Annu. Rev. Nutr. 2001. 21:231–54

THE ROLE OF APOLIPOPROTEIN A-IV IN THE REGULATION OF FOOD INTAKE

Patrick Tso,[1] Min Liu,[1] Theodore John Kalogeris[2] and Alan BR Thomson[3]

[1]*Department of Pathology, University of Cincinnati, Cincinnati, Ohio 45267; e-mail: tsopp@email.uc.edu; lium@email.uc.edu*

[2]*Department of Surgery, Louisiana State University Medical Center, Shreveport, Louisiana 71130; e-mail: tkalog@lsumc.edu*

[3]*Department of Medicine, University of Alberta, Alberta T6G 2C2 Canada; e-mail: thomson@ualberta.ca*

Key Words obesity, chylomicron, intestine, satiety, Sprague Dawley rats

■ **Abstract** Apolipoprotein A-IV (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 the circulating apo A-IV. Intestinal apo A-IV synthesis is markedly stimulated by fat absorption and appears not to be mediated by the uptake or reesterification of fatty acids to form triglycerides. Rather, the 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. The inhibition of food intake by apo A-IV is mediated centrally. The stimulation of intestinal synthesis and the 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. Chronic ingestion of a high-fat diet blunts the intestinal apo A-IV response to lipid feeding and may explain why the chronic ingestion of a high-fat diet predisposes both animals and humans to obesity.

CONTENTS

INTRODUCTION

Apolipoprotein A-IV (Apo A-IV) was discovered over 22 years ago (88), but its physiological role was not firmly established until recently. Apo A-IV is a protein secreted only by the small intestine in humans (5, 35). In rodents, both the small intestine and the liver secrete apo A-IV; however, the small intestine is the major organ responsible for the circulating apo A-IV (30). It has been demonstrated that apo A-IV production by the small intestine is stimulated by active lipid absorption (5, 10, 40, 47). Hayashi et al (39) demonstrated that the stimulation of apo A-IV production by lipid feeding is associated with the formation of chylomicrons. They further demonstrated that the stimulation of apo A-IV production by fat absorption can be abolished by a Pluronic surfactant called Pluronic L-81 (L-81), a potent inhibitor of the formation of chylomicrons (92).

In vitro studies have demonstrated roles for apo A-IV in lipoprotein metabolism (10a, 22, 26, 32, 85). There has been no direct evidence, however, to demonstrate that apo A-IV plays such roles in vivo. Apo A-IV has been shown in vivo to protect apo E–deficient mice from developing atherosclerosis despite the fact that they have a plasma lipid profile that is atherogenic, i.e. increased total plasma cholesterol with no significant change in high-density-lipoprotein cholesterol (21a). This protective effect is further demonstrated by the ability of apo A-IV to protect against diet-induced atherosclerotic lesions (18). These studies demonstrate that both human and mouse apo A-IV protect against atherosclerosis. This might be due to the protective effect of apo A-IV has against lipid oxidation (75). Although there are many exciting proposed functions of apo A-IV, this review focuses on its unique role in regulating short- and long-term food intake, possibly as the satiety factor that is released by the gastrointestinal tract following the ingestion of fat.

MECHANISMS OF FAT ABSORPTION

Before a discussion of the role of apo A-IV in mechanisms of fat-induced satiety can take place, it is necessary to briefly discuss the process of fat absorption by the small intestine and the intestinal synthesis and secretion of lipoproteins and apolipoproteins.

Dietary Lipids

Dietary lipids provide as much as 30%–40% of the daily caloric intake in the Western diet. The daily intake of lipids by humans in the Western world ranges between 60–100 g. Triacylglycerol (TG) is the major dietary fat in humans. The major long-chain fatty acids (FAs) present in the diet are palmitate (16:0), stearate (18:0), oleate (18:1), linoleate (18:2), and linolenate (18:3). In most infant diets, fat becomes an even more important source of calories. In human milk and in human formulas, as much as 50% of the total calories are present as fat (36). In human milk, there is also an abundance of medium-chain FAs. The human small intestine is presented daily with other lipids, such as phospholipids (PLs), cholesterol, and plant sterols. Both PL and cholesterol are major constituents of bile. In humans, the biliary PL is a major contributor of luminal PL, and as much as 11–20 g of biliary PL enters the small intestinal lumen daily, whereas the dietary contribution is only between 1 and 2 g (12, 66). The small intestinal epithelium undergoes rapid turnover, thus contributing to the luminal PL and cholesterol. The predominant sterol in the Western diet is cholesterol. Plant sterols account for 20%–25% of total dietary sterol (34, 90), but they are poorly absorbed.

Luminal Digestion and Uptake of Dietary Lipids

Lipid digestion begins in the stomach. Lipase activity has been reported to be present in human gastric juice (17). In humans, the gastric lipase activity is mainly contributed by the stomach, with the highest activity detected in the fundus of the stomach. Human gastric lipase has a pH optimum ranging from 3.0 to 6.0 and is therefore called acid lipase. It hydrolyzes medium-chain TG (predominantly 8- to 10-carbon chain length) better than long-chain TG (53). The main hydrolytic products of gastric lipase are diacylglycerols and free FAs (70, 76). Gastric lipase does not hydrolyze PL or cholesteryl esters. Gastric lipases play an important role in the absorption of lipids in newborns. It is interesting that in rodents, the main gastric lipase activity is derived from the lipase secreted by the salivary glands and is therefore termed lingual lipase. Though derived from different areas in humans and rodents, and therefore termed differently, gastric and lingual lipase is the same enzyme.

In the stomach, lipid is emulsified (broken into small oil droplets) and enters the small intestinal lumen as fine droplets less than 0.5 μm in diameter (16, 80). The combined action of bile and pancreatic juice brings about a marked change in the physical and chemical form of the luminal lipid emulsion. Pancreatic lipase is secreted into the duodenum and hydrolyses TG to form 2-monoacylglycerol and FAs (15, 58, 81). The most potent gastrointestinal hormone that stimulates the release of enzymes by the pancreas is cholecystokinin (82), and cholecystokinin-A receptor has been demonstrated to be present in the pancreas (78). In vitro studies using purified pancreatic lipase have demonstrated a potent inhibitory effect of bile salts on the lipolysis of TG at a concentration that is above the critical micellar concentration (9, 62). The inhibitory effect of bile salt, then, is physiological

because its concentration in the duodenum is normally higher than what is typically needed to observe an inhibitory effect. Why, then, is pancreatic lipase so efficient in digesting TG? The pancreas secretes another protein that counteracts this inhibition, and this factor is colipase.

Colipase was first isolated by Morgan et al (62) from the pancreatic juice of rats. The structure and mechanism of action of colipase have been elucidated by Maylie et al (59) and Börgstrom et al (13). Colipase acts by attaching to the ester bond region of the TG molecule. In turn, the lipase binds strongly to the colipase by electrostatic interactions, thereby allowing the hydrolysis of the TG by the lipase molecule (24).

Most dietary cholesterol is free sterol, with only 10%–15% being esters. Cholesteryl esters entering the small intestine are hydrolyzed before free cholesterol can be absorbed. The enzyme involved in hydrolyzation is called cholesterol esterase (EC 3.1.1.13—also called carboxylic ester hydrolase, monoglycerol hydrolase, bile salt–dependent lipase, or sterol ester hydrolase), and it is secreted by the pancreatic acinar cells. The digestion of PL occurs in the small intestine. In bile, PL [predominantly phosphatidylcholine (PC)] is found in mixed micelles along with cholesterol and bile salts. Once in the intestinal lumen, the luminal PC distributes between the mixed micelles and the TG droplets; however, PC tends to favor the micellar phase over the oil phase. PC is hydrolyzed by pancreatic phospholipase A_2 (EC 3.1.1.4) to yield FA and lysoPC.

