Adaptive Response of Apolipoprotein A-I and A-IV mRNA in Residual Ileum after Massive Small Bowel Resection in Rats

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Summary The response of apolipoproteins, which are synthesized mainly in the small intestine, after small bowel resection has not been documented. In this study, we investigated the effect of small bowel resection on the expression of apolipoprotein mRNA in the residual ileum in rats. Wistar rats underwent either an 85% jejunoileal resection or a sham-operation. Plasma concentrations of total and HDL-cholesterol, apolipoprotein A-I and A-IV were measured on days 0, 3, 6, 9, 12, and 16 (surgery on day 1). The abundances of apolipoprotein mRNA in the residual ileum and liver on day 16 were determined. Plasma levels of total and HDL-cholesterol and apolipoprotein A-I and A-IV in resected rats were significantly lower than in sham-operated rats on days 3 and 6. Resected rats showed a significant increase in ileal apolipoprotein A-I (1.2-fold) and A-IV (3.2-fold) mRNA compared with sham-operated animals. Hepatic apolipoprotein mRNA were the same between two groups. These data suggest that the residual ileum adapts to jejunoileal resection by selective increases in apolipoprotein A-I and A-IV expression at a pretranslational stage. The recoveries of apolipoprotein A-I and A-IV in plasma appear to depend, at least in part, on the increased expression of these apolipoproteins in the residual ileum.

Key Words apolipoprotein A-I, apolipoprotein A-IV, mRNA, small intestine, small bowel resection, rats

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, apo A-I and A-IV are synthesized mainly in the small intestine and to a lesser extent in the liver (10). The small intestine is capable of adaptive changes in its mucosal structure and function. For example, massive small bowel resection, which results in a severe malabsorptive state known as the short bowel syndrome, is an established animal model of the intestinal adaptation seen after disease or surgery (11). The short bowel syndrome after massive small bowel resection is characterized by malabsorption, diarrhea and body weight loss as a result of a marked decrease in the intestinal absorptive surface area. In response to small bowel resection, the mucosa in the residual small intestine becomes hyperplastic with tall villi, deep crypts, and enhanced absorption per unit length of intestine (12). These changes result in greater absorptive capacity in the residual small intestine, with diminution of malabsorptive nutritional losses. The mucosal adaptation to small bowel resection is stimulated by several factors including circulating hormones and luminal nutrients (13, 14). 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 response of apolipoproteins which are synthesized mainly in the small intestine.

In this study, to obtain further information about the intestinal adaptation to small bowel resection, we have investigated the effect of resection on the steady-state levels of apo A-I and A-IV mRNA in the residual intestine and liver, and compared these mRNA levels with plasma levels of apo A-I and A-IV.

MATERIALS AND METHODS

Animals, diets, and study protocol. Male Wister rats (Japan SLC, Hamamatsu, Japan), 5 weeks old at the start of the experiment, were housed in individual cages in a temperature-controlled (23 ± 2°C) room under a 12-h light-dark cycle (light: 8:00–20:00 h). They were allowed free access to water and to a purified diet (25.0% casein, 64.7% sucrose, 5.0% corn oil, 4.0% mineral mixture, 1.0% vitamin mixture, 0.2% choline chloride) as previously described (15). This diet is used as a standard rat diet in our laboratory since it yields maximal growth rates. After 7 days of acclimation, rats weighing 150 ± 2 g (n = 12) were divided into two groups of 6 resected and 6 sham-operated rats (“day 0”), and all rats were deprived of food for 24 h before surgery. They were anesthetized by intraperitoneal injection of Nembutal (sodium pentobarbital 35 mg/kg body wt; Abbott Laboratories, North Chicago, IL). The resected rats underwent 85% jejunooileal resection. This operation resulted in removal of the bowel from a point 1 cm distal to the ligament of Treitz to a point 6 cm proximal to the ileocecal valve. In sham-operated rats, the abdominal cavity was exposed for the same length of time as required for the resection. The rats were not allowed food for the first 24 h postoperatively, and then they were fed the standard diet for 14 days ad libitum. Blood samples were collected from a tail vein on days 0, 3, 6, 9, 12, and 16 for determination of the
levels of total and HDL-cholesterol, and apo A-I and A-IV.  

