Gastrointestinal Satiety Signals
IV. Apolipoprotein A-IV

Patrick Tso, William Sun, and Min Liu
Department of Pathology, University of Cincinnati, Ohio 45267

Tso, Patrick, William Sun, and Min Liu. Gastrointestinal Satiety Signals. IV. Apolipoprotein A-IV. Am J Physiol Gastrointest Liver Physiol 286: G885–G890, 2004; 10.1152/ajpgi.00511.2003.—The focus of this article is to review evidence that apolipoprotein A-IV (apo A-IV) acts as a satiety factor. Additionally, information regarding the general involvement of apo A-IV in the regulation of food intake and body weight is stated. Apo A-IV is a glycoprotein synthesized by the human intestine. In rodents, both the small intestine and liver secrete apo A-IV, but the small intestine is the major organ responsible for circulating apo A-IV. There is now solid evidence that the hypothalamus, especially the arcuate nucleus, is another active site of apo A-IV expression. Intestinal apo A-IV synthesis is markedly stimulated by fat absorption and does not appear to be mediated by the uptake or reesterification of fatty acids to form triglycerides. Rather, the local formation of chylomicrons acts as a signal for the induction of intestinal apo A-IV synthesis. Intestinal apo A-IV synthesis is also enhanced by a factor from the ileum, probably peptide tyrosine-tyrosine (PYY). The inhibition of food intake by apo A-IV is mediated centrally. The stimulation of intestinal synthesis and secretion of apo A-IV by lipid absorption are rapid; thus apo A-IV likely plays a role in the short-term regulation of food intake. Other evidence suggests that apo A-IV may also be involved in the long-term regulation of food intake and body weight, as it is regulated by both leptin and insulin. Chronic ingestion of a high-fat diet blunts the intestinal as well as the hypothalamic apo A-IV response to lipid feeding. It also suppresses apo A-IV gene expression in the hypothalamus. Whereas it is tempting to speculate that apo A-IV may play a role in diet-induced obesity, we believe the confirmation of such a proposal awaits further experimental evidence.

REGULATION OF INTESTINAL APO A-IV SYNTHESIS AND SECRETION
To gain a better understanding of the physiological role of apo A-IV on food intake, it is important to have a good understanding of how the synthesis and secretion of apo A-IV are regulated in the enterocytes. As mentioned earlier, intestinal apo A-IV production is stimulated by fat absorption. Hayashi et al. (15) showed that the stimulation of apo A-IV production by fat absorption is not related to the digestion, uptake, or reesterification of absorbed monoacylglycerol and fatty acids to form triacylglycerol. Rather, it is dependent on the formation of CM. Thus Hayashi et al. (15) found that when CM formation was abolished by a Pluronic surfactant called Pluronic L-81 (L-81), fat absorption failed to stimulate apo A-IV production by the enterocytes despite the accumulation of large amounts of lipids in the endoplasmic reticulum of the enterocytes (31). The stimulation of intestinal apo A-IV synthesis and secretion by CM formation and/or secretion is further supported by the studies of Kalogeris et al. (19) who demonstrated that with duodenal infusion of fatty acids with varying chain length, significant increases in lymph triacylglycerol and apo A-IV output were observed only in response to long-chain fatty acids (14:0, 18:0, 18:1, 18:2, 20:4) but not to the intraduodenal infusion of short- or medium-chain fatty acids (4:0, 8:0, 12:0). This pattern of lymphatic output of apo A-IV agrees with data on mucosal apo A-IV synthesis. The important question for which we do not currently have an answer is how the formation and/or secretion of CM stimulate the synthesis and secretion of apo A-IV. Is this an intracellular event or an extracellular event? We do know whether the stimulation of intestinal apo A-IV synthesis and secretion by fat absorption involve the stimulation of the vagus nerve because vagotomy does not affect this physiological process.