Much of our current understanding of the uptake of dietary lipids has been derived from the pivotal work of Hofmann & Borgstrom (43, 44), who identified and emphasized the importance of micellar solubilization of lipid in the uptake of lipid digestion products by enterocytes. Although for a long time uptake of lipids was believed to be passive, a number of more recent studies have raised questions about the validity of this concept. Work by Stremmel et al (86, 87) has raised the possibility that some lipids, especially FAs, may be taken up by enterocytes by carrier-mediated processes. A number of other proteins, including GP330 (also called megalin) (55, 101), CD36 (1, 2), caveolin (64), and FA transporter (1, 37), also bind lipids. Recently, Stahl et al (84) reported having identified a FA transport protein, FATP4 (a member of the family of the FA transport proteins), being abundantly expressed in the intestinal enterocytes, with most of the protein associated with the apical brush border membrane. Considerable excitement has been raised over the ability of these transporters to reduce fat absorption, a potential magic bullet to combat obesity. However, further studies are critical to support such a view.

Formation and Secretion of Chylomicrons

2-Monoacylglycerol and FA reconstitute to form TG, mainly via the monoacylglycerol pathway (46, 57). The enzymes involved present in a complex called "triglyceride synthetase" (46). Some of the absorbed lysoPC is reacylated to form

PC (65). Cholesterol is transported almost exclusively by the lymphatic system, mainly as esterified cholesterol. The rate of esterification of cholesterol regulates the rate of its lymphatic transport (25). The TG, PL, cholesterol, and cholesteryl esters, together with apolipoproteins, are packaged to form chylomicrons. Chylomicrons are secreted by enterocytes, via a process called exocytosis, and carried by the lymphatic system before being emptied into the circulatory system.

INTESTINAL APOLIPOPROTEIN SECRETION

Chylomicrons are secreted by the small intestine and have the following proteins associated with them: apolipoprotein apo A-I, apo A-IV, apo B-48, and the apo Cs (Figure 1). Of the various apolipoproteins, only apo A-IV synthesis and secretion is markedly stimulated by fat absorption (two- to threefold) (39, 40).

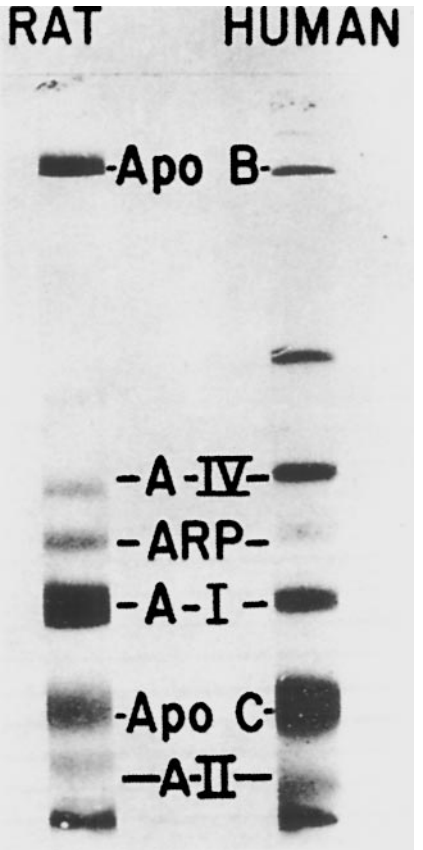

Figure 1 Sodium dodecyl sulfate polyacrylamide gels (5.6%) of apolipoproteins of rat lymph chylomicrons and human chylous rine chylomicrons. ARP = apo E. A dye front is apparent at the bottom of each gel. [Reproduced with permission from Bisgaier & Glickman (10)].

REGULATION OF INTESTINAL APO A-IV SYNTHESIS AND SECRETION

Lymphatic apo A-IV secretion by the gastrointestinal tract displays a circadian rhythm with peak output occurring before feeding and just before the first half of the dark period (Figure 2) (30). This pattern is closely correlated with lymphatic, triglyceride, PL, and cholesterol outputs. Bile diversion reduces lymphatic outputs of apo A-IV by 67%, cholesterol by 81%, and both triglyceride and PL by 90%. Moreover, bile diversion completely abolishes the circadian rhythm in outputs of apo A-IV (Figure 2) (30). Davidson et al (19) has demonstrated that bile diversion significantly reduces apo A-IV synthesis by the intestinal mucosa. Thus, an intact enterohepatic circulation is necessary for both normal basal lymphatic output of apo A-IV and its circadian rhythm.

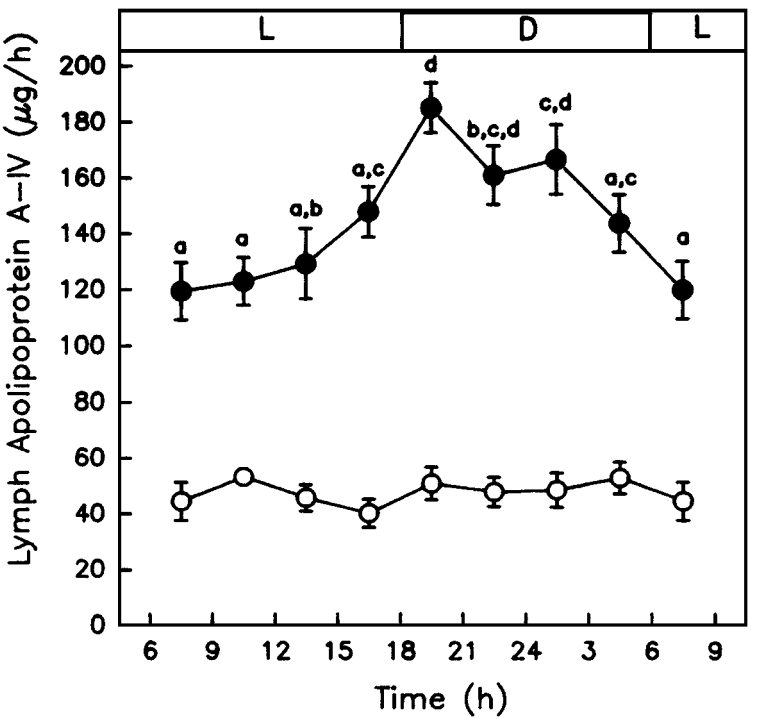

Figure 2 Lymph apo A-IV output in 24-h fasted rats with bile diversion (open circles) or without bile diversion (closed circles). Seven rats were used for this study. Values are expressed as the mean plus or minus the standard error. Different letters show that the difference is significant, with $P < 0.05$. L, light period; D, dark period. [Reproduced with permission from Fukagawa et al (30)].

In vivo studies have demonstrated that synthesis and secretion of apo A-IV by the gastrointestinal tract is regulated by the absorption of fat, as well as by a peptide secreted by the lower small intestine, probably peptide YY (49).

Lipid Absorption

A number of us have demonstrated that intestinal lipid absorption stimulates the synthesis and secretion of apo A-IV (5, 20, 40, 51). Of the major apolipoproteins produced by the gastrointestinal tract (A-I, A-IV, B-48), only apo A-IV is stimulated by lipid absorption (39, 40). Kalogeris et al (47) have demonstrated that intestinal lymphatic transport of apo A-IV increases in a graded fashion with increasing steady state levels of intestinal triglyceride transport. This increase in apo A-IV secretion might be due to graded increases in intestinal mucosal apo A-IV synthesis along a proximal-distal gradient. Evidence thus far suggests that increased synthesis of apo A-IV by fat absorption in adult rats is by a pretranslational mechanism (5).

As previously discussed, intestinal fat absorption involves multiple steps, and knowing which steps of fat absorption are involved in stimulating intestinal apo A-IV synthesis and secretion is crucial. Several lines of evidence support the notion that assembly and transport of chylomicrons is necessary for stimulating intestinal apo A-IV synthesis. Evidence of this first came from studies using L-81. Animals infused with lipid plus L-81 showed no change in intestinal lipid digestion or uptake. The absorbed lipid accumulated in the intestinal mucosa but was not transported (no change in uptake) into lymph (92, 93). It has been further demonstrated that L-81 preferentially blocks chylomicron formation and, thus, intestinal apo A-IV secretion and synthesis, but not the secretion of very-low-density lipoproteins (94). Hayashi et al (40) observed that the increase in apo A-IV synthesis and secretion by the small intestinal mucosa was blocked when L-81 was infused. However, when L-81 was removed, lymphatic lipid transport immediately increased as the accumulated mucosal lipid was cleared from the mucosa as chylomicrons. Removal of L-81 similarly reverses the blockade of apo A-IV transport. A lag period of about 120 min between the maximal output of lipid and that of A-IV (lipid occurring first) with reversal of L-81 blockage suggests that lipid transport stimulates apo A-IV synthesis and secretion (40).