On the last day of the experiment ("day 16"), the animals were decapitated, and 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. The mucosa was scraped off with a slide glass and immediately plunged into liquid nitrogen, and stored at −80°C for RNA extraction. The liver was excised and immediately plunged into liquid nitrogen, and stored at −80°C for analyses of mRNA.

The study protocol 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 total cholesterol and HDL cholesterol levels were measured by enzymatic methods using Cholesterol C-Test and HDL-Cholesterol-Test, respectively (Wako Pure Chemical Industries, Osaka, Japan).

**Immunoblotting for plasma apo A-I and A-IV quantitation.** Whole plasma samples (0.5 and 1.0 µl for apo A-I and apo A-IV, respectively) were subjected to 12% (apo A-I) or 7.5% (apo A-IV) SDS-PAGE under reducing conditions (16), and then electrophoretically transferred to nitrocellulose membrane (Hybond C extra, Amersham International plc., Buckinghamshire, U.K.) in a semidy electroblotting apparatus (Nippon Eido, Tokyo, Japan) at constant current of 170 mA for 1 h. The blotting buffer contained 125 mmol/liter Tris, 960 mmol/liter glycine in 20% (v/v) methanol, pH 8.3. The membrane was then incubated in a blocking solution of 5% (w/v) skim milk in Tris-buffered saline (20 mmol/liter Tris-HCl, 150 mmol/liter NaCl, pH 7.4), followed by the incubation for 1 h with a 1:500 dilution of the rabbit anti-rat apo A-I serum (a gift from Dr. F. Horio, Nagoya University, Nagoya, Japan) or a 10 µg/ml of the anti-human apo A-IV monoclonal antibody (Boehringer Mannheim, Mannheim, Germany) in the blocking solution containing 0.05% (w/v) of Tween 20. The membrane was then washed three times with Tris-buffered saline containing 0.05% (w/v) of Tween 20. Next, the membrane was incubated for 1 h with a 1:2,000 dilution of a second antibody of goat anti-rabbit IgG conjugated with horseradish peroxidase (Biomedical Technologies Inc., Stoughton, MA) for apo A-I or a 1:500 dilution of the chicken anti-mouse IgG conjugated with horseradish peroxidase (Chemicon International Inc., Temecula, CA) for apo A-IV 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) as recommended by the manufacturer except that the exposure time to X-ray film was 30 and 180 s for apo A-I and apo A-IV, respectively. The relative quantities of apo A-I and apo A-IV were estimated by densitometry scanning (Dual-Wavelength Flying-Spot Scanner CS-9000, Shimadzu, Kyoto, Japan).

**RNA isolation and Northern blot analysis.** Total RNA was isolated by the acid guanidium-phenol-chloroform method (17) from ileal mucosa and liver.
Samples of total RNA (10 μg/lane) were electrophoresed on denaturing 2.2 mol/liter formaldehyde, 1% agarose gel (18), and transferred to a 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 (15). The probe was 3'-labeled using a nonradioisotopic system, DIG Oligonucleotide Tailing Kit (Boehringer Mannheim), 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. After the detection, each filter was then sequentially rehybridized with the DIG-labeled apo A-IV probe of the 54-base oligonucleotide (15) and the DIG-labeled rat β-actin cDNA (19), and the detection was carried out similarly. For hepatic RNA, blots were also rehybridized with the DIG-labeled apo E probe of the 54-base oligonucleotide (15).

Statistical analysis. Results are expressed as means±SEM and the statistical comparison of the mean was done by Student’s t-test. Values in the text are means±SEM.

RESULTS

Body weights on the last day of the experiment (day 16) were 239±5 and 170±3 g for sham-operated and resected rats, respectively, and there was a significant difference between the two groups (p<0.001). Diarrhea was observed in all of the resected rats from days 2 to 7.

