Regulation of Intestinal and Hypothalamic Apolipoprotein A-IV

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This review discusses the regulation of the intestinal and hypothalamic apolipoprotein A-IV (apo A-IV) gene and protein expression. Apo A-IV is a glycoprotein secreted together with triglyceride-rich lipoproteins by the small intestine. Intestinal apo A-IV synthesis is stimulated by fat absorption, probably mediated by chylomicron formation. This stimulation of intestinal apo A-IV synthesis is attenuated by intravenous leptin infusion. Chronic ingestion of a high-fat diet blunts the intestinal apo A-IV in response to dietary lipid. Intestinal apo A-IV synthesis is also stimulated by members of the pancreatic polypeptide family, including peptide YY (PYY), neuropeptide Y (NPY), and pancreatic polypeptide (PP). Recently, apo A-IV was demonstrated to be present in the hypothalamus as well. Hypothalamic apo A-IV level was reduced by food deprivation and restored by lipid feeding. Intracerebroventricular administration of apo A-IV antiserum stimulated feeding and decreased the hypothalamic apo A-IV mRNA level, implying that feeding is intimately regulated by endogenous hypothalamic apo A-IV. Central administration of NPY significantly increased hypothalamic apo A-IV mRNA levels in a dose-dependent manner. Exp Biol Med 228:1181–1189, 2003

Key words: apolipoprotein A-IV; lipid; intestine; hypothalamus

Discovered in the late 1970s (1), apo A-IV is a protein secreted by the enterocytes in association with triglyceride (TG)-rich lipoproteins (chylomicrons). In humans, apo A-IV is a 46-kDa protein that is synthesized by the small intestine only. In rodents, apo A-IV is a smaller protein with a molecular weight of 43-kDa, and is synthesized by both the intestine and liver, although the intestine synthesizes the majority of the circulating apo A-IV (2, 3). Recently, we found that apo A-IV is also present in the hypothalamus. Numerous studies have demonstrated that intestinal and hypothalamic apo A-IV mRNA and protein levels are highly regulated. This review discusses the regulation of apo A-IV production in both the intestine and the hypothalamus.

Regulation of Intestinal apo A-IV Synthesis and Secretion

Lipid Absorption. Intestinal lipid absorption stimulates the synthesis and secretion of apo A-IV (4–7). Kalogeris *et al.* (8) reported that intestinal lymphatic transport of apo A-IV increases in a gradient fashion with increasing steady-state levels of intestinal TG transport. Evidence thus far suggests that increased synthesis of apo A-IV by fat absorption in adult rats is by transcriptional control (4). What is presently unclear is whether different types of TG (eg, TG containing saturated, monounsaturated, or polyunsaturated fatty acids) are equally effective in stimulating the secretion of apo A-IV by the small intestinal epithelial cells.

Intestinal fat absorption involves multiple steps, any of which could be involved in stimulating intestinal apo A-IV synthesis and secretion. Several lines of evidence support the notion that the assembly and transport of chylomicrons is the critical step for stimulating intestinal apo A-IV synthesis. Hayashi *et al.* (6) infused animals simultaneously with lipid and Pluronic L-81 (L-81, a hydrophobic surfactant that specifically and reversibly blocks chylomicron formation). No change in intestinal lipid digestion or uptake was observed. The absorbed lipid accumulated in the intestinal mucosa as mostly TG. However, the infusion of L-81 blocked the increase in apo A-IV synthesis and secretion by the small intestinal mucosa (6). When L-81 was removed, chylomicron secretion resumed and the accumulated mucosal lipid was cleared from the mucosa as chylomicrons. Reversing the blockage of chylomicron formation by removing L-81 also resulted in increased lymphatic apo A-IV secretion by the small intestine.

Further evidence that lymphatic apo A-IV output depends upon chylomicron transport comes from a study in which fatty acids differing in chain length were infused into the duodenum of rats (9). Infusion of fatty acids of 14 or more carbons (myristic, C-14; oleic, C-18; and arachidonic, C20) resulted in lipids being transported mainly by the lymphatic route as chylomicrons. Importantly, this also resulted in marked stimulation of lymphatic apo A-IV secretion. In

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contrast, intraduodenal infusion of medium- and short-chain fatty acids (caprylic, C-8 and butyric, C-4) resulted in lipids being primarily transported in the portal vein, and this elicited a negligible apo A-IV response (10). This finding in rats differs from the studies of Gonzalez-Vallina *et al.* (11) using neonatal swine. Intestinal infusion of medium- (C-8 and C-10) and long-chain TG mixtures in neonatal swine increased jejunal apo A-IV mRNA expression and synthesis (11). Whether the relationship between chylomicron and apo A-IV synthesis and secretion is common to all species or developmental stages is currently unclear. For example, little is known about how the formation of chylomicrons is signal-transduced to stimulate intestinal apo A-IV synthesis; or whether the signal-transduction event takes place in the enterocytes. However, we do know that it does not involve the central nervous system, as vagotomy has no effect on the stimulation of intestinal apo A-IV synthesis by intestinal fat absorption (9).