Further evidence that lymphatic apo A-IV output is dependent on chylomicron transport is derived from rat studies that examined the intestinal synthesis and lymphatic secretion of apo A-IV in response to intestinal infusion of FAs that differed in chain length. Infusion of long-chain FAs (myristic, C-14; oleic, C-18; and arachidonic, C-20), which are transported via the lymph in chylomicrons, stimulated synthesis and output of apo A-IV, whereas medium- and short-chain FAs (caprylic, C-8 and butyric, C-4), primarily transported as free FAs in the portal vein, elicited a negligible A-IV response (50). These findings in rats differ

from those in neonatal swine where similar increases in jejunal apo A-IV mRNA expression and synthesis in response to infusions of both medium (C8:0 and C10:0) and long-chain triglyceride mixtures were observed (33). Results of the rat studies strongly support the hypothesis that some aspect of chylomicron formation and secretion is required to stimulate apo A-IV synthesis, and thus secretion (39), but contradict the recent findings of the neonatal swine studies. Therefore, it is currently unclear whether the relationship between chylomicron and apo A-IV synthesis and secretion is common to all species or developmental stages. An extremely interesting question worth investigating is how the formation of chylomicrons is signal transduced to stimulate intestinal apo A-IV synthesis.

Peptide YY

It has recently been determined that the formation and secretion of chylomicrons is not the only mechanism by which apo A-IV synthesis and secretion is stimulated. We administered duodenal infusions of graded doses of TG to rats and quantified both regional lipid distribution and mucosal synthesis of apo A-IV at various sites along the intestine (47). It was determined that despite significant amounts of lipid found only in the proximal half of the small intestine, apo A-IV synthesis was stimulated in the proximal three quarters of the gut, even in segments where there was a negligible amount of lipid. This raised an interesting question of whether factors other than lipid transport are independent of the presence of lipid, which is itself capable of stimulating apo A-IV production by the gut. To address this, a series of experiments were performed comparing the effects of proximal versus distal intestinal infusion of lipid on apo A-IV synthesis. Kalogeris et al (50) found that duodenal lipid infusion elevated both apo A-IV synthesis and mRNA levels two- to threefold compared with control infusions of glucose-saline in the jejunum, and that ileal apo A-IV synthesis and mRNA levels were unaffected. Previous work from our laboratory demonstrated that duodenal lipid infusion only negligibly affected the amount of lipid reaching the ileum, which suggests that insufficient exposure of lipid to the distal gut thereby inhibited a change in apo A-IV secretion.

In contrast to this finding, 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 (a segment of intestine isolated luminally from the rest of the gastrointestinal tract) demonstrated the following interesting findings: (*a*) Ileally infused lipid elicits an increase in proximal jejunal apo A-IV synthesis independent of the presence of jejunal lipid, and (*b*) both the ileum and more distal sites may be involved in the stimulation. These results strongly suggest that there is a signal in the distal gut that 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 (54, 56), decreased intestinal motility

and transit (56, 83), and decreased pancreatic secretion (38). Ileal nutrient also inhibits food intake (61, 99). The mechanism for these effects has been collectively termed the ileal brake (83) and appears to be related to the release of one or more peptide hormones from the distal intestine (6, 7, 45, 71–73, 79). These effects have traditionally been considered operative only in the abnormal delivery of undigested nutrients to the distal gut, such as the malabsorptive state (83). However, growing evidence supports the notion that nutrient reaches the distal gut, even under normal conditions, because of rapid gastric emptying during the early phases of food ingestion (54, 61, 77). We recently studied the intraluminal and mucosal distribution of a bolus of [^{3}H]triolein-labeled Intralipid (0.5 ml of a 20% emulsion) fed by gavage. By 15–30 min, radiolabeled lipid was spread evenly throughout the gut, with 10%–15% of the load recovered in the ileum and cecum combined. The presence of substantial amounts of lipids in these distal sites persisted for at least 4 h after food ingestion. 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 as well by 30 min after feeding with a gastric lipid load (77). 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.

The most likely peptide to mediate the phenomenon of ileal brake is peptide tyrosine-tyrosine (PYY), which is a member of the peptide family that includes pancreatic polypeptide, neuropeptide Y, and fish pancreatic peptide Y (52). PYY is synthesized by the endocrine cells in the ileum and large intestine (3, 6, 41, 89, 91) and is released in response to intestinal nutrients, especially long-chain FAs (41). However, PYY may not be the only mediator of the ileal brake. For example, perfusion of fat in the intestine produces a greater suppression of pentagastrin-stimulated acid secretion than does PYY (79), indicating that the enterogastrone effect of fat is mediated by more than one factor.

We now have evidence that PYY stimulates jejunal apo A-IV synthesis and secretion. Continuous intravenous infusion of physiological doses of PYY elicits significant increases in both synthesis and lymphatic transport of apo A-IV in rats (48). Kalogeris et al (48) further demonstrated that the stimulation of jejunal apo A-IV synthesis by PYY is probably translationally rather than transcriptionally controlled because the apo mRNA level is not altered but synthesis is markedly stimulated. Furthermore, Kalogeris et al (48) showed that vagotomy failed to block the stimulation of jejunal apo A-IV synthesis by fat absorption (Figure 3). However, when lipid was infused directly into the ileum, vagotomy totally abolished an increase in jejunal apo A-IV synthesis (Figure 4). Thus, the stimulation of jejunal apo A-IV synthesis and secretion by fat absorption is mediated by a factor (probably PYY), which, in turn, acts centrally to send a signal via the vagus

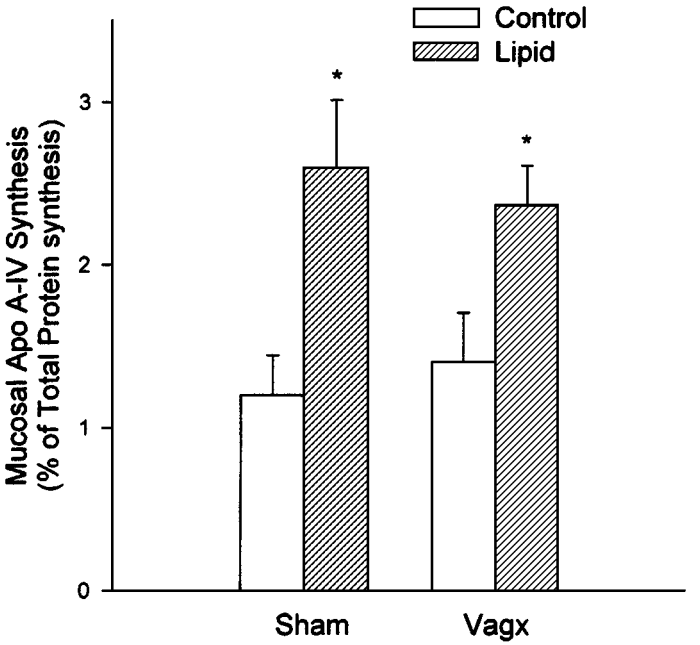

Figure 3 Vagotomy has no effect on the stimulation of apo A-IV synthesis in proximal jejunum by duodenal infusion of triacylglycerol emulsion. Vagotomized (Vagx) or sham-vagotomized rats equipped with duodenal infusion cannulas received continuous 8-h duodenal infusions of glucose saline control) or lipid (triolein emulsion, 40 μmol per h). Values are means plus or minus standard errors for four rats (sham, control), six rats (vagx, control), five rats (sham, lipid), and seven rats (vagx, lipid). (Asterisk) Significant effect of lipid infusion on apo A-IV synthesis ($P < 0.01$). [Reproduced with permission from Kalogeris et al (48)].

nerve to the gut. We believe this to be the first demonstration of a gastrointestinal hormone involved in the control of expression and secretion of an intestinal apolipoprotein, thus bringing together two areas of research in gastrointestinal physiology.