The time course of changes in plasma cholesterol concentration in sham-operated and resected rats are shown in Fig. 1. On days 3, 6, and 16, plasma total cholesterol increased significantly in the resected group compared to the sham-operated group (p<0.05). The HDL-cholesterol concentration also increased in the resected group, but the difference was not significant. Fig. 1. Changes in plasma total and HDL-cholesterol concentrations in sham-operated and resected rats for 16 days. Each point is the mean±SEM of six rats. Values in resected group with asterisk are significantly different (p<0.05) from values in sham-operated group at each time point.

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Fig. 2. Representative immunoblots of plasma from sham-operated (Sham) and resected (Resection) rats on days 0, 3, 6, 9, 12, and 16. Whole plasma (0.5 and 1.0 µl for apo A-I and A-IV, respectively) were subjected to 12% (apo A-I) or 7.5% (apo A-IV) SDS-PAGE under reducing condition, and then electrophoretically transferred to a nitrocellulose membrane (Hybond C extra). The rabbit anti-rat apo A-I serum (a gift from Dr. Fumihiko Horio) and the goat anti-rabbit IgG conjugated with horseradish peroxidase (Biomedical Technologies) were used as the primary and secondary antibodies, respectively, for the detection of apo A-I. The anti-human apo A-IV monoclonal antibody (Boehringer Mannheim) and the chicken anti-mouse IgG conjugated with horseradish peroxidase (Chemicon International) were used as the primary and secondary antibodies, respectively, for the detection of apo A-IV. Identification of the antigen-antibody complex was performed using Renaissance Western Blot Chemiluminescence Reagent (DuPont NEN Research Products).

and HDL-cholesterol concentrations were significantly lower in resected rats than in sham-operated rats. There was no significant difference between the two groups on other days measured.

Representative immunoblots of plasma on days 0, 3, 6, 9, 12, and 16 are shown...
Fig. 3. Effect of massive small bowel resection on the relative quantities of plasma apo A-I and A-IV, as estimated by the densitometry scanning of immunoblots (see Fig. 2). Chart A and B show the time course of changes in plasma concentrations of apo A-I and A-IV, respectively. Results are the means ± SEM of six rats. The value at each time point is expressed relative to the average value in sham-operated rats on day 0 which is taken as 100. Values in the resected group with an asterisk are significantly different (p < 0.05) from values in the sham-operated group at each time point.

The blots had the expected sizes of approximately 28 and 46 kDa for apo A-I and apo A-IV, respectively. Figure 3A and B show the time course of changes in plasma levels of apo A-I and A-IV, respectively, as estimated by densitometry scanning. The value of each time point is expressed relative to the average value in sham-operated rats on day 0 which was normalized to 100. The relative concentrations of apo A-I and A-IV in plasma on days 3 and 6 were significantly decreased in resected rats compared with sham-operated rats. The degree of plasma apo A-IV decrease in resected rats appeared to be more prominent than apo A-I.

The representative Northern blots of total RNA in the ileal mucosa of sham-operated and resected rats on the last day of the experiment are shown in Fig. 4A, and Fig. 4B shows the relative quantities of apo A-I and A-IV mRNA. The
Fig. 4. Effect of massive small bowel resection on the relative quantities of ileal apo A-I and A-IV mRNA. Chart A shows the representative Northern blots of ileal RNA in sham-operated and resected rats. Upper, middle, and lower charts show the transcripts of apo A-I, apo A-IV, and β-actin, respectively. The relative concentrations of apo A-I and A-IV mRNA in the ileum are shown in chart B. Results are the mean ± SEM of six rats. The abundance of apo A-I and A-IV mRNA is normalized by comparing each value of β-actin mRNA, and the value on resected group is expressed relative to the mean value of sham-operated rats which is taken as 100.

