ApoA-IV: Current and emerging roles in intestinal lipid metabolism, glucose homeostasis, and satiety

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RUNNING TITLE: ApoA-IV: role in lipid metabolism, glucose homeostasis, and satiety

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KEYWORDS: apolipoprotein A-IV (apoA-IV); chylomicron; intestinal lipid transport; lymph fistula mouse model; satiety; diabetes; obesity; cardiovascular disease; glucose homeostasis

ABBREVIATIONS: apolipoprotein A-IV (apoA-IV); apolipoprotein A-I (apoA-I); apolipoprotein B-48 (apoB-48); apolipoprotein C-III (apoC-III); knockout (KO); wild-type (WT); very-low density lipoprotein (VLDL); high-density lipoprotein (HDL); cholecystokinin (CCK); nucleus of the solitary tract (NTS); subdiaphragmatic vagal deafferentation (SDA); intracerebroventricular (ICV); glial fibrillary acidic protein (GFAP); arcuate nucleus (ARC); peptide tyrosine-tyrosine (PYY); corticosterone (CORT); Neuropeptide Y (NPY); α-Melanocyte-stimulating hormone (α-MSH); proopiomelanocortin...
(POMC); melanocortin type 3 and 4 receptors (MC3/4-R); agouti-related protein (AgRP);
metallothionein-II (MT-II); Roux-en-Y gastric bypass (RYGB)

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ABSTRACT:

Apolipoprotein A-IV (apoA-IV) is secreted by the small intestine on chylomicrons into intestinal lymph in response to fat absorption. Many physiological functions have been ascribed to apoA-IV, including a role in chylomicron assembly and lipid metabolism, a mediator of reverse-cholesterol transport, an acute satiety factor, regulator of gastric function, and finally, a modulator of blood glucose homeostasis. The purpose of this review is to update our current view of intestinal apoA-IV synthesis and secretion and the physiological roles of apoA-IV in lipid metabolism and energy homeostasis, and to underscore the potential for intestinal apoA-IV to serve as a therapeutic target for the treatment of diabetes and obesity related disease.
**Introduction**

Apolipoprotein A-IV (apoA-IV) was discovered more than 30 years ago as a major protein component of lymph chylomicrons in the post-prandial state (31, 90, 99). Human apoA-IV is found in the apoA-I/C-III/A-IV gene cluster on chromosome 11 (43). This gene cluster is essential for lipoprotein metabolism and the maintenance of plasma lipid levels, and as such, is a modulator of cardiovascular disease risk. In humans, apoA-IV is synthesized only by the small intestine; in rodents a small amount is also synthesized by the liver (31, 51, 84). The jejunum is the major site of apoA-IV synthesis, but it is also produced in the duodenum and ileum (42).

ApoA-IV is secreted into the lymph on chylomicrons in response to lipid feeding. During subsequent metabolism of chylomicrons in the periphery, approximately 25% of the apoA-IV is transferred to HDL in the plasma and the rest is found in the lipoprotein-free fraction (24, 69). Therefore, the presence of apoA-IV in the periphery (on chylomicrons, circulating on HDL, and free in the plasma) is linked to intestinal lipid absorption and chylomicron packaging.

Many physiological functions have been ascribed to apoA-IV, including a role in chylomicron assembly and lipid metabolism, a mediator of reverse-cholesterol transport, an acute satiety factor, regulator of gastric function, and finally, a modulator of blood glucose homeostasis. This review will highlight the evidence for these physiological roles of apoA-IV, as well as emerging evidence of a role for intestinal apoA-IV in mediating chronic disease.

**Role of apoA-IV in chylomicron assembly and lipid absorption**

Nearly 40 years after the first description of apoA-IV, a considerable body of evidence links apoA-IV to intestinal triglyceride absorption and secretion. ApoA-IV is responsive to nutritional status, and dietary fat absorption results in increased apoA-IV synthesis and secretion in a dose-dependent manner (39–42). Dietary fat absorption also results in increased plasma apoA-IV levels.
(32), and in patients with malabsorptive disorders (such as chronic pancreatitis) apoA-IV levels in plasma do not rise in response to a fatty meal (45). ApoA-IV production requires chylomicron synthesis, and is upregulated in response to dietary long chain fatty acids (the absorption of which requires chylomicron synthesis), but not when short chain fatty acids are absorbed through the portal blood (44). Blocking chylomicron secretion inhibits the rise in apoA-IV synthesis induced by fat absorption (88). Finally, apoA-IV co-localizes with apo-B in the chylomicron secretory pathway (50).