Apo A-IV is synthesized mainly by intestinal villus epithelial cells (12), and its synthesis is higher in the jejunum than in the ileum (13). In the jejunum, all epithelial cells of the villi were found to synthesize apo A-IV, such that lipid infusion increased the apo A-IV content in these cells. Importantly, no apo A-IV immunostaining was observed in the cells of the crypt (Fig. 1A). In the ileum, apo A-IV was observed mainly in cells occupying the upper portion of the villi (Fig. 1G). Following lipid infusion, apo A-IV was observed in all epithelial cells of the upper and lower villi. These results suggest that all epithelial cells in the jejunal and ileal villi have the capability to synthesize apo A-IV, and that this synthesis is markedly stimulated by the absorption of fat (Fig. 1B and 1H). The distribution of apo A-IV observed along the small intestine suggests that most lipid absorption as chylomicrons normally occurs in the jejunum. When the intestinal epithelial cells were examined under high magnification, apo A-IV existed as granules concentrated mainly in the supranuclear region of the cell (Fig. 1D). After lipid infusion, apo A-IV staining spread throughout the entire cytoplasm (Fig. 1E). These findings probably reflect the transport of apo A-IV together with chylomicrons in Golgi-derived vesicles.

Leptin. Leptin is a peptide hormone synthesized and secreted by white adipocytes. Administration of exogenous leptin decreases food intake and increases energy expenditure across all mammals tested. Plasma leptin levels are elevated in obesity and/or when animals are maintained on a high-fat diet (14, 15). Recently, we demonstrated that stimulated levels of intestinal apo A-IV induced by active fat absorption were significantly attenuated by intravenous leptin infusion (12). Specifically, apo A-IV immunostaining during intraduodenal lipid infusion was reduced by an average of 50% in the jejunum and 35% in the ileum ($P < 0.05$ for each) in animals infused intravenously with leptin (Fig. 1C, 1F, and 1I). This is consistent with the findings of Morton *et al.* (16), who observed that leptin reduced apo

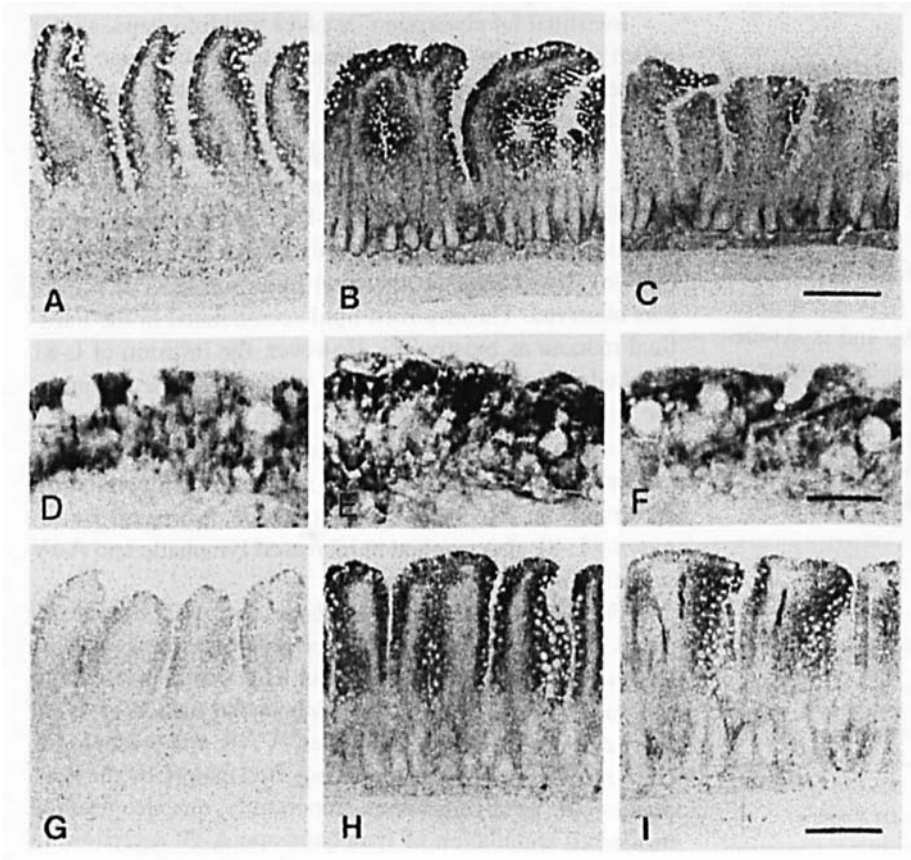

Figure 1. (A–F) Apo A-IV immunostaining in rat proximal jejunum and (G–I) proximal ileum after 4 h of lipid and leptin infusion. (A, D, G) glucose–saline intraduodenal (ID) infusion + saline intravenous (IV) infusion in control rats. (B, E, H) 5% lipid ID infusion + saline IV infusion in experimental rats. (C, F, I) 5% lipid ID infusion + leptin IV infusion in experimental rats. Bars: A–C, G - I = 200 μm; D–F = 25 μm.

A-IV transcript levels 90 min following ingestion of a pure fat meal.