abundance of apo A-I and A-IV mRNA was normalized by comparing each value with that of β-actin mRNA, and the value on resected group was expressed relative to the mean value of sham-operated rats which was taken as 100. The resected rats showed a significant increase in ileal apo A-I (1.2-fold) and A-IV (3.2-fold) mRNA compared with the sham-operated animals. In contrast, the levels of apo A-I, A-IV,
DISCUSSION

The data obtained in this study clearly indicate that the ileum adapts to massive small bowel resection by selective increases in apo A-I and A-IV mRNA. Fifteen days after the resection ("day 16"), the apo A-IV mRNA in the residual ileum showed a 3.2-fold increase compared with the control whereas the apo A-I mRNA increased only 1.2-fold. The non-parallel increases in apo A-I and A-IV mRNA suggest that the adaptation of apo A-I and A-IV mRNA to the small bowel resection is gene-specific. After massive small bowel resection, the residual ileum undergoes mucosal hyperplasia with increased cell turnover resulting in villus enlargement (11). If the cause of increases in apo A-I and A-IV mRNA in the residual ileum of resected rats had been an increase in number of finally differentiated enterocytes which located in villus and expressed these apolipoproteins, the degree of increases in apo A-I and A-IV mRNA would have been the same.

Though the mechanism for the increase in apo A-I and A-IV mRNA in the residual ileum after massive small bowel resection is still unclear, there are two possible explanations. The first is that the apo A-IV mRNA in the residual ileum increases in response to increased absorption of dietary fat by enterocytes. There are a number of reports which suggest that the intestinal expression of apo A-IV is regulated by an event associated with the absorption of dietary fat. Hayashi et al.

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reported that apo A-IV and chylomicron output in mesenteric lymph increased dramatically after ingestion of dietary lipid, and that this was associated with increased synthesis and secretion of apo A-IV by the small intestine. Elshourbagy et al. (10) and Apfelbaum et al. (21) also showed that apo A-IV synthesis in the rat small intestine increased in response to dietary triacylglycerol and might be under pretranslational control. Thus, if the enterocytes in the residual ileum adapt to massive small bowel resection by increasing fat absorption, this could elevate the apo A-IV mRNA level in the residual ileum. Gordon et al. (22), however, showed that acute triacylglycerol feeding results in a rapid increase in apo A-IV mRNA in the rat small intestine without a corresponding increase in apo A-I mRNA. We observed that the degree of the increase in apo A-I mRNA was less prominent than in apo A-IV mRNA in the residual ileum. The response of apo A-I mRNA to massive small bowel resection may be not associated with fat absorption. Another possibility is that there may be humoral factors which will regulate the adaptation of apo A-I and A-IV mRNA to massive small bowel resection. Mucosal adaptation to bowel resection is known to be stimulated by circulating hormones. Enteroglucagon is considered as the enterotrophic humoral factor involved in the intestinal adaptation to bowel resection. A number of reports showed increased plasma levels of enteroglucagon (14, 23, 24) and increased levels of ileal proglucagon mRNA (25, 26) after massive small bowel resection in rats. Peptide YY (PYY) has also been postulated to be a trophic factor involved in intestinal adaptation (27, 28). These hormones are synthesized in the L-cells located in the lower bowel (29, 30). Further studies are necessary to test whether these enterotrophic hormones stimulate apo A-I and A-IV expression during intestinal adaptation.

Plasma levels of apo A-I and A-IV decreased 2 to 5 days after massive small bowel resection, followed by the recovery to the control levels. The increases in apo A-I and A-IV mRNA in the residual ileum on the last day of the experiment suggest that the recoveries of plasma apo A-I and A-IV at least partly depend on the increased expression of these apolipoproteins in the residual ileum. The observation that the degree of plasma apo A-IV decrease was more prominent than that of apo A-I may be because the apo A-IV gene is expressed mainly in the small intestine whereas a considerable amount of apo A-I gene is expressed in the liver in addition to the small intestine. Elshourbagy et al. (10) showed that the apo A-IV and A-I mRNA levels in the liver were 12 and 36%, respectively, of that found in the small intestine, although mRNA for both apolipoproteins were most abundant in the small intestine.