INTRAVENOUS INFUSION OF APO A-IV INHIBITS FOOD INTAKE

That apo A-IV may play a role in the regulation of food intake is supported by a series of physiological studies that examined (*a*) the effect that intravenous administration of intestinal lymph derived from fasted rats had on food intake, and (*b*) the effect that intestinal lymph derived from rats actively absorbing fat had on food intake. Experimental rats were implanted with indwelling intravenous

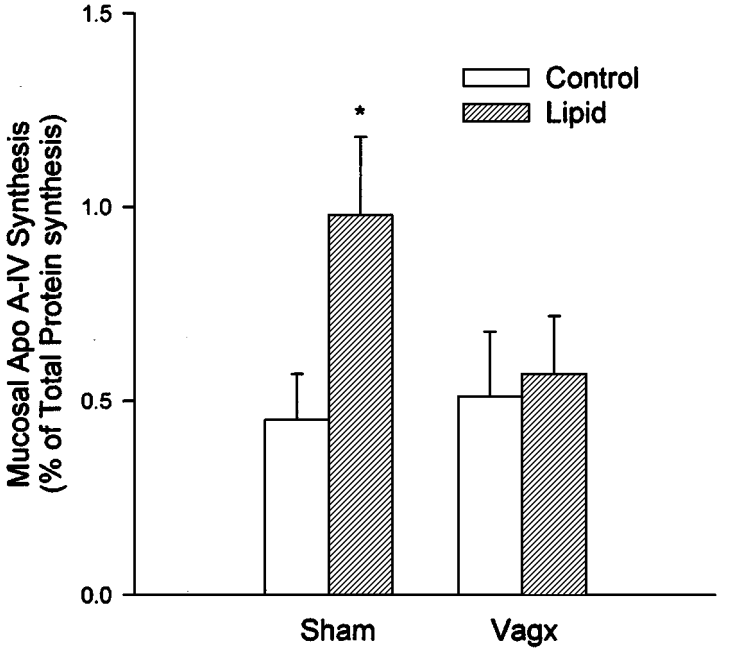

Figure 4 Vagotomy prevents increase in jejunal apo A-IV synthesis in a jejunal Thiry-Vella fistula elicited by ileal lipid infusion. Vagotomized (Vagx) or sham-vagotomized rats equipped with jejunal Thiry-Vella fistula and ileal infusion cannulas received continuous 8-h ileal infusions of either glucose-saline (control) or lipid emulsion (lipid). Mucosal apo A-IV synthesis in the Thiry-Vella segment was then measured. Values are mean plus or minus the standard error for six rats (sham, control), five rats (sham, lipid), six rats (vagx, control), and eight rats (vagx, lipid). (Asterisk) Significant effect of lipid ($P = 0.001$). [Reproduced with permission from Kalogeris et al (48)].

infusion cannula and allowed to recover for 1 week before feeding studies began. After a 24-h fasting period, various intestinal lymph samples were taken from donor rats and solutions of apo A-IV and apo A-I infused intravenously to determine whether food intake was affected by a nonpurified diet. Fujimoto et al (27) demonstrated that rats intravenously infused with a physiological dose of saline through the indwelling right atrial catheter ate 3.90 $\pm$ 0.40 g during the first 30 min of refeeding. Infusion of food-deprived intestinal lymph collected from donor lymph-fistula rats had little effect on food intake compared with the saline-infused rats deprived of food for 24 h. In contrast, the intestinal lymph collected from rats that were actively absorbing lipid markedly suppressed food intake during the first 30 min of refeeding ($P < 0.01$). Suppression of food intake was not observed in the subsequent 30 min of refeeding.

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 chylomicrons) caused the inhibition of food intake. To test this hypothesis in rats deprived of food for 24 h, 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 Intralipid (20 g/liter) in saline containing 42 μmol of triglyceride and 3.1 μmol of PL (a composition comparable to the lymph collected during active lipid absorption) was infused intravenously, food intake was not suppressed. This result indicated that the effect of chylous lymph on food intake was not caused by its lipid content.

Fujimoto et al (27) 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 (40). L-81 blocks the stimulation of apo A-IV production by the small intestine during lipid absorption. Fujimoto et al (27) 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 did. 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 (27) further studied the effect of apo A-IV–deficient chylous lymph on feeding. The chylous lymph treated with normal goat serum suppressed food intake significantly in the first 30 min of feeding. In contrast, chylous lymph that was treated with apo A-IV antiserum had no effect on food intake—the rats consumed an amount of food similar to that consumed by the saline controls. Fujimoto et al demonstrated that the composition of the chylous lymph treated with apo A-IV antiserum was not altered by the treatment, except that the apo A-IV had been removed. In contrast, lymph treated with apo A-I antiserum was just as effective as the untreated lymph in inhibiting food intake.

Apo A-IV or apo A-I (200 μg) dissolved in 2 ml of physiological saline was infused intravenously in 24-h food-deprived rats. An amount of apo A-IV (200 μg) comparable to that present in 2 ml of lymph collected from rats actively absorbing lipid suppressed food intake significantly and to the same extent as the chylous lymph collected during 6–8 h of lipid infusion. 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 effect food intake. No nonphysiological reactions, such as sedation, ataxia, or hyperthermia, were observed after apo A-IV and chylous lymph infusion. These studies led Fujimoto et al (27) to first propose that apo A-IV is a circulating signal released by the small intestine in response to fat feeding and is likely the mediator for the anorectic effect of a lipid meal. This function is unique to apo A-IV and is not shared by apo A-I, although all of the functions that are ascribed to apo A-IV in the in vitro studies can also be performed by apo A-I.

TABLE 1 Food intake in 24-h fasted rats after infusion of 2 ml of test solution through indwelling atrial catheter[a]

	Food Consumption After Refeeding (g)	
Test Solutions	**0–30 Min**	**30–60 Min**
Control (physiological saline)	3.90 ± 0.40	1.31 ± 0.33
Apolipoprotein A-IV		
60 μg	3.35 ± 0.46	1.13 ± 0.31
135 μg	2.14 ± 0.16[b]	1.16 ± 0.26
200 μg	0.90 ± 0.18[b,c]	1.10 ± 0.19
Apolipoprotein A-I		
200 μg	3.90 ± 0.48	1.20 ± 0.25

[a]Values are means plus or minus the standard error. Five rats were treated in each group.

[b]$P < 0.01$ compared with values for saline control.

[c]$P < 0.01$ compared with value for 135 μg of apolipoprotein A-IV.

CENTRAL ADMINISTRATION OF APO A-IV ALSO INHIBITS FOOD INTAKE

The hypothalamus is a potential site where apo A-IV can elicit an inhibition of food intake because it is intimately involved in regulating food intake and energy metabolism (102). Fujimoto et al (28) reported that administration of apo A-IV into the third cerebroventricular of rats decreased food intake in a dose-dependent manner and with a potency that was ~50-fold higher than intravenous administration (Figure 5). In contrast, apo A-I had no effect on food intake when infused into the third ventricle. When goat anti-rat apo A-IV serum was administered into the third ventricle at 11:00 h (during the light phase when rats usually do not eat), all tested rats began to eat. Thus, Fujimoto et al (28) proposed that apo A-IV antiserum removes any endogenous apo A-IV present.

Evidence suggests that de novo synthesis of apo A-IV in the brain is unlikely (23). Fujimoto et al (28, 29) proposed that apo A-IV (or perhaps a fragment thereof) released by the small intestine may traverse the blood-brain barrier and act in the central nervous system. Using electroimmunosassay, they supported this argument by demonstrating that intact apo A-IV, or a fragment thereof, was present in the third ventricular cerebrospinal fluid. Furthermore, they demonstrated that the concentration of apo A-IV immunoreactivity in the third ventricular cerebrospinal fluid increased as a result of lipid feeding. Later, using immunohistochemical technique, Fukagawa et al (31) demonstrated that specific staining for apo A-IV in astrocytes and tanycytes appears throughout both white and gray matter. The granular nature and perinuclear distribution of apo A-IV immunoreactivity suggests that apo A-IV may be contained in perinuclear organelles or vesicles. The demonstration of immunoreactive apo A-IV in tanycytes does not necessarily