Leptin is an important component of lipid homeostasis since its levels in the circulation are directly correlated with the amount of fat in the body (15). Thus, circulating leptin increases as an individual becomes more obese. Because of this, a potential consequence of becoming obese may be an attenuated intestinal apo A-IV response to ingested lipids due to increased circulating leptin levels. Consumption of a high-fat diet initially increases plasma apo A-IV levels in humans. Over time, however, this increase disappears (17). It is tempting to speculate that this apparent autoregulation of apo A-IV in response to chronic ingestion of a high-fat diet may, in fact, be related to the elevation of circulating leptin. Another clinical observation indirectly supporting a role for leptin in intestinal apo A-IV synthesis is the indifference in plasma apo A-IV levels between obese and lean subjects with non-insulin dependent diabetes mellitus (NIDDM) (18). That plasma apo A-IV did not become elevated may be due to increased plasma leptin levels.

Circadian Factors. Lymphatic apo A-IV secretion by the gastrointestinal tract is closely tied with the intestinal secretion of lipoproteins during both fasting and fat absorption. When the circadian rhythm of lymphatic apo A-IV output was examined, Fukagawa *et al.* (2) reported that secretion increased just before feeding and peaked midway through the dark period (top line, Fig. 2). This pattern closely correlates with lymphatic triglycerides (TG), phospholipids (PL), and cholesterol (CL) outputs (2).

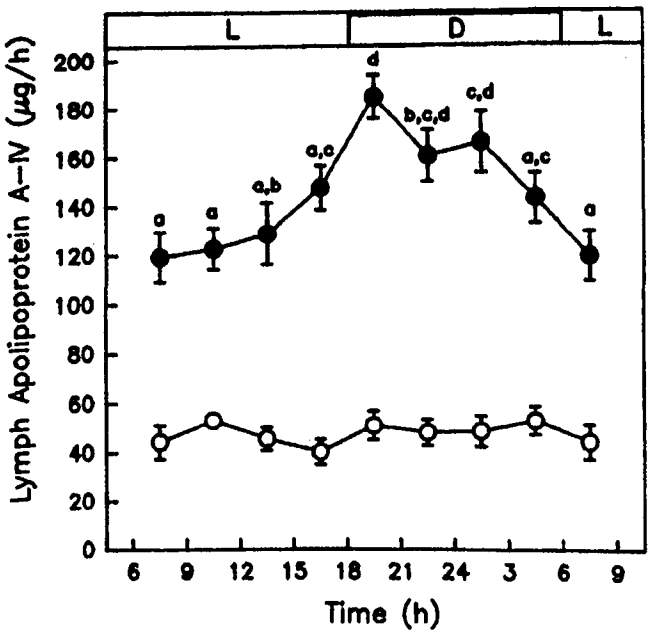

Figure 2. Lymph apo A-IV output in 24 h fasted rats with or without bile diversion (●); lymph apo A-IV output in fasted rats (µg/h) (○), lymph apo A-IV output in fasted rats with bile diversion (µg/h). Seven rats were used for this study. Values are expressed as mean ± SE. Difference letters show that the difference is significant with $P < 0.05$ (Reproduced with permission from Ref. 2).

Davidson *et al.* (19) have demonstrated that bile diversion significantly reduces apo A-IV synthesis by the intestinal mucosa. Bile diversion reduces lymphatic outputs of apo A-IV by 67%, CL by 81%, and both TG and PL by 90%. Bile diversion not only reduces lymphatic apo A-IV secretion, but it also abolishes the circadian rhythm output of apo A-IV (bottom line, Fig. 2) (2). Thus, intact enterohepatic circulation is necessary for both normal basal lymphatic output of apo A-IV and its circadian rhythm.

Pancreatic Polypeptide Family: Peptide YY (PYY), Neuropeptide Y (NPY), and Pancreatic Polypeptide (PP). All members of the pancreatic polypeptide family, PYY, NPY, and PP, are related to nutrient homeostasis. PYY is synthesized in endocrine cells in the ileum and colon (20) and is released into the circulation during a meal (21) or during lipid infusion into the distal jejunum (22). PP is secreted from endocrine cells in the islets of Langerhans (23) and is also released during ingestion of a meal via enhanced activity of the vagus nerves (24). NPY is found in neurons in the central and the peripheral nervous system (25), and in the brain it potently stimulates food intake (26).

Recently, it has been determined that fat absorption in the ileum can stimulate intestinal apo A-IV synthesis and secretion in the jejunum without involving jejunal chylomicron formation and secretion. This strongly implies that a signal generated in the ileum feeds back to the jejunum to stimulate the apo A-IV system. Kalogeris *et al.* (10) compared the effects of proximal versus distal intestinal infusion of lipid on jejunal and ileal apo A-IV synthesis. They found that duodenal lipid infusion elevated both apo A-IV synthesis and mRNA levels only in the jejunum. In contrast, lipid infused into the ileum stimulated both ileal and jejunal apo A-IV synthesis. Subsequent experiments performed in rats with jejunal or ileal Thiry-Vella fistulas (a segment of intestine luminally isolated from the rest of the gastrointestinal (GI) tract) demonstrated that ileally infused lipid elicits an increase in proximal jejunal apo A-IV synthesis independent of the presence of jejunal lipid. These results also strongly suggest that a signal is released by the distal gut and is capable of stimulating apo A-IV synthesis in the proximal gut. These findings have important physiological implications. The distal intestine is known to play an important role in the control of GI function. Nutrient, especially lipid, delivered to the ileum results in the inhibition of gastric emptying (27, 28), decreased intestinal motility and transit (28, 29), and decreased pancreatic secretion (30). Ileal nutrient infusion also inhibits food intake (31, 32).