Massive small bowel resection causes severe malabsorption as a result of a drastic decrease in the intestinal absorptive surface area. Though the diet used in this study contained no cholesterol, the resected rats showed lower cholesterol levels in plasma as a result of decreased re-absorption of cholesterol excreted in the bile. The transient decrease in plasma HDL-cholesterol levels after the resection may be associated with decreased delivery of apo A-I and A-IV from the residual ileum into the plasma. These apolipoproteins seem to be important determinants
for HDL-cholesterol levels in plasma. Actually, Li et al. (31) reported a marked decrease in plasma HDL-cholesterol levels in mice whose apo A-I gene was inactivated by gene targeting.

In conclusion, we propose that the rat ileum adapts to massive small bowel resection by selective increases in apo A-I and A-IV expression at a pretranslational stage. These increases may, at least in part, contribute to the recoveries of plasma apo A-I and A-IV levels.

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REFERENCES

1) Breslow, J. L. (1988): Apolipoprotein genetic variation and human disease. *Physiol. Rev.*, 68, 85–132.
2) Swaney, J. B., Braithwaite, F., and Eder, A. A. (1977): Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry*, 16, 271–278.
3) Rothblat, G. H., and Phillips, M. C. (1982): Mechanism of cholesterol efflux from cells. *J. Biol. Chem.*, 257, 4775–4782.
4) DeLamatre, J., Wolfbauer, G., Phillips, M. C., and Rothblat, G. H. (1986): Role of apolipoproteins in cellular cholesterol efflux. *Biochim. Biophys. Acta*, 875, 419–428.
5) Stein, O., Stein, Y., Lefevre, M., and Roheim, P. S. (1986): The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from an ether analog of phosphatidylcholine. *Biochim. Biophys. Acta*, 878, 7–13.
6) Steinmetz, A., Barbaras, R., Ghali, M., Clavel, V., Fruchart, J. C., and Ailhaud, G. (1990): Human apolipoprotein A-IV binds to apolipoprotein A-I/A-II receptor sites and promotes cholesterol efflux from adipose cells. *J. Biol. Chem.*, 265, 7859–7863.
7) Fielding, C. J., Shore, V. G., and Fielding, P. E. (1972): A protein co-factor of lecithin: Cholesterol acyltransferase. *Biochim. Biophys. Res. Commun.*, 46, 1493–1498.
8) Chen, C. H., and Albers, J. J. (1985): Activation of lecithin: Cholesterol acyltransferase by apolipoprotein E-2, E-3, and A-IV isolated from human plasma. *Biochim. Biophys. Acta*, 836, 279–285.
9) Steinmetz, A., and Utermann, G. (1985): Activation of lecithin: Cholesterol acyltransferase by human apolipoprotein A-IV. *J. Biol. Chem.*, 260, 2258–2264.
10) Elshourbagy, N. A., Boguski, M. S., Liao, W. S. L., Jefferson, L. S., Gordon, J. I., and Taylor, J. M. (1985): Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. *Proc. Natl. Acad. Sci. U.S.A.*, 82, 8242–8246.
11) Booth, C. C., Evans, K. T., and Menzies, T. (1989): Intestinal hypertrophy following partial resection of the small bowel in the rat. *Br. J. Surg.*, 46, 403–410.
12) Dowling, R. H. (1982): Small bowel adaptation and its regulation. *Scand. J. Gastroenterol.*, 17, 53–74.
13) Bristol, J. B., and Williamson, R. C. N. (1988): Mechanisms of intestinal adaptation. *Pediatr. Surg. Int.*, 4, 233–241.

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14) Sagor, G. R., Al-Mukhtar, M. Y. T., Ghatei, M. A., Wright, N. A., and Bloom, S. R. (1982): The effect of altered luminal nutrition on cellular proliferation and plasma concentrations of enteroglucagon and gastrin after small bowel resection in the rat. Br. J. Surg., 69, 14–18.

