-IV proteins (Experiment 2). (A) The representative immunoblots of ileal protein in sham-operated (Lane 1), resected (Lane 2), and resected plus biliary diverted (Lane 3) rats. (B) The relative concentrations of apo A-I and A-IV in the ileum. Results are the mean ± SEM of six rats. The values on resected and resected plus biliary diverted rats were expressed relative to the mean value of sham-operated rats, which was taken as 100. Results not sharing a common letter are significantly different ($P < 0.05$). N.S., not significant.

sult suggests that the increase in apo A-IV gene expression after massive small bowel resection is at least partly independent of the uptake of dietary lipids since the rats were deprived of food for 18 and 24 hr pre- and postoperatively, respectively. It is yet debatable whether the dietary lipids induce the additional increase in apo A-IV mRNA postresection. Comparing the effect of small bowel resection on apo A-IV mRNA in the residual ileum of rats on the oral nutrition with the case of rats on the total parenteral nutrition would shed light on the question. The unchanged apo A-I mRNA levels in the ileum postresection in this study are in conflict with our previous observation that showed that the small but significant increase (1.2-fold) in apo A-I mRNA in the residual ileum of fed rats

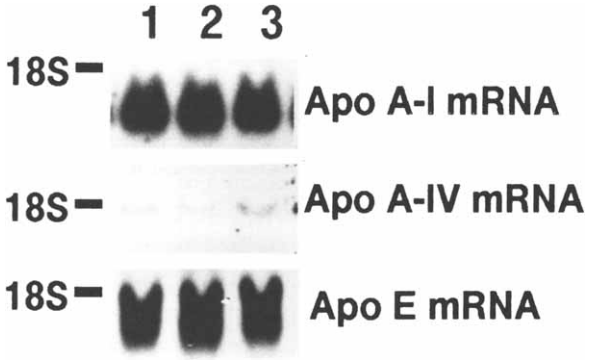

Figure 5. Representative Northern blots of hepatic RNA from sham-operated (Lane 1), resected (Lane 2), and resected plus biliary diverted (Lane 3) rats (Experiment 2). Apo A-I (*upper*), apo A-IV (*middle*), and E (*lower*) mRNAs are shown. The position of 18S ribosomal RNA is shown.

at 15 days after the resection (16). In the present study, however, the rats were sacrificed at 24 hr postresection under fasting condition. So the response of ileal apo A-I mRNA may require relatively longer period after the resection and/or depend on the luminal nutrients.

The second finding was obtained by *in situ* hybridization histochemistry. The area in which apo A-IV mRNA was detected (i.e., the upper part of villi in the ileum) was not extended by the small bowel resection, and the intensity of apo A-IV mRNA signal was increased postresection. These results suggest that the terminally differentiated enterocytes in the upper part of ileal villi respond to the resection with the upregulation of apo A-IV gene expression in the individual cells and that the number of cells that express the apo A-IV mRNA is not increased by the resection. Apo A-I mRNA expression was observed in the enterocytes from villus base to the tip, whereas apo A-IV mRNA limited to the upper part of villi. Thus the ileal enterocytes have the different expression patterns of apo A-I and A-IV mRNA along with the differentiation and migration of the cells.

The third is that the increase in apo A-IV mRNA in the residual ileum postresection was completely abolished by concurrent biliary diversion. This result suggests that the upregulation of ileal apo A-IV gene expression after the small bowel resection in fasted rats is mediated by biliary constituents that would normally be absorbed in the proximal intestine. The ~4-fold higher apo A-IV mRNA levels in the jejunum, compared with those in the ileum at time 0, observed in the Experiment 1 (representative Northern blots in Fig. 1, data not shown) agree with Apfelbaum *et al.* (17), who showed the 5-fold higher synthesis rates and translational apo A-IV mRNA levels in the jejunum. The increase in ileal apo A-IV mRNA after the proximal resection to the levels seen in the jejunum suggest a reserved function of the ileum. Since the postresectional increase in ileal apo A-IV mRNA completely abolished by the biliary diversion in Experiment 2, the lower apo A-IV mRNA levels in the normal ileum compared with the jejunum may represent the regulation of apo A-IV gene expression by the biliary constituents that would be absorbed proximally. The biliary constituents would stimulate the apo A-IV gene expression rather by its direct effect on the enterocytes than by its indirect effects via the solubilization and absorption of luminal nutrients since the response of apo A-IV gene expression was observed in fasted rats in which the contribution of dietary factors could be negligible. We have previously observed the drastic decrease in apo A-IV mRNA in the ileum of chronic (7 days) biliary diverted rats (16). In the present study, however, the apo A-IV mRNA levels in the residual ileum were the same between sham-operated and resected plus biliary diverted rats at 24 hr postsurgery. The reduction of apo A-IV gene expression would require prolonged deficiency of biliary constituents, or the apo A-IV gene expression in the residual ileum would be at least partly modulated by the bile-independent factors.

Mucosal adaptation to bowel resection is known to be stimulated by circulating hormones. Enteroglucagon is considered the enterotrophic humoral factor involved in the intestinal adaptation to bowel resection. A number of reports showed increased plasma levels of enteroglucagon (24–26) and increased levels of ileal proglucagon mRNA (27, 28) after massive small bowel resection in rats. Peptide YY has also been postulated to be a trophic factor involved in intestinal adaptation (29, 30). It still remains unclear whether these enterotrophic hormones stimulate apo A-IV gene expression during intestinal adaptation. Cholecystokinin (CCK) seems to be another enterotrophic hormone involved in the intestinal adaptation. Watanapa *et al.* (31) reported the increase in plasma CCK concentration after massive proximal small bowel resection in rats, and Fine *et al.* (32) demonstrated that CCK has the trophic effect on the small intestine indirectly, probably through stimulation of pancreatic secretion. CCK, however, may be excluded from the involvement in the upregulation of apo A-IV gene expression after the bowel resection, because Miyasaka *et al.* (33) reported that the biliary diversion for 7 days in rats increased the plasma CCK concentration, and we demonstrated the diminished apo A-IV mRNA level in the ileum under the same condition (16).

Ochoa *et al.* (34) demonstrated that the apo A-IV gene expression was activated by HNF-4 and repressed by Ear3/COUP-TF using transient expression assay in Caco-2 and HepG2 cells. They suggested that the apo A-IV gene expression is modulated by the orphan ligand members of the superfamily of nuclear hormone receptors. Therefore, it is of interest to investigate whether the biliary constituents that modulate the intestinal apo A-IV gene expression are the physiological effectors which influence HNF-4 and Ear3/COUP-TF intracellular concentrations.

Further investigations will be necessary to identify the biliary constituents responsible for the regulation of apo A-IV gene expression in the ileum, and to clarify the regulatory mechanism for the apo A-IV gene expression by the biliary constituents on the cellular and molecular bases.

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