Evidence strongly suggests that PYY is the signal that stimulates jejunal apo A-IV synthesis and secretion. Continuous intravenous infusion of physiological doses of PYY elicits significant increases in both apo A-IV synthesis and lymphatic transport in fasting rats (9). Kalogeris *et al.* (9) demonstrated that the stimulation of jejunal apo A-IV synthesis by PYY is probably translationally, not transcriptionally, controlled, since the apo mRNA level was unaltered

but synthesis was markedly stimulated. Furthermore, Kalogeris *et al.* (9) showed that vagotomy failed to block the stimulation of jejunal apo A-IV synthesis by fat absorption (Fig. 3). However, when lipid was infused directly into the ileum, vagotomy completely abolished the increase in jejunal apo A-IV synthesis (Fig. 4). Thus, stimulation of jejunal apo A-IV synthesis and secretion by fat absorption in the ileum is mediated by a factor, probably PYY, which acts centrally via the vagus nerve to send a reflex signal to the gut to stimulate jejunal apo A-IV synthesis. We believe this to be the first demonstration of a GI hormone involved in the control of expression and secretion of an intestinal apolipoprotein, thus bringing together 2 areas of research in GI physiology.

Recently we investigated whether members of the PP family other than PYY also stimulate intestinal apo A-IV synthesis. We assessed the effects of intravenous (IV) infusion of NPY and PP on apo A-IV synthesis in the small intestine by immunohistochemistry, and compared them with the effect of PYY. We found that apo A-IV immunostaining increased markedly following IV infusion (200 pmol/kg/h) of PYY (Fig. 5B and 5F), NPY (Fig. 5C and 5G), and PP (Fig. 5D and 5H) compared with the saline control (Fig. 5A and 5E), and this was true in both the jejunum and the ileum. Quantitative analysis revealed that apo A-IV immunostaining increased approximately 2-fold in the jejunum (Fig. 6A) and 10-fold in the ileum (Fig. 6B) following PYY ($P < 0.05$ and $P < 0.01$, respectively), NPY ($P < 0.01$ in both jejunum and ileum), and PP ($P < 0.05$ in both jejunum and ileum) infusion. There were no significant differences among the PYY, NPY, or PP groups in either the jejunum or ileum. To investigate the pathway of the effect, we also administered NPY by intracerebroventricular (ICV) infusion and analyzed apo A-IV in the small intestine. Figure 7 depicts the quantitative analysis of apo AIV immunostaining in the proximal jejunum and the proximal ileum after 4 h of ICV infusion of NPY. ICV NPY increased ileal apo AIV approximately 5-fold ($P < 0.05$). The increase of jejunal apo AIV by ICV infusion of NPY was not statistically significant.

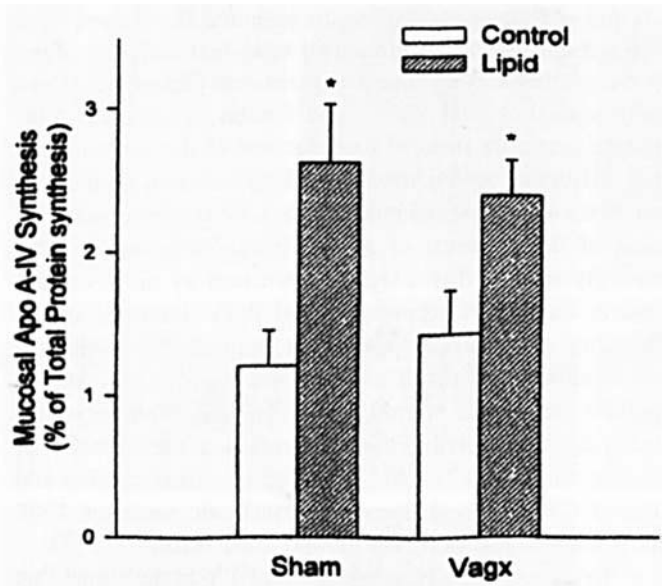

Figure 3. Vagotomy has no effect on the stimulation of apo A-IV synthesis in proximal jejunum by duodenal infusion of triacylglycerol emulsion. Vagotomized (Vagx) or sham-vagotomized rats equipped with duodenal infusion cannulas received continuous 8 h duodenal infusions of glucose saline (control) or lipid (triolein emulsion, 40 micromoles per h). Values are means ± SE for 4 rats (sham, control), 6 rats (vagx, control), 5 rats (sham, lipid), and 7 rats (vagx, lipid). *Significant effect of lipid infusion on apo A-IV synthesis ($P < 0.01$) (Reproduced with permission from Ref. 8).