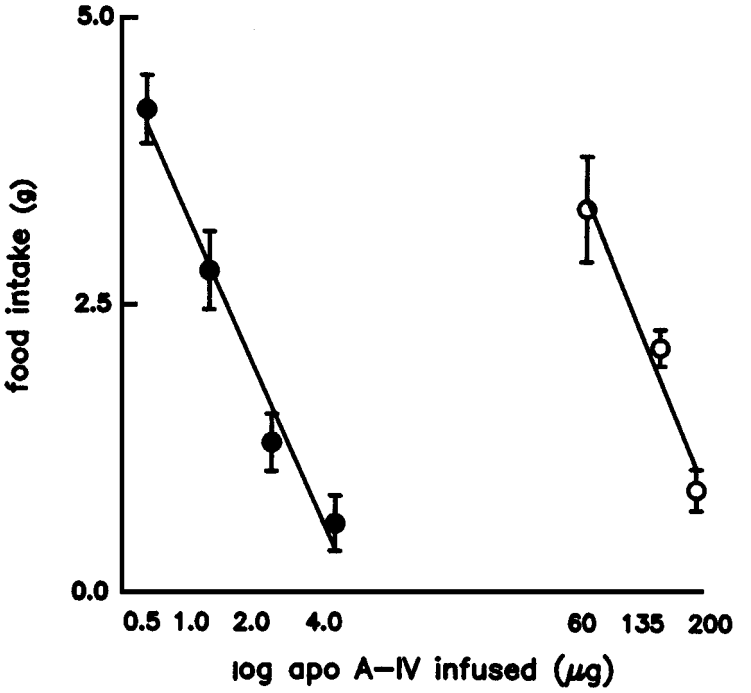

Figure 5 The relationship between the suppression of food intake during the first 30 min of refeeding in 24-h fasted rats and the logarithm of the dose in micrograms of apo A-IV infused into the third ventricle (closed circles) or intravenously (open circles). Five animals were studied at each dose, and the values are expressed as mean plus or minus the standard error. [Reproduced with permission from Fujimoto et al (28)].

indicate that there is a selective uptake mechanism because tanycytes take up a variety of neurotransmitters and nonmetabolizable amino acids. The presence of apo A-IV immunostaining in astrocytes does indicate selective uptake of this apolipoprotein by astrocytes. Whether astrocytes are involved in satiety mechanisms associated with lipid feeding is unknown. Extremely preliminary data from our laboratory implies that the brain, particularly the hypothalamus, has apo A-IV mRNA and is therefore capable of synthesizing apo A-IV protein. This interesting finding is being investigated actively in our laboratory.

IS APO A-IV A SHORT-TERM SATIETY FACTOR?

Although there is compelling evidence that apo A-IV can acutely inhibit food intake during the ingestion of a lipid meal, the temporal relationship between intestinal synthesis and secretion of apo A-IV and satiety has to be considered. The question

is whether the increase in plasma levels of apo A-IV in response to lipid feeding is rapid enough and of sufficiently large magnitude to elicit satiety. Rodriguez et al (77) demonstrated that when a gastric bolus of 0.5 ml of an Intralipid solution (200 g/liter) containing 100 mg of triglyceride was fed to rats, there was a significant increase in plasma apo A-IV within 15 min, and the increment remained significant until 30 min following the ingestion of a meal. These changes in plasma apo A-IV concentration were similar to those observed by Fujimoto et al (27), in which the intravenous administration of apo A-IV produced a significant, dose-dependent inhibition of food intake. Rodriguez et al (77) therefore concluded that the increase in plasma levels of apo A-IV produced in response to lipid meals 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.

POTENTIAL ROLE OF APO A-IV IN THE LONG-TERM CONTROL OF FOOD INTAKE

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 gain. First, it has been demonstrated that intravenous administration of apo A-IV decreases food intake in rats given free access to food (29). This suggests that exogenously administered apo A-IV controls food intake under ad libitum feeding conditions. In rats with free access to food, central administration of apo A-IV antiserum stimulated feeding during the light cycle (28). Similar studies conducted during the dark phase, when rats actively eat, may help clarify the role of apo A-IV in the control of individual meals. Second, evidence shows that there is a link between the regulation of food intake and the circadian rhythm of lymph and serum apo A-IV. Fukagawa et al (30) reported that in rats given free access to food, both serum and intestinal lymph apo A-IV exhibited a circadian rhythm that was significantly higher during the dark hours than during the light hours. When Fukagawa et al (30) examined the serum apo A-IV level in food-deprived rats, they found it to exhibit the same circadian rhythm as in fed rats. However, for all time points, the serum apo A-IV level was significantly higher in rats given free access to food compared with food-deprived rats. This indicated that although ad libitum feeding greatly increased the levels of serum apo A-IV, it did not change the pattern of its inherent circadian rhythm. That serum apo A-IV increased during the dark phase, which corresponds to the most active feeding period of rats, suggests a physiological role for apo A-IV in the regulation of food intake.

Third, Morton et al (63) reported down-regulation of intestinal apo A-IV mRNA levels by leptin. Leptin is a protein made by the adipose tissue and is believed to signal the hypothalamus in the brain (a center for the regulation of food intake and energy metabolism) of how much fat is in the body. Leptin decreases food intake and increases energy metabolism (102). The discovery of leptin by Zhang

et al (103) has energized the field of obesity. Preliminary data suggest that leptin decreases apo A-IV synthesis and secretion by the small intestinal epithelial cells. It has also been demonstrated that intestinal apo A-IV synthesis and secretion is up-regulated by insulin in both rodents and humans (8, 11). Energy homeostasis in the body is accomplished by a high integrated and redundant neurohumoral system that prevents the effect of short-term fluctuations in energy balance on fat mass (102). Insulin and leptin are hormones that are secreted in proportion to body adiposity, and they play a critical role in this energy homeostasis. Because leptin and insulin seem to regulate intestinal apo A-IV synthesis, apo A-IV may be involved in the long-term regulation of food intake and body weight. This interesting hypothesis is currently just speculation and must be proven experimentally.

Another point worthy of consideration is that PYY, released by the L cells located in the ileum and the colon, stimulates the production of apo A-IV by the jejunum and the ileum. Several laboratories have demonstrated that PYY is released in response to fat bile salts, glucose, short-chain FAs such as butyrate, and amino acids (4, 74, 95). Thus, the inhibition of food intake by apo A-IV is not just limited to the absorption of long-chain FAs; it could also be initiated by other nutrients and macromolecules in the lumen of the ileum and the colon. This exciting area of apo A-IV research requires further exploration.

THE ROLE OF APO A-IV ON UPPER GASTROINTESTINAL FUNCTION

Another possible mechanism of action by which apo A-IV inhibits food intake is by affecting gastric motility as well as gastric acid secretion. Okumura et al demonstrated that intracisternal injections of purified apo A-IV inhibited gastric acid secretion (67, 68) and gastric motility (69) in rats in a dose-dependent manner. The doses of apo A-IV chosen for these studies were thought to reproduce the levels of apo A-IV measured in cerebrospinal fluid after lipid feeding (28). Intravenous infusion of similar doses of apo A-I did not elicit gastric responses. This is important because Fujimoto et al (27) demonstrated that intravenous infusion of purified apo A-IV inhibited food intake in a dose-dependent manner, but administration of similar doses of apo A-I had no effect on food intake. Additionally, Fujimoto et al (29) demonstrated that apo A-IV administered into the third ventricle inhibited food intake in a dose-dependent manner but that apo A-I did not. Thus, the studies of Okumura et al (67–69) corroborate the observations made by Fujimoto et al (27, 29). As proposed by Okumura et al (67), apo A-IV acts as an enterogastrone, i.e. a humoral mediator released by the intestine that mediates the humoral inhibition of gastric acid secretion as well as motility by the ingestion of fat. Currently, it is not clear if the effects of apo A-IV on food intake is directly linked to its effects on gastric function. Apo A-IV could directly

influence central feeding mechanisms; alternatively, feeding could be affected by the effect of apo A-IV on gastric function, particularly via the inhibition of gastric emptying (60).

Preliminary data seem to indicate that intravenous infusion of apo A-IV also affects intestinal motility as well as the activity of the vagal afferents (H Raybould, personal communication).

EFFECT OF CHRONIC HIGH-FAT FEEDING ON INTESTINAL APO A-IV SYNTHESIS

The effect that chronic ingestion of a high-fat diet has on intestinal apo A-IV synthesis is interesting, but the mechanisms involved are unclear. Previous studies have determined that acute administration of a lipid meal results in a marked increase in apo A-IV levels and stimulation of apo A-IV synthesis in both the jejunum and the ileum (5). In this same study, Apfelbaum et al (5) determined that chronic consumption of a high-fat diet (30 g/100 g by weight of diet as fat) resulted in the stimulation of apo A-IV synthesis and increased mRNA levels in the jejunum but not the ileum. Does the ileum, then, become less responsive to lipid after chronic ingestion of a high-fat diet, or does the adaptation of the digestive and absorptive process result in fat no longer reaching the ileum? This question warrants further investigation.