Although it is well accepted that fat absorption stimulates intestinal apo A-IV synthesis and secretion, we asked what maintains the basal apo A-IV (fasting) output? Lymphatic apo A-IV secretion by the gastrointestinal tract displays a circadian rhythm with output starting to increase before feeding and peaking in the middle of the dark period (top line of Fig. 1) (12). This pattern is closely correlated with lymphatic triacylglycerol, phospholipid, and cholesterol outputs. Bile diversion reduces lymphatic output of apo A-IV by 67%, cholesterol by 81%, and both triacylglycerol and phospholipid by 90%. Moreover, bile diversion completely abolishes the circadian rhythm in outputs of apo A-IV (bottom line of Fig. 1) (12). Davidson et al. (6) have demonstrated that bile diversion significantly reduces apo A-IV synthesis by the intestinal mucosa. Thus an understanding of how the synthesis and secretion of apo A-IV are regulated in the enterocytes. As mentioned earlier, intestinal apo A-IV production is stimulated by fat absorption. Hayashi et al. (15) showed that the stimulation of apo A-IV production by fat absorption is not related to the digestion, uptake, or reesterification of absorbed monoacylglycerol and fatty acids to form triacylglycerol. Rather, it is dependent on the formation of CM. Thus Hayashi et al. (15) found that when CM formation was abolished by a Pluronic surfactant called Pluronic L-81 (L-81), fat absorption failed to stimulate apo A-IV production by the enterocytes despite the accumulation of large amounts of lipids in the endoplasmic reticulum of the enterocytes (31). The stimulation of intestinal apo A-IV synthesis and secretion by CM formation and/or secretion is further supported by the studies of Kalogeris et al. (19) who demonstrated that with duodenal infusion of fatty acids with varying chain length, significant increases in lymph triacylglycerol and apo A-IV output were observed only in response to long-chain fatty acids (14:0, 18:0, 18:1, 18:2, 20:4) but not to the intraduodenal infusion of short- or medium-chain fatty acids (4:0, 8:0, 12:0). This pattern of lymphatic output of apo A-IV agrees with data on mucosal apo A-IV synthesis. The important question for which we do not currently have an answer is how the formation and/or secretion of CM stimulate the synthesis and secretion of apo A-IV. Is this an intracellular event or an extracellular event? We do know whether the stimulation of intestinal apo A-IV synthesis and secretion by fat absorption involve the stimulation of the vagus nerve because vagotomy does not affect this physiological process.

Although it is well accepted that fat absorption stimulates intestinal apo A-IV synthesis and secretion, we asked what maintains the basal apo A-IV (fasting) output? Lymphatic apo A-IV secretion by the gastrointestinal tract displays a circadian rhythm with output starting to increase before feeding and peaking in the middle of the dark period (top line of Fig. 1) (12). This pattern is closely correlated with lymphatic triacylglycerol, phospholipid, and cholesterol outputs. Bile diversion reduces lymphatic output of apo A-IV by 67%, cholesterol by 81%, and both triacylglycerol and phospholipid by 90%. Moreover, bile diversion completely abolishes the circadian rhythm in outputs of apo A-IV (bottom line of Fig. 1) (12). Davidson et al. (6) have demonstrated that bile diversion significantly reduces apo A-IV synthesis by the intestinal mucosa. Thus an
In contrast, infusion of lipid to the ileum stimulated both ileal and jejunal apo A-IV synthesis. Subsequent experiments in rats equipped with jejunal or ileal Thiry-Vella fistulas (segment of intestine isolated luminaly from the rest of the gastrointestinal tract) demonstrated the following interesting findings: 1) ileally infused lipid elicits an increase in proximal jejunal apo A-IV synthesis independent of the presence of lipid in the jejunum, and 2) both the ileum and more distal sites may be involved in the stimulation. These results strongly suggest that a signal produced and secreted by the distal gut 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 gastrointestinal function. Nutrient (especially lipid) delivered to the ileum results in the inhibition of gastric emptying (21), decreased intestinal motility and transit (30), and decreased pancreatic secretion (14). Ileal nutrient also inhibits food intake (25, 33). The mechanism(s) for these effects has been collectively termed the “ileal brake” (30), which have traditionally been considered operative only in the abnormal delivery of undigested nutrients to the distal gut, such as the malabsorptive state (30). However, growing evidence seems to indicate that nutrient reaches the distal gut, even under normal conditions, because of rapid gastric emptying during the early phases of food ingestion (21, 29). We recently studied the intraluminal and mucosal distribution of a bolus of 3H-triolein-labeled intralipid (0.5 ml of a 20% emulsion) administered by gavage. By 15–30 min, radiolabeled lipid spread throughout the entire gut, with 10–15% of the load recovered in the ileum and cecum combined. When we examined apo A-IV synthesis in the small intestine, we discovered that stimulation had occurred rapidly (between 15–30 min) and that it had occurred throughout the entire intestine including the ileum. Significant stimulation of lymphatic output and plasma levels of apo A-IV occurred by 30 min after the feeding of a gastric lipid load (29). Consequently, it appears that a much greater length of intestine is involved in the absorption of lipid and in the control of gastric and upper gut functions, even under normal conditions, than has been previously recognized. Thus the ileal brake may play an important role in the normal control of gut function and control of lipid absorption.