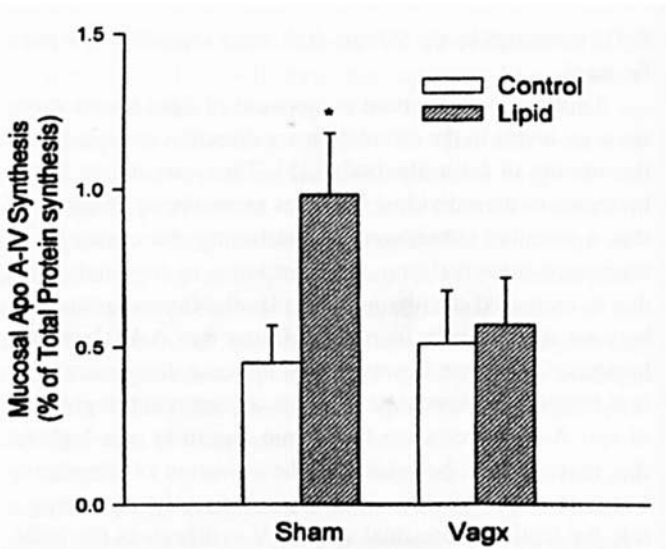

Figure 4. Vagotomy prevents increase in jejunal apo A-IV synthesis in a jejunal Thiry-Vella fistula elicited by ileal lipid infusion. Vagotomized or sham-vagotomized rats equipped with jejunal Thiry-Vella fistula and ileal infusion cannulas received continuous 8 h ileal infusions of either glucose-saline (Control) or lipid emulsion (Lipid). Mucosal apo A-IV synthesis in the Thiry Vella segment was then measured. Values are mean ± SE for 6 rats (sham, control), 5 rats (sham, lipid), 6 rats (vagx, control), and 8 rats (vagx, lipid). *Significant effect of lipid ($P < 0.001$) (Reproduced with permission from Ref. 9).

Effect of Chronic Consumption of a High-Fat Diet on Intestinal apo A-IV Synthesis. The effect that chronic high-fat consumption has on intestinal apo A-IV synthesis is interesting, but the mechanisms involved are presently unclear. Weinberg *et al.* (17) first reported that human plasma apo A-IV levels adapt in response to prolonged consumption of fat. Humans chronically consuming a high-fat diet initially developed significantly elevated plasma apo A-IV levels, which disappeared over time. Investigators therefore concluded that intestinal apo A-IV production is autoregulated in response to diets high in fat.

Apfelbaum *et al.* (4) reported a 1.6- to 1.8-fold increase in jejunal apo A-IV synthesis in rats fed a 30% (wt/wt) fat diet for 6 weeks compared with rats consuming a fat-free diet for 3 weeks. Apfelbaum *et al.* also found that, unlike acute experiments using bolus intragastric injections of corn oil, chronic high-fat consumption had no effect on ileal apo A-IV synthesis. Does the ileum, then, become less responsive to lipid following chronic consumption of a high-fat diet, or does the adaptation of the digestive and absorptive

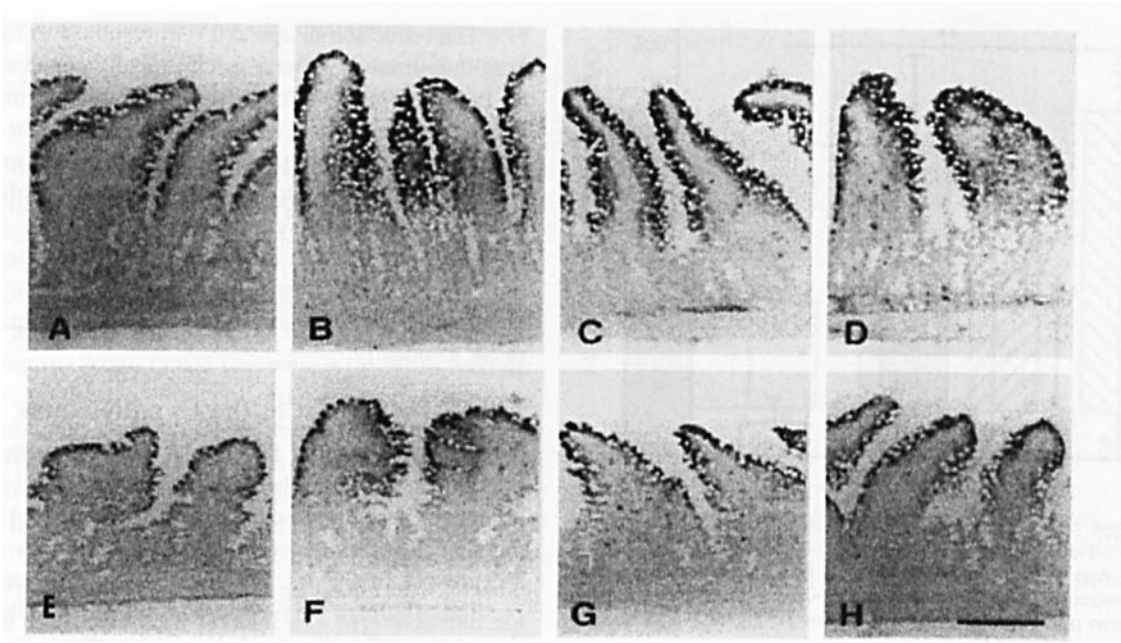

Figure 5. Apolipoprotein A-IV (apo AIV) immunohistochemistry in (A–D) rat proximal jejunum and (E–H) proximal ileum after 4 h of IV infusion (200 pmol/kg/h) of peptide YY (PYY), neuropeptide Y (NPY), or pancreatic polypeptide (PP). (A, E) Saline, (B, F) PYY, (C, G) NPY, and (D, H) PP. Bar = 200 μm.