In humans, the 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 (97) but disappears during the second week, thus leading investigators to conclude that there is autoregulation 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. Our preliminary data shows that plasma leptin increases dramatically in rats chronically consuming a diet high in fat and while becoming obese. It is interesting that their intestinal apo A-IV responses to lipid feeding were also attenuated. Morton et al (63) demonstrated that intravenous administration of leptin reduces apo A-IV mRNA levels of the small intestine after a lipid load. Our laboratory has also confirmed that leptin greatly suppressed the stimulation of apo A-IV synthesis and secretion by the small intestine in conscious rats (21). It is tempting to speculate that this apparent autoregulation of apo A-IV in response to chronic ingestion of high fat is related to elevated circulating leptin.

Reduction of apo A-IV in response to lipid feeding in both animals and humans that chronically consume a high-fat diet may be physiologically and clinically important because it may explain in part why increasing dietary fat content accelerates the development of obesity. The conclusion that increased consumption of a high-fat diet leads to an increase in body fat is evidenced by numerous experiments

with varying diets and species. Excellent reviews on this subject have come from the laboratories of Bray & Popkin (14), Hill et al (42), Warwick et al (96), and West & York (100). Further investigation of why chronic ingestion of a high-fat diet attenuates an apo A-IV response to lipid feeding is extremely important to our understanding of why a high-fat diet predisposes both animals and humans to obesity.

CONCLUSIONS AND FUTURE DIRECTIONS

Intestinal apo A-IV is a protein stimulated by dietary lipid that has a potentially important physiological role in the integrated control of digestive function and ingestive behavior. It also has a presumed role in cholesterol and lipoprotein metabolism. The role of apo A-IV in the regulation of upper gut function and satiety needs further investigation. For instance, what molecular form of apo A-IV is involved—free monomer, homodimeric (98), high-density-lipoprotein bound, or apo A-IV–derived bioactive peptides? Additional information is urgently needed to help us understand how apo A-IV works in the central nervous system to modify food intake and gastric motility and secretion. Is apo A-IV being made by the central nervous system? If so, where and by what cells? If the brain makes apo A-IV, is it physiologically regulated? Is it regulated in the same way as intestinal apo A-IV? What is the relationship between circulating apo A-IV and brain apo A-IV? Is there a receptor for apo A-IV? If so, where is it located? Is it physiologically regulated? These questions and others need addressing before a comprehensive understanding of the physiology of apo A-IV can be achieved.

Understanding the mechanism of stimulation of apo A-IV synthesis by the formation and secretion of chylomicrons and peptide YY is another area that warrants further investigation. There is evidence that apo A-IV synthesis is stimulated in both the jejunum and ileum when exposed to fat. Lipid exposed to the ileum also results in PYY secretion, which further stimulates the synthesis and release of apo A-IV by the jejunum. Several studies have demonstrated that apo A-IV biosynthesis stimulated by lipid absorption is mediated by a different molecular mechanism than stimulation by PYY because the mRNA levels increase markedly during lipid stimulation but remain the same during PYY stimulation. Future investigation of the mechanisms of regulation of apo A-IV synthesis by lipid absorption, PYY, leptin, chronic consumption of a high-fat diet, and other currently unknown factors will pose both a challenge and a reward in enhancing our understanding of diet-induced obesity.

ACKNOWLEDGMENT

This work was supported by grants from the National Institutes of Health DK 53444, DK 54504, DK 56863, and DK 56910.

LITERATURE CITED

1. Abumrad NA, Coburn C, Ibrahimi A. 1999. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPm. *Biochim. Biophys. Acta* 18:4–13
2. Abumrad NA, Sfeir Z, Connelly MA, Coburn C. 2000. Lipid transporters: membrane transport systems for cholesterol and fatty acids. *Curr. Opin. Clin. Nutr. Metab. Care* 3:255–62
3. Adrian TE, Bacarese HA, Smith HA, Chohan P, Manolas KJ, Bloom SR. 1987. Distribution and postprandial release of porcine peptide YY. *J. Endocrinol.* 113:11–14
4. Anini Y, Fu-Cheng X, Cuber JC, Kervran A, Chariot J, Roz C. 1999. Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. *Pfugers Arch.* 438:299–306
5. Apfelbaum TF, Davidson NO, Glickman RM. 1987. Apolipoprotein A-IV synthesis in the rat intestine: regulation by dietary triglyceride. *Am. J. Physiol. Gastrointest. Liver Physiol.* 252:G662–66
6. Aponte GW, Fink AS, Meyer JH, Tatemoto K, Taylor IL. 1985. Regional distribution and release of peptide YY with fatty acids of different chain length. *Am. J. Physiol. Gastrointest. Liver Physiol.* 249:G745–50
7. Aponte GW, Park K, Hess R, Garcia R, Taylor IL. 1989. Meal-induced peptide tyrosine-tyrosine inhibition of pancreatic secretion in the rat. *FASEB J.* 3:1949–55
8. Attia N, Touzani A, Lahrichi M, Balafrej A, Kabbaj O, Girard-Globa A. 1997. Response of apolipoprotein A-IV and lipoproteins to glycaemic control in young people with insulin-dependent diabetes mellitus. *Diabet. Med.* 14(3):242–47
9. Benzonana G, Desnuelle P. 1968. Action of some effectors on the hydrolysis of long-chain triglycerides by pancreatic lipase. *Biochim. Biophys. Acta* 164:47–58
10. Bisgaier CL, Glickman RM. 1983. Intestinal synthesis, secretion, and transport of lipoproteins. *Annu. Rev. Physiol.* 45:625–36

10a. Bisgaier CL, Sachdev OP, Lee ES, Williams KJ, Blum CB, Glickman RM. 1987. Effect of lecithin:cholesterol acyl transferase on distribution of apolipoprotein A-IV among lipoproteins of human plasma. *J. Lipid Res.* 28:693–703

11. Black DD, Ellinas H. 1992. Apolipoprotein synthesis in newborn piglet intestinal explants. *Pediatr. Res.* 32:553–58
12. Borgström B. 1974. Fat digestion and absorption. *Biomembranes* 4B:555–620
13. Borgström B, Erlanson-Albertson C, Wieloch T. 1979. Pancreatic colipase-chemistry and physiology. *J. Lipid Res.* 20:805–16
14. Bray GA, Popkin BM. 1998. Dietary fat does affect obesity. *Am. J. Clin. Nutr.* 68:1157–73
15. Brockerhoff H. 1968. Substrate specificity of pancreatic lipase. *Biochim. Biophys. Acta* 159:296–303
16. Carey MC, Small DM, Bliss CM. 1983. Lipid digestion and absorption. *Annu. Rev. Physiol.* 45:651–77
17. Carriere F, Barrowman JA, Verger R, Laugier R. 1993. Secretion and contribution of lipolysis of gastric and pancreatic lipases during a test meal in humans. *Gastroenterology* 105:876–78
18. Cohen RD, Castellani LW, Qiao JH, Van Lenten BJ, Lusis AJ, Reue K. 1997. Reduced aortic lesions and elevated high density lipoprotein level in transgenic mice overexpressing mouse apolipoprotein A-IV. *J. Clin. Invest.* 99:1906–16
19. Davidson NO, Kollmer ME, Glickman RM. 1991. Apolipoprotein B synthesis in rat small intestine: regulation by dietary

triglyceride and biliary lipid. *J. Lipid Res.* 27:30–39

20. Delamatre JG, Roheim PS. 1983. The response of apolipoprotein A-IV to cholesterol feeding in rats. *Biochim. Biophys. Acta* 751:210–17
21. Doi T, Liu M, Seeley RJ, Woods SC, Kalogeris TJ, Tso P. 2000. The effect of leptin on intestinal apolipoprotein AIV synthesis and secretion. *Gastroenterology* 118:A71 (Abstr.)