We believe one likely peptide to mediate the phenomenon of ileal brake is peptide tyrosine-tyroline (PYY), which is a member of the peptide family including pancreatic polypeptide and neuropeptide Y. PYY is synthesized by endocrine cells in the ileum and large intestine (2) and is released in response to intestinal nutrients, especially long-chain fatty acids (17).

We now have evidence that PYY stimulates jejunal apo A-IV synthesis and secretion. Continuous intravenous infusion of physiological doses of PYY (thus maintaining a circulating level of PYY comparable with that observed during fat absorption) elicits significant increases in both synthesis and lymphatic transport of apo A-IV in rats (18). Kalogeris et al. (18) further demonstrated that the stimulation of jejunal apo A-IV synthesis by PYY is probably translationally controlled rather than transcriptionally controlled, because the apo mRNA level is not altered, but synthesis is markedly stimulated. However, unlike the stimulation by fat absorption, vagotomy totally abolished an increase in jejunal apo A-IV synthesis stimulated by ileal lipid absorption. PYY has been demonstrated recently by Batterham et al. (4) to play an important role in the inhibition of food intake. They concluded from their study that peptide YY(3–36) is released from the gastrointestinal tract postprandially in proportion to the calorie content of a meal, and it inhibits food intake and reduces weight gain in rats. PYY(3–36) also inhibits food intake in mice but not in Y2 receptor (Y2R)-null mice, which suggests that the anorectic effect requires the Y2R. In humans, infusion of normal postprandial concentrations of PYY(3–36) significantly decreases appetite and reduces food intake by 33% over 24 h. Thus postprandial elevation of PYY(3–36) may act through the arcuate nucleus (ARC) Y2R to inhibit feeding in a gut-hypothalamic pathway. With what we know about apo A-IV and with the recent finding of Batterham et al. (4), it is questioned whether the PYY effect is mediated partially or fully via apo A-IV. A critical experiment to test this concept would be to study the effect of PYY on food intake in apo A-IV knockout animals.

Fig. 1. Lymph apolipoprotein A-IV (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). Values are expressed as means ± SE. Different letters show that the difference is significant (P < 0.05).
INTRAVENOUS INFUSION OF APO A-IV INHIBITS FOOD INTAKE

Fujimoto et al. (9) were the first to report that apo A-IV is a satiety factor secreted by the gut following fat absorption and that this unique function of apo A-IV is not shared by gut apo AI. In 24-h fasted rats, various intestinal lymph samples were taken from donor rats and were infused intravenously to determine their effect on food intake. Fujimoto et al. (9) demonstrated that intestinal lymph collected from rats that were actively absorbing lipid markedly suppressed food intake during the first 30 min of refeeding. In contrast, infusion of fasting intestinal lymph had very little effect on food intake compared with the saline-infused rats. Suppression of food intake was not observed in the subsequent 30 min of refeeding either.