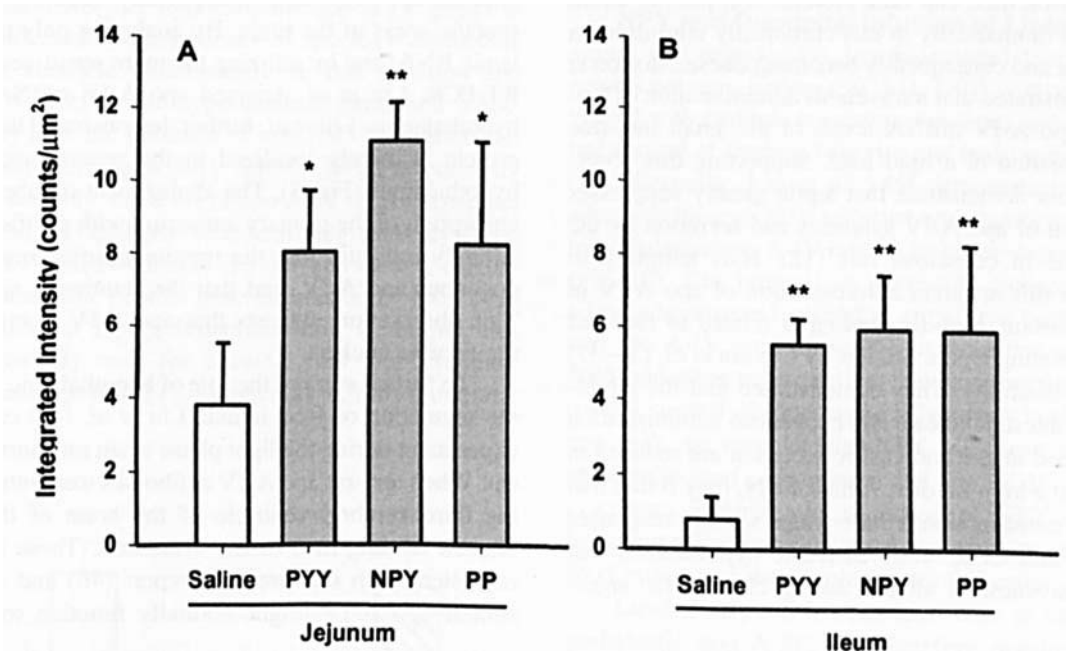

Figure 6. Quantitative analysis of apo A-IV immunostaining in rat proximal jejunum (A) and proximal ileum (B) after IV infusion of PYY, NPY, or PP. Each bar is the mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus corresponding values of the saline group.

process result in fat no longer reaching the ileum? This question warrants further investigation.

Recently, Kalogeris and Painter (33) examined the effect of daily fat supplementation on intestinal gene expression and protein synthesis and plasma levels of apo A-IV. Male rats were administered bolus injections of 3.6 ml of either saline or Intralipid (20% fat emulsion) intragastrically for 0, 1, 2, 4, 8, or 16 days. They found that plasma apo A-IV showed an initial 40% increase in rats fed the fat emulsion compared with saline-injected controls. Continued daily fat feeding produced a plasma apo A-IV response that rapidly and progressively diminished, such that by day 4, plasma apo A-IV was the same in both the fat- and saline-fed groups. Jejunal mucosal apo A-IV synthesis and mRNA levels also showed a time-dependent reduction in response to fat feeding. There was no correlation between plasma TG levels and intestinal apo A-IV synthesis or plasma apo A-IV (33).

Consequently, both rodent and human data suggest that intestinal apo A-IV synthesis and secretion become less

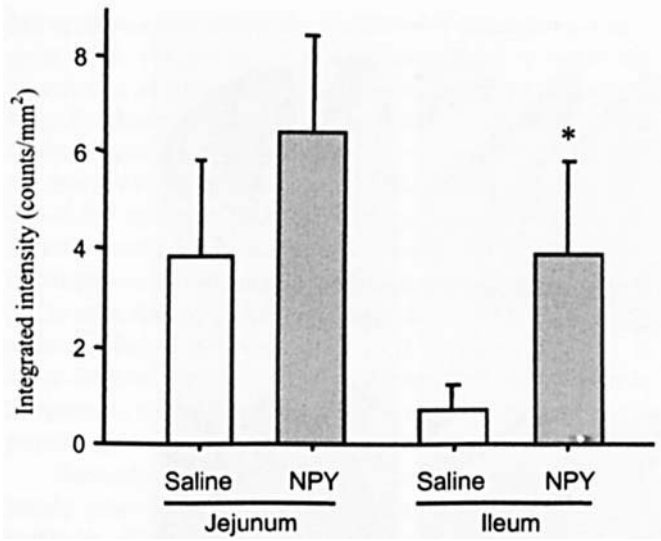

Figure 7. Apo AIV immunostaining in rat proximal jejunum (A) and proximal ileum (B) after NPY intracerebroventricular (ICV) infusion. Each column and vertical bar shows mean ± SD. $^*P < 0.05$ versus corresponding values of saline group.

responsive to lipid feeding following the chronic consumption of a high-fat diet. Our data indicate that plasma leptin also increases dramatically in rats chronically consuming a diet high in fat and consequently becoming obese. Morton *et al.* (16) demonstrated that intravenous administration of leptin reduces apo A-IV mRNA levels of the small intestine following ingestion of a lipid load. Supporting this observation, our data demonstrate that leptin greatly suppresses the stimulation of apo A-IV synthesis and secretion by the small intestine in conscious rats (12). It is tempting to speculate that this apparent autoregulation of apo A-IV in response to chronic high-fat feeding is related to elevated levels of circulating leptin. Studies by Covasa *et al.* (34–37) support this possibility. They demonstrated that the inhibitory effect of intestinal oleate and exogenous administration of CCK on food intake and gastric secretion are reduced in rats adapted to a high-fat diet. Additionally, they found that prolonged fat consumption reduces vagal sensory responses to both lipid and CCK. They therefore hypothesized that vagal responsiveness is altered during chronic fat ingestion (34).