21a. Duverger N, Tremp G, Caillaud JM, Emmanuel F, Castro G, et al. 1996. Protection against atherogenesis in mice mediated by human apolipoprotein A-IV. *Science* 273:966–68

22. Dvorin E, Gorder NL, Benson DM, Gotto AM. 1988. Apolipoprotein A-IV. A determinant for binding and uptake of high density lipoproteins by rat hepatocytes. *J. Biol. Chem.* 261:15714–18
23. Elsbourhagy NA, Walker DW, Paik YK, Boguski MS, Freeman M, et al. 1987. Structure and expression of the human apolipoprotein A-IV gene. *J. Biol. Chem.* 262:7973–81
24. Erlanson-Albertsson C. 1992. Pancreatic colipase. Structural and physiological aspects. *Biochim. Biophys. Acta* 1125:1–7
25. Field FJ, Cooper AD, Erickson SK. 1982. Regulation of rabbit intestinal acyl coenzyme A-cholesterol acyltransferase in vivo and in vitro. *Gastroenterology* 83:873–80
26. Fielding CJ, Shore VG, Fielding PE. 1972. A protein cofactor of lecithin: cholesterol acyltransferase. *Biochem. Biophys. Res. Commun.* 46:1493–98
27. Fujimoto K, Cardelli JA, Tso P. 1992. Increased apolipoprotein A-IV in rat mesenteric lymph after lipid meal acts as a physiological signal for satiation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 262:G1002–6
28. Fujimoto K, Fukagawa K, Sakata T, Tso P. 1993. Suppression of food intake by apolipoprotein A-IV is mediated through the central nervous system in rats. *J. Clin. Invest.* 91:1830–33
29. Fujimoto K, Machidori H, Iwakiri R, Yamamoto K, Fujisaki J, et al. 1993. Effect of intravenous administration of apolipoprotein A-IV on patterns of feeding, drinking and ambulatory activity of rats. *Brain Res.* 608:233–37
30. Fukagawa K, Gou HM, Wolf R, Tso P. 1994. Circadian rhythm of serum and lymph apolipoprotein A-IV in ad libitum-fed and fasted rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 267: R1385–90
31. Fukagawa K, Knight DS, Hamilton HA, Tso P. 1995. Immunoreactivity for apolipoprotein A-IV in tanycytes and astrocytes of rat brain. *Neurosci. Lett.* 199:17–20
32. Goldberg IJ, Scheraldi CA, Yacoub LK, Saxena U, Bisgaier CL. 1990. Lipoprotein C-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. *J. Biol. Chem.* 265:4266–72
33. Gonzalez-Vallina R, Wang H, Zhan R, Berschneider HM, Lee RM, et al. 1996. Lipoprotein and apolipoprotein secretion by a newborn piglet intestinal cell line (IPEC-1). *Am. J. Physiol. Gastrointest. Liver Physiol.* 271:G249–59
34. Gould RG, Jones RJ, LeRoy GV, Wissler RW, Taylor CB. 1969. Absorbability of β-sitosterol in humans. *Metabolism* 18: 652–62
35. Green PHR, Glickman RM, Riley JW, Quinet E. 1980. Human apolipoprotein A-IV. Intestinal origin and distribution. *J. Clin. Invest.* 65:911–19
36. Hamosh M. 1979. The role of lingual lipase in neonatal fat digestion. In *Development of Mammalian Absorptive Process, Ciba Found. Symp. 70*, ed. K Elliot, J Whelan, pp. 69–98. Amsterdam: Excerpta Med.
37. Harmon CM, Luce P, Beth AH, Abumrad NA. 1991. Labeling of adipocyte membranes by sulfo-N-succinimidyl derivatives of long-chain fatty acids: inhibition

of fatty acid transport. *J. Membr. Biol.* 121:261–68

38. Harper AA, Hood JCA, Mushens J. 1979. Inhibition of external pancreatic secretion by intracolonic and intraileal infusions in the cat. *J. Physiol.* 292:445–54
39. Hayashi H, Nutting DF, Fujimoto K, Cardelli JA, Black D, Tso P. 1990. Fat feeding increases size, but not number, of chylomicrons produced by small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 259:G709–19
40. Hayashi H, Nutting DF, Fujimoto K, Cardelli JA, Black D, Tso P. 1990. Transport of lipid and apolipoproteins A-I and A-IV in intestinal lymph of the rat. *J. Lipid. Res.* 31:1613–25
41. Hill FLC, Zhang T, Gomez G, Greeley GH Jr. 1991. Peptide YY, a new gut hormone. *Steroids* 56:77–82
42. Hill JO, Lin D, Yakubu F, Peters JC. 1992. Development of dietary obesity in rats: influence of amount and composition of dietary fat. *Int. J. Obes.* 16:321–33
43. Hofmann AF, Borgstrom B. 1962. Physico-chemical state of lipids in intestinal content during their digestion and absorption. *Fed. Proc.* 21:43–50
44. Hofmann AF, Borgstrom B. 1964. The intraluminal phase of fat digestion in man: the lipid content of the micellar and oil phases of intestinal content obtained during fat digestion and absorption. *J. Clin. Invest.* 43:247–57
45. Jin H, Gai L, Lee K, Chang TM, Li P, et al. 1993. A physiological role of peptide YY on exocrine pancreatic secretion in rats. *Gastroenterology* 105:208–15
46. Johnston JM, Rao GA, Lowe PA. 1967. The separation of the α-glycerophosphate and monoglyceride pathways in the intestinal biosynthesis of triglycerides. *Biochim. Biophys. Acta* 137:578–80
47. Kalogeris TJ, Fukagawa K, Tso P. 1994. Synthesis and lymphatic transport of intestinal apolipoprotein A-IV in response to graded doses of triglyceride. *J. Lipid Res.* 35:1141–51
48. Kalogeris TJ, Holden VR, Tso P. 1999. Stimulation of jejunal synthesis of apolipoprotein A-IV by ileal lipid infusion is blocked by vagatomy. *Am. J. Physiol. Gastrointest. Liver Physiol.* 277: G1081–87
49. Kalogeris TJ, Qin X, Chey WY, Tso P. 1998. PYY stimulates synthesis and secretion of intestinal apolipoprotein AIV without affecting mRNA expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* 275:G668–74
50. Kalogeris TJ, Tsuchiya T, Fukagawa K, Wolf R, Tso P. 1996. Apolipoprotein A-IV synthesis in proximal jejunum is stimulated by ileal lipid infusion. *Am. J. Physiol. Gastrointest. Liver Physiol.* 270:G277–86
51. Krause BR, Sloop CH, Castle CK, Roheim PS. 1981. Mesenteric lymph apolipoproteins in control and thinyl estradiol-treated rats: a model for studying apolipoproteins of intestinal origin. *J. Lipid Res.* 22:610–19
52. Larhammar D, Söderberg C, Blomgvist AG. 1993. Evolution of the neuropeptide Y family of peptides. In *The Biology of Neuropeptide Y and Related Peptides*, ed. WF Colmers, C Wahlestadt, pp. 1–41. Totoa, NJ: Humana
53. Liao TH, Hamosh P, Hamosh M. 1984. Fat digestion by lingual lipase: mechanism of lipolysis in the stomach and upper small intestine. *Pediatr. Res.* 18:402–9
54. Lin HC, Doty JE, Reedy TJ, Meyer JH. 1990. Inhibition of gastric emptying of sodium oleate depends upon length of intestine exposed to the nutrient. *Am. J. Physiol. Gastrointest. Liver Physiol.* 259: G1031–36
55. Lundgren S, Carling T, Hjälm G, Juhlin C, Rastad J, et al. 1997. Tissue distribution of human gp330/megalin, a putative Ca^{2+}-sensing protein. *J. Histochem. Cytochem.* 45:383–92
56. MacFarlane A, Kinsman R, Read NW.