The fact that food intake was inhibited in rats that received intestinal lymph from donor rats actively absorbing fat indicates that one or more factors may be involved in the inhibition of food intake. Because the lipid content of lymph increased as much as 10–15 times during fat absorption, it was hypothesized that the additional lipid in the chylous lymph (in the form of CM) caused the inhibition of food intake. To test this hypothesis, a separate group of rats was infused intravenously with a diluted intralipid solution that mimicked the lipid content of the chylous lymph. When 2 ml of this intralipid in a saline mixture (a composition comparable with the lymph collected during active lipid absorption) were infused intravenously, food intake was not suppressed. This result indicated that the factor in chylous lymph affecting food intake was not its lipid content.

Fujimoto et al. (9) reasoned that if it was not the lipid component of the chylous lymph that inhibited food intake, then it must be apo A-IV because it is the only apolipoprotein secreted by the small intestine that is markedly stimulated by lipid feeding (16). L-81 blocks the stimulation of apo A-IV production by the small intestine during lipid absorption. Fujimoto et al. (9) therefore tested whether lymph from rats that were fed lipid and L-81 had any effect on food intake. Lymph from L-81-treated rats did not inhibit food intake. However, lymph collected from rats that did not receive L-81 had inhibited food intake. This suggests that apo A-IV is probably the factor in chylous lymph that is responsible for the inhibition of food intake.

Fujimoto et al. (9) then removed apo A-IV from chylous lymph by immunoprecipitation and found that the treated chylous lymph was no longer effective in inhibiting food intake. To confirm that it was the apo A-IV in the chylous lymph that was inhibiting food intake, 200 μg of apo A-IV or apo A-I dissolved in 2 ml of physiological saline were infused intravenously in 24-h food-deprived rats. apo A-IV (200 μg; an amount comparable to that present in 2 ml of lymph collected from rats actively absorbing lipid) suppressed food intake significantly and equally as potent as the chylous lymph. The inhibition of food intake by apo A-IV was shown to be dose dependent (Table 1). Conversely, 200 μg of apo A-I did not affect food intake, suggesting that this function is unique to apo A-IV and is not shared by apo A-I, although all of the known functions that are ascribed to apo A-IV in vitro studies are also performed by apo A-I (Table 1).

Table 1. Food intake in 24-h fasted rats after infusion of 2-ml test solution through indwelling arterial catheter

<table>
<thead>
<tr>
<th>Test Solutions</th>
<th>Food Consumption After Refeeding, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–30 min</td>
</tr>
<tr>
<td>Control</td>
<td>3.90±0.40</td>
</tr>
<tr>
<td>Apolipoprotein A-IV, μg</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>3.35±0.46</td>
</tr>
<tr>
<td>135</td>
<td>2.14±0.16*</td>
</tr>
<tr>
<td>200</td>
<td>0.90±0.18**</td>
</tr>
<tr>
<td>Apolipoprotein A-I, 200 μg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.90±0.48</td>
</tr>
</tbody>
</table>

Values are means ± SE. Five rats were treated in each group. *P < 0.01 compared with values for saline control. †P < 0.01 compared with value for 135-μg apo A-IV.

CENTRAL ADMINISTRATION OF APO A-IV ALSO INHIBITS FOOD INTAKE

The hypothalamus is one potential site where apo A-IV might elicit an inhibition of food intake because it is intimately involved in regulating food intake and energy metabolism. Fujimoto et al. (10) reported that administration of apo A-IV into the 3rd cerebroventricle of rats decreased food intake in a dose-dependent manner and with a potency that was ~50-fold higher than intravenous administration. In contrast, apo A-I had no effect on food intake when infused into the 3rd ventricle. When goat anti-rat apo A-IV serum was administered into the 3rd ventricle at 1100 (during middle of the light phase when rats usually do not eat), all of the rats began to eat. Thus Fujimoto et al. (10) proposed that apo A-IV antiserum removes the endogenous apo A-IV present and thus stimulates food intake. This antibody experiment has been recently replicated by Liu et al. (22).