The reduction in apo A-IV in response to lipid feeding in both animals and humans chronically consuming a high-fat diet may be physiologically and clinically important, as it may explain why it leads to the development of obesity. Increased consumption of a high-fat diet leading to an increase in body fat is supported by numerous studies utilizing different species consuming various diets (38–42). Understanding why chronic ingestion of fat attenuates the intestinal apo A-IV response may provide us with a clue to better understand why it also predisposes both animals and humans to obesity.

Apo A-IV Present in the Hypothalamus

Today, we know that apo A-IV is also present in the hypothalamus, a site that is intimately involved in the regulation of energy homeostasis (43). Liu *et al.* (43) provided the first direct evidence that apo A-IV mRNA and protein are present in the rat hypothalamus. These studies refute previous findings by Elshourbagy *et al.* (44) that rat brain does not synthesize apo A-IV. Elshourbagy *et al.* (44) extracted total brain RNA and analyzed it by RNA dot blotting, a method that may not have been sufficiently sensitive to detect apo A-IV mRNA, especially when it is localized in specific areas of the brain. By analyzing only the hypothalamic RNA, and by utilizing the more sensitive competitive RT-PCR, Liu *et al.* detected apo A-IV mRNA in the rat hypothalamus. Liu *et al.* further demonstrated that apo A-IV protein is mainly localized in the arcuate nucleus of the hypothalamus (Fig. 8). The abolishment of labeling by preabsorption of the primary antiserum with purified apo A-IV strongly indicates that the immunoreactive material is endogenous apo A-IV, and that the staining is specific (45). This observation suggests that apo A-IV is synthesized in the arcuate nucleus.

To further explore the role of hypothalamic apo A-IV in the regulation of food intake, Liu *et al.* (43) conducted an experiment during the light phase when rats normally do not eat. When anti-rat apo A-IV antibodies were introduced into the third cerebral ventricle of the brain of these rats, it elicited feeding in 6 of the 7 animals. These findings are consistent with our previous report (46) and suggest that central apo A-IV might normally function to inhibit the

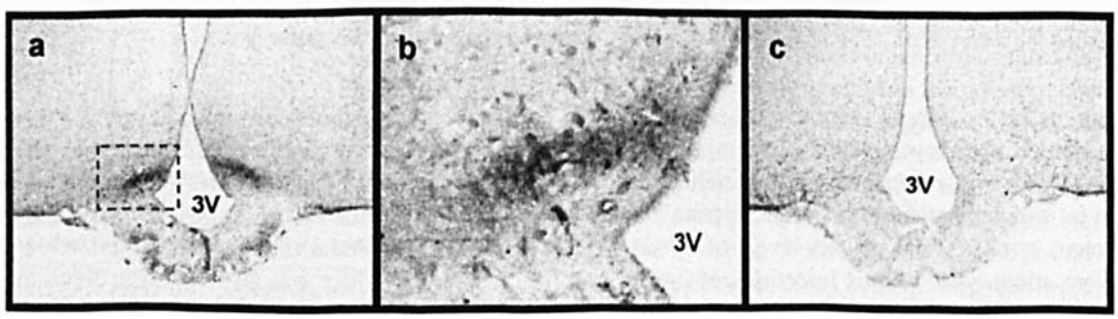

Figure 8. Immunohistochemical detection of apo A-IV in rat hypothalamus. (a) A section incubated in goat anti-rat apo A-IV serum. Strong gray cellular and cytoplasmic staining are evident in the hypothalamus (× 20 amplification). (b) A section, outlined in (a), with amplified image (× 100) to enable finer localization of apo A-IV staining. (c) A section incubated in goat anti-rat apo A-IV serum after preabsorption with 2.3 μM purified apo A-IV (20 ×).

onset of feeding. If so, this would distinguish apo A-IV from other so-called satiety signals such as CCK, which limit meal size but have no effect on meal initiation (47).

Regulation of Hypothalamic apo A-IV

Fasting and Lipid Refeeding. Recently, Liu *et al.* demonstrated that the level of apo A-IV mRNA in the hypothalamus is influenced markedly by an animal's nutritional status. Fasting, for example, caused a marked reduction in apo A-IV gene expression in both the hypothalamus and the jejunum. When fasted rats were allowed to feed on chow (a low-fat diet), only a very small effect was found on hypothalamic apo A-IV mRNA levels. However, when fasted animals were gavage-fed with lipid (5 ml of 20% of lipid emulsion), hypothalamic apo A-IV mRNA levels were restored to the same levels observed in *ad libitum*-fed animals (Fig. 9) (43). Presently, it is unclear how lipid feeding regulates hypothalamic apo A-IV expression (ie, whether it is a direct or indirect effect). Nonetheless, hypothalamic and intestinal apo A-IV respond similarly to fasting and lipid feeding.