1983. The ileal brake: ileal fat slows small bowel transit and gastric emptying in man. *Gut* 24:471–72
57. Manganaro F, Kuksis A. 1986. Purification and preliminary characterization of 2-monoacylglycerol acyltransferase from rat intestinal villus cells. *Can. J. Biochem. Cell Biol.* 63:341–47
58. Mattson FH, Beck LW. 1956. The specificity of pancreatic lipase on the primary hydroxyl groups of glycerides. *J. Biol. Chem.* 219:735–40
59. Maylie MF, Charles M, Gache C, Desnuelle P. 1971. Isolation and partial identification of a pancreatic colipase. *Biochim. Biophys. Acta* 229:286–89
60. McHugh PR, Moran TH. 1985. The stomach: a conception of its dynamic role in satiety. *Prog. Psychobiol. Physiol. Psychol.* 11:197–232
61. Meyer JH, Elashoff JD, Doty JE, Gu YG. 1994. Disproportionate ileal digestion on canine food consumption. A possible model for satiety in pancreatic insufficiency. *Dig. Dis. Sci.* 39:1014–24
62. Morgan RGH, Barrowman J, Borgström B. 1969. The effect of sodium taurodeoxycholate and pH on the gel filtration behaviour of rat pancreatic protein and lipases. *Biochim. Biophys. Acta* 175:65–75
63. Morton NM, Emilsson V, Liu YL, Cawthorne MD. 1998. Leptin action in intestinal cells. *J. Biol. Chem.* 273:26194–201
64. Murata M, Peränen J, Schreiner R, Wieland F, Kurzchalia TV, Simons K. 1995. VIP21/caveolin is a cholesterol-binding protein. *Proc. Natl. Acad. Sci. USA* 92:10339–43
65. Nilsson A. 1968. Intestinal absorption of lecithin and lysolecithin by lymph fistula rats. *Biochim. Biophys. Acta* 152:379–90
66. Northfield TC, Hofmann AF. 1975. Biliary lipid output during three meals and an overnight fast. *Gut* 16:1–11
67. Okumura T, Fukagawa K, Tso P, Taylor IL, Pappas TN. 1994. Intracisternal injection of apolipoprotein A-IV inhibits gastric secretion in pylorus-ligated conscious rats. *Gastroenterology* 107:1861–64
68. Okumura T, Fukagawa K, Tso P, Taylor IL, Pappas TN. 1995. Mechanisms of action of intracisternal apolipoprotein A-IV in inhibiting gastric acid secretion in rats. *Gastroenterology* 109:1583–88
69. Okumura T, Fukagawa K, Tso P, Taylor IL, Pappas TN. 1996. Apolipoprotein A-IV acts in the brain to inhibit gastric emptying in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* 270:G49–53
70. Paltauf F, Esfandi F, Holasek A. 1974. Stereospecificity of lipases. Enzymatic hydrolysis of enantiomeric alkyl diacylglycerols by lipoprotein lipase, lingual lipase, and pancreatic lipase. *FEBS Lett.* 40:119–23
71. Pappas TN, Debas HT, Chang AM, Taylor IL. 1986. Peptide YY release by fatty acids is sufficient to inhibit gastric emptying in dogs. *Gastroenterology* 91:1386–89
72. Pappas TN, Debas HT, Taylor IL. 1985. Peptide YY: metabolism and effect pancreatic secretion in dogs. *Gastroenterology* 89:1387–92
73. Pappas TN, Debas HT, Taylor IL. 1986. Enterogastrone-like effect of peptide YY is vagally mediated in the dog. *J. Clin. Invest.* 77:49–53
74. Plaisancie P, Dumoulin V, Chayvialle JA, Cuber JC. 1996. Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. *J. Endocrinol.* 151:421–29
75. Qin X, Swertfeger DK, Zheng S, Hui DY, Tso P. 1998. Apolipoprotein AIV: a potent endogenous inhibitor of lipid oxidation. *Am. J. Physiol.* 274:H1836–40. *Heart Circ. Physiol.*
76. Roberts IM, Montgomery RK, Carey MC. 1984. Rat lingual lipase: partial purification, hydrolytic properties and comparison with pancreatic lipase. *Am. J. Physiol. Gastrointest. Liver Physiol.* 247:G385–93

77. Rodriguez MD, Kalogeris TJ, Wang XL, Wolf R, Tso P. 1997. Rapid synthesis and secretion of intestinal apolipoprotein A-IV after gastric fat loading in rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 272:R1170–77
78. Rosenzweig SA, Miller LJ, Jamieson JD. 1983. Identification and localization of cholecystokinin-binding sites on rat pancreatic plasma membranes and acinar cells: a biochemical and autoradiographic study. *J. Cell Biol.* 96:1288–97
79. Savage AP, Adrian TE, Carolan G, Chattarjee VK, Bloom SR. 1987. Effects of peptide YY (PYY) on mouth to caecum intestinal transit time and on the rate of gastric emptying in healthy volunteers. *Gut* 28:166–70
80. Senior JR. 1964. Intestinal absorption of fats. *J. Lipid Res.* 5:495–521
81. Simmonds WJ. 1972. Fat absorption and chylomicron formation. In *Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism*, ed. GJ Nelson, pp. 705–43. New York: Wiley Intersci.
82. Solomon TE. 1994. Control of exocrine pancreatic secretion. In *Physiology of the Gastrointestinal Tract*, ed. LR Johnson, pp. 1173–207. New York: Raven. 3rd ed.
83. Spiller RC, Trotman IF, Higgens BE, Ghatel MA, Grimble GK, et al. 1984. The ileal brake-inhibition of jejunal motility after ileal fat perfusion in man. *Gut* 25:365–74
84. Stahl A, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, et al. 1999. Identification of the major intestinal fatty acid transport protein. *Mol. Cell.* 4:299–308
85. Stein O, Stein Y, Lefevre M, Roheim P. 1986. The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and lipsomes derived from an ether analog of phosphatidylcholine. *Biochim. Biophys. Acta* 878:7–13
86. Stremmel W. 1988. Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *J. Clin. Invest.* 82:2001–10
87. Stremmel W, Strohmeyer G, Borchard F, Kochwa S, Berk PD. 1985. Isolation and partial characterization of a fatty acid binding protein in rat liver plasma membranes. *Proc. Natl. Acad. Sci. USA* 82:4–8
88. Swaney JB, Braithewaite F, Eden HA. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry* 16:271–78
89. Tatemoto K. 1982. Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. *Proc. Natl. Acad. Sci. USA* 79:2514–18
90. Taylor CB, Gould RG. 1967. A review of human cholesterol metabolism. *Arch. Pathol.* 84:2–14
91. Taylor IL. 1985. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. *Gastroenterology* 88:731–37
92. Tso P, Balint JA, Bishop MB, Rodgers JB. 1981. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. *Am. J. Physiol.* 241:G487–97
93. Tso P, Balint JA, Rodgers JB. 1980. Effect of hydrophobic surfactant (Pluronic L-81) on lymphatic lipid transport in the rat. *Am. J. Physiol. Gastrointest. Liver Physiol.* 239:G348–53
94. Tso P, Drake DS, Black DD, Sabesin SM. 1984. Evidence for separate pathways of chylomicron and very low-density lipoprotein assembly and transport by rat small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 247:G599–610
95. Vu MK, Verkijk M, Muller ES, Biemond I, Lamers CB, Masclee AA. 1999. Medium chain triglycerides activate distal but not proximal gut hormones. *Clin. Nutr.* 18:359–63
96. Warwick ZS, Bowen KJ, Synowski SJ. 1997. Learned suppression of intake based on anticipated calories: cross-nutrient comparisons. *Physiol. Behav.* 62:1319–24
97. Weinberg RB, Dantzker C, Patton CS.

1990. Sensitivity of serum apolipoprotein A-IV levels to changes in dietary fat content. *Gastroenterology* 98:17–24

98. Weinberg RB, Spector MS. 1985. The self-association of human apolipoprotein A-IV. Evidence for an in vivo circulating dimeric form. *J. Biol. Chem.* 260:14279–86
99. Welch I, Saunders K, Read NW. 1985. Effect of ileal and intravenous infusions of fat emulsions on feeding and satiety in human volunteers. *Gastroenterology* 89: 1293–97
100. West DB, York B. 1998. Dietary fat, genetic predisposition, and obesity: lessons from animal models. *Am. J. Clin. Nutr.* 67:505–12S
101. Willnow TE, Goldstein JL, Orth K, Brown MS, Herz J. 1992. Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. *J. Biol. Chem.* 267:26172–80
102. Woods SC, Seeley RJ, Porte DJ, Schwartz MW. 1998. Signals that regulate food intake and energy homeostasis. *Science* 280:1378–83
103. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–32