15) Sonoyama, K., Nishikawa, H., Kiriyama, S., and Niki, R. (1995): Apolipoprotein mRNA in liver and intestine of rats is affected by dietary beet fiber or cholestyramine. J. Nutr., 125, 13–19.

16) Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680–685.

17) Chomozynski, P., and Sacchi, N. (1987): Single-step method of RNA isolation by acid guanidium thiocyanate-henol-chloroform extraction. Anal. Biochem., 162, 156–159.

18) Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989): Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 7.37–7.52.

19) Nudel, U., Zakut, R., Shani, M., Neuman, S., Levi, Z., and Yaffe, D. (1983): The nucleotide sequence of the rat cytoplasmic β-actin gene. Nucl. Acids Res., 11, 1759–1771.

20) Hayashi, H., Nutting, D. F., Fujimoto, K., Cardelli, J. A., Black, D., and Tso, P. (1990): Transport of lipid and apolipoprotein A-I and A-IV in intestinal lymph of rat. J. Lipid Res., 31, 1613–1625.

21) Apfelbaum, T. F., Davidson, N. O., and Glickman, R. M. (1987): Apolipoprotein A-IV synthesis in rat intestine: Regulation by dietary triglyceride. Am. J. Physiol., 252, G662–G666.

22) Gordon, J. I., Smith, D. P., Alpers, D. H., and Strauss, A. W. (1982): Cloning of a complementary deoxyribonucleic acid encoding a portion of rat intestinal pre-apolipoprotein AIV messenger ribonucleic acid. Biochemistry, 21, 5424–5431.

23) Al-Mukhtar, M. Y. T., Sagor, G. R., Ghatei, M. A., Bloom, S. R., and Wright, N. A. (1983): The role of pancreatico-biliary secretions in intestinal adaptation after resection, and its relationship to plasma enteroglucagon. Br. J. Surg., 70, 398–400.

24) Jacobs, L. R., Bloom, S. R., and Dowling, R. H. (1981): Response of plasma and tissue levels of enteroglucagon immunoreactivity to intestinal resection, lactation and hyperphagia. Life Sci., 29, 2003–2007.

25) Fuller, P. J., Beveridge, D. J., and Taylor, R. G. (1993): Ileal proglucagon gene expression in the rat: Characterization in intestinal adaptation using in situ hybridization. Gastroenterology, 104, 459–466.

26) Taylor, R. G., Verity, K., and Fuller, P. J. (1990): Ileal glucagon gene expression: Ontogeny and response to massive small bowel resection. Gastroenterology, 99, 724–729.

27) Adrian, T. E., Savage, A. P., Fuessl, H. S., Wolke, K., Besterman, H. S., and Bloom, S. R. (1987): Release of peptide YY (PYY) after resection of small bowel, colon or pancreas in man. Surgery, 101, 715–719.

28) Goodlad, R. A., Ghatei, M. A., Domin, J., Bloom, S. R., Gregory, H., and Wright, N. A. (1989): Plasma enteroglucagon, peptide YY and gastrin in rats deprived of luminal nutrition, and after urogastrone-EGF administration: A proliferative role for PYY in the intestinal epithelium? Experientia, 45, 168–169.

29) Ali-Rachedi, A., Varndell, I. M., Adrian, T. E., Gapp, D. A., Van Noorden, S., Bloom, S. R., and Polak, J. M. (1984): Peptide YY (PYY) immunoreactivity is co-stored with
glucagon-related immunoreactants in endocrine cells of the gut and pancreas. *Histochemistry*, 80, 487–491.

30) Bottcher, G., Sjolund, K., Ekblad, E., Hakanson, R., Schwartz, T. W., and Sundler, F. (1984): Coexistence of peptide YY and glicentin immunoreactivity in endocrine cells of the gut. *Regul. Pept.*, 8, 261–266.

31) Li, H., Reddick, R. L., and Maeda, N. (1993): Lack of apo A-I is not associated with increased susceptibility to atherosclerosis in mice. *Arterioscler. Thromb.*, 13, 1814–1821.