The question of whether the brain synthesizes apo A-IV was carefully examined by Liu and his colleagues. They reported in 2001 that both apo A-IV mRNA and protein are present in the hypothalamus (22). Using immunohistochemistry, Liu et al. (23) observed that apo A-IV is mainly concentrated in an area of the ARC of the hypothalamus (Fig. 2A). This labeling is specific, because it can be abolished by the preabsorption of the primary antiserum with purified apo A-IV (Fig. 2C). This finding is significant, because it clearly demonstrates that in addition to the small intestine, the ARC of the hypothalamus also synthesizes apo A-IV. The ARC is an important site for the regulation of both food intake and energy homeostasis (34).

There is evidence that hypothalamic apo A-IV is physiologically regulated. Using a quantitative method (competitive RT-PCR), Liu et al. (22) demonstrated that hypothalamic apo A-IV mRNA is physiologically regulated. As shown in Fig. 3, the hypothalamic apo A-IV mRNA content in rats fasted for 28 h was significantly lower than that of ad libitum-fed animals (P < 0.01). However, refeeding with lipid restored the suppressed apo A-IV level to levels observed in ad libitum-fed animals (Fig. 3). In addition to fasting and feeding of lipid, hypothalamic apo A-IV is also regulated diurnally. Hypothalamic apo A-IV mRNA, and protein levels increase during the light phase, peaking at 0900 (3 h after lights on), and gradually decrease during the dark phase with a nadir at 2100 (3 h after lights off). Thus the diurnal rhythm of hypothalamic apo A-IV parallels the feeding patterns of the rat.

Is apo A-IV an enterogastrone, a hormone secreted by the small intestine that inhibits gastric motility and acid secretion?
The evidence for this is largely derived from the work of Okumura et al. (27). They first demonstrated that intracisternal administration of apo A-IV inhibits gastric acid secretion in a dose-dependent manner. Thus they proposed that apo A-IV is an enterogastrone produced by the small intestine in response to fat feeding to inhibit gastric acid secretion. In 1995, Okumura and colleagues (28) demonstrated that in addition to gastric acid secretion, intracisternal administration of apo A-IV also inhibits gastric motility. A particularly interesting study reported in 2002 by Glatzle et al. (13) indicates that CM components inhibit gastric motor function in the rat via a cholecystokinin-1 (formerly called CCK-A) receptor-mediated pathway. According to their study, it appears that CMs, or their products, release CCK by the enteroendocrine cells, which, in turn, activates the CCK-1 receptors on vagal afferent nerve fiber terminals. Judging from the information provided from their earlier study, the factor involved may potentially be apo A-IV. It would therefore be extremely interesting to determine whether intravenously infused apo A-IV would stimulate the contraction of the gallbladder and the secretion of enzymecher pancreatic secretion, two very well known functions of apo A-IV.

To summarize, it appears that apo A-IV is an enterogastrone secreted by the small intestine in response to lipid feeding, and this may be partially or totally responsible for its satiety effect. In addition to its role as a satiety factor, it may also stimulate the secretion of CCK. If these roles of apo A-IV prove valid, it would imply that apo A-IV plays an important role in the absorption of nutrients, because it not only limits the delivery of nutrients from the stomach by slowing down gastric emptying (thus its role as an enterogastrone), but it may also be involved in the preparation of the gastrointestinal tract to digest, absorb, and deliver the absorbed nutrients to the body by the enterocytes. That apo A-IV is involved in the formation of CM was recently confirmed by the finding (24) that over-expression of apo A-IV enhances the transport of lipid by newborn swine intestinal epithelial cells. Determining whether the absorption of lipid is compromised in apo A-IV knockout animals would provide a definitive answer to whether apo A-IV is important to the formation of CM.