Treatment with apo A-IV Antibody. Liu *et al.* (43) also determined whether hypothalamic apo A-IV gene expression is affected by the presence of apo A-IV antibodies in the third ventricle. Interestingly, in this situation apo A-IV mRNA level in the hypothalamus decreased markedly. Exactly how the presence of apo A-IV antiserum decreases hypothalamic apo A-IV mRNA levels is currently unclear. Presumably, the level of apo A-IV in cerebrospinal fluid (reduced by antibody treatment) regulates hypothalamic apo A-IV mRNA levels. To our knowledge, regulation of hypothalamic peptide gene expression by local peptide concentration in cerebrospinal fluid is unique, described previously only for cocaine- and amphetamine-regulated transcript (CART). Lambert *et al.* (48) found that

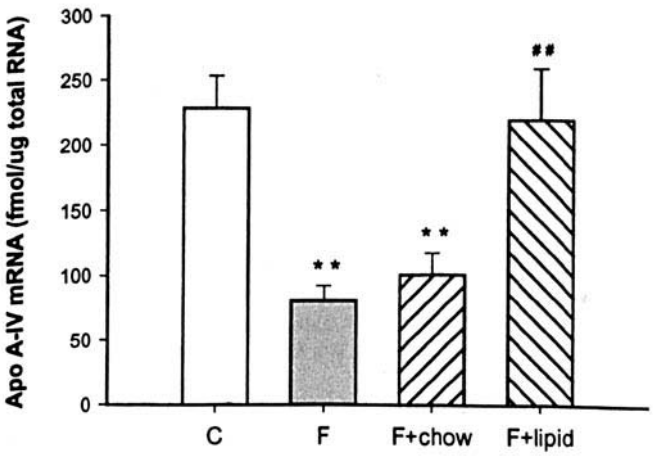

Figure 9. Analysis of relative changes in hypothalamic apo A-IV mRNA level by competitive RT-PCR. C = *ad libitum* fed animals; F = fasting; F+C = fasting followed by chow refeeding; F+L = fasting followed by Intralipid emulsion infused by gavage. Values are means ± SE (n = 6). Compared to *ad libitum*-fed animals, the control, $^{**}P < 0.01$; and compared to fasting, $^{\#\#}P<0.01$.

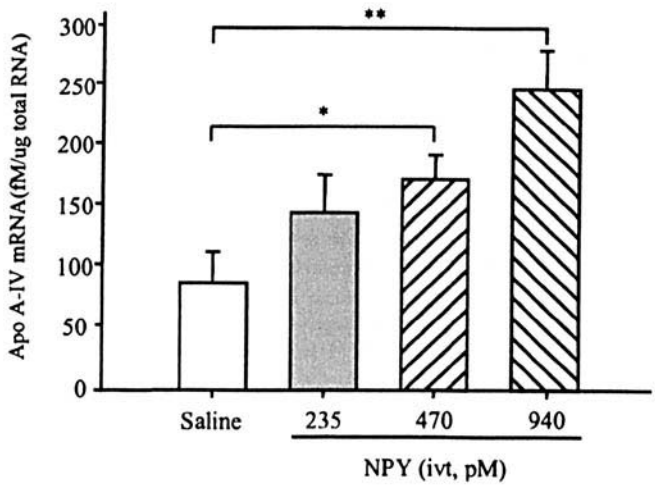

Figure 10. Effect of centrally administered NPY on hypothalamic apo A-IV gene expression determined by competitive RT-PCT. Values are means ± SE (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with saline group.

intracerebroventricular introduction of antibodies against CART resulted in feeding (an observation similar to ours) and injection of the peptide itself inhibited food intake.

NPY and Duodenal Infusion of Lipid. NPY, a 36-amino acid peptide (49), has widespread distribution in the central nervous system (50, 51). NPY is thought to be involved with multiple regulatory functions including the central control of feeding behavior and body weight regulation (52). Recently, Liu *et al.* (45) demonstrated that central administration of NPY to fasted rats significantly increased hypothalamic apo A-IV mRNA in a dose-dependent manner (Fig. 10). This presents an interesting dichotomy, as NPY, when administered centrally, stimulates both food intake and apo A-IV production. We propose that apo A-IV and NPY and other neuropeptides function together to maintain overall control of food intake. Therefore, in the absence of apo A-IV, we speculate that NPY will cause an even bigger increase in food consumption and obesity. If our hypothesis is correct, central administration of NPY in apo A-IV knockout mice should stimulate an even greater increase in food intake and the development of obesity.

Lipid absorption is associated with an increase in hypothalamic apo A-IV. We therefore questioned whether centrally administered NPY further increases the already-elevated apo A-IV mRNA level in the arcuate nucleus of lipid-infused rats. We determined that it did not (Fig. 9), and speculated that it might be explained by the following. First, perhaps hypothalamic apo A-IV mRNA levels reach their maximum possible levels following NPY administration or duodenal lipid infusion. Second, maybe NPY is ineffective at stimulating apo A-IV mRNA when lipid is processed by the gut.

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