**HOW QUICKLY DOES APO A-IV ACT?**

Apo A-IV likely acts quickly. First, consider the temporal relationship between the intestinal synthesis and secretion of apo A-IV and satiety immediately following the ingestion of food. Increases of plasma levels of apo A-IV in response to lipid feeding are rapid and of sufficient magnitude to elicit satiety. Rodriguez et al. (29) demonstrated that a gastric bolus of 0.5 ml of a 200 g/l Intralipid solution (containing 100 mg of triglyceride) fed to rats significantly increased plasma apo A-IV between 15 and 30 min following the ingestion of a meal. The changes in plasma apo A-IV concentration observed by Rodriguez et al. (29) were similar to those observed by Fujimoto et al. (9), in which the intravenous administration of apo A-IV produced a significant, dose-dependent inhibition of food intake. Rodriguez et al. (29) therefore concluded that the increase in plasma levels of apo A-IV produced in response to a lipid meal was sufficiently quick and large enough to produce satiety, thereby supporting a role for apo A-IV in the short-term control of food intake in rats. Other indirect evidence that apo A-IV acts quickly is derived from the study of Liu et al. (22) who observed that the introduction of apo A-IV antibodies into the 3rd ventricle of the rat quickly resulted in feeding during the middle of the light phase, a period when rats normally do not eat. Thus it would appear that the production and the action of apo A-IV are sufficiently fast and therefore capable of playing a role as a satiety factor in the regulation of food intake.
DOES APO A-IV PLAY A ROLE IN THE REGULATION OF FOOD INTAKE AND BODY WEIGHT?

Evidence from a number of experiments suggests that apo A-IV may be involved in the long-term regulation of food intake and body weight. First, it has been demonstrated that intravenous administration of apo A-IV decreases food intake in rats given free access to food (11). This suggests that exogenously administered apo A-IV controls food intake under ad libitum feeding conditions. Second, Liu and his colleagues (unpublished data) have shown that an inverse relationship exists between food intake and the expression of hypothalamic apo A-IV. Third, Liu and colleagues (unpublished data) recently found that the intestine of obese rats no longer responded to fasting and feeding of a high-fat diet. In other words, the apo A-IV response to lipid feeding in these rats was significantly blunted. Furthermore, the hypothalamic expression of apo A-IV was markedly suppressed in rats chronically fed a high-fat diet compared with rats fed a low-fat or chow diet. Finally, Liu and colleagues (unpublished data) demonstrated that hypothalamic apo A-IV failed to respond to lipid feeding in diet-induced obese rats. In humans, chronic consumption of a high-fat diet significantly elevates plasma apo A-IV levels. This elevation is observed during the first week of high fat consumption (32) but disappears during the second week, thus leading to the conclusion that there is autorregulation of intestinal apo A-IV production in response to diets high in fat. Consequently, both rodent and human data suggest that intestinal apo A-IV synthesis and secretion become less responsive to fat after chronic high-fat diet consumption. Of course we do not know how human hypothalamic apo A-IV expression responds to the ingestion of lipid in lean and obese subjects. It is therefore important to gain a better understanding of the molecular mechanism responsible for diminishing the response to lipid feeding when animals become fat after chronic feeding of a high-fat diet. Does this diminished response pertain to all instances of diet-induced obesity? What would be the findings of studies involving genetically obese animals?

The discovery of leptin has energized the field of obesity. Leptin is a protein made by adipose tissue believed to signal the hypothalamus (a center for the regulation of food intake and energy metabolism) as to how much fat is in the body. Leptin decreases food intake and increases energy metabolism (34). Morton et al. (26) reported downregulation of intestinal apo A-IV mRNA levels by leptin. This observation was also confirmed in vivo by Doi et al. (7). It has been recently demonstrated that intestinal apo A-IV synthesis and secretion are upregulated by insulin in both rodents and humans (3, 5). Energy homeostasis in the body is accomplished by a highly integrated and redundant neurohumoral system that prevents the effect of short-term fluctuations in energy balance on fat mass (34). Insulin and leptin are hormones secreted in proportion to body adiposity and play a critical role in this energy homeostasis. Because leptin and insulin both regulate intestinal apo A-IV synthesis, it is reasonable to propose that apo A-IV is involved in the long-term regulation of food intake and body weight. To fully understand the role of apo A-IV in the regulation of energy intake and body weight, it is important to know how both intestinal and hypothalamic apo A-IV are regulated. We believe that hypothalamic apo A-IV is the more important player in the overall regulation of food intake and body weight regulation.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-32288, DK-53444, DK-59630, and DK-063907.

REFERENCES