Despite this considerable evidence linking apoA-IV with triglyceride absorption and chylomicron formation, in vivo models with gain or loss of apoA-IV (apoA-IV transgenic or KO mice), do not result in a loss of triglyceride secretion, or in the secretion of triglyceride-deficient chylomicron particles (1, 46, 47, 97). This dichotomy between the apparent role of apoA-IV in intestinal triglyceride packaging and secretion and its lack of effect in genetically modified mice has led to two prevailing conclusions: 1) that apoA-IV plays a substantive role in triglyceride secretion, or 2) that its main physiological role may be extra-intestinal. We will discuss both.

Evidence supporting the role of apoA-IV in intestinal triglyceride absorption and packaging is significant. In newborn swine enterocytes (IPEC-1 cells) the overexpression of apoA-IV greatly enhances the secretion of triglyceride, cholesterol ester, and phospholipid in chylomicron/VLDL particles (28, 62, 95, 100). This role of apoA-IV in IPEC-1 cells appears to rely on a region of apoA-IV (residues 344-354), the presence of which stimulates the secretion of larger, more triglyceride-rich lipoproteins in a dose-dependent manner (62). This role of apoA-IV may be due to its ability to stabilize the expanding lipid/aqueous interface during lipoprotein synthesis, resulting in the formation of larger triglyceride-rich chylomicrons. For apoA-IV to facilitate the expansion of triglyceride-rich lipoproteins, it is likely that it would interact with apoB in the endoplasmic reticulum (as part of the triglyceride secretory pathway). Studies in both COS and McA-RH7777
hepatoma cells have shown that the ER-retained apoA-IV (tagged with the ER retention signal KDEL) inhibited the early stages of triglyceride-rich lipoprotein assembly (23) and that overexpression of WT apoA-IV is able to modulate the secretory trafficking of apoB leading to an enhancement of particle expansion and triglyceride secretion (95). In addition to this work in cell culture, work by Simon and Weinberg suggests that apoA-IV expression mediates the expression of other triglyceride packaging and nutrient sensing genes in the intestine, which may act to regulate regional lipid absorption (83).

Despite this evidence, most in vivo studies show that the total loss of apoA-IV or the transgenic overexpression of apoA-IV do not result in a loss or gain of triglyceride secretion, nor in the secretion of triglyceride-deficient chylomicron particles. Using the conscious in vivo lymph fistula model to directly assess intestinal lipoprotein secretion [Figures 1 and 2], apoA-IV KO mice have been shown to have no change in dietary lipid absorption, or in the cumulative secretion of radiolabeled triglyceride into lymph (under both an acute bolus acute dose or continuous intraduodenal lipid infusion paradigms)(47). In mice expressing high levels of human apoA-IV, lipid absorption was measured using a variety of labeled metabolic tracers and there was no difference found (1). These data suggest apoA-IV is not required for normal dietary lipid absorption.

Though these in vivo studies show that apoA-IV may not be required for triglyceride absorption (since the amount of triglyceride absorbed and secreted was the same between models), the loss of apoA-IV in apoA-IV KO mice does result not only in the secretion of larger chylomicrons from the intestine but these chylomicrons were less rapidly cleared from plasma than WT chylomicrons [Figures 3 and 4](46). This suggests that apoA-IV has a previously unknown role in regulating chylomicron metabolism and is therefore important in regulating plasma lipid metabolism. It should also be noted the chylomicrons derived from the apoA-IV null animals also metabolized more slowly than those derived from WT animals, suggesting that apoA-IV may not be
freely interchangeable between the free and the HDL-bound compartment with the circulating chylomicrons.

This dichotomy in findings between cell culture and *in vivo* models is not only a conundrum, but also remains an important unresolved issue - especially in light of the importance of apoA-IV in mediating cardiovascular disease risk. Over-expression of apoA-IV protects transgenic mice from atherosclerosis caused by either apoE-deficiency, LDL receptor deficiency or cholesterol feeding (8, 16, 72). Interestingly, apoA-IV knock-out (KO) may also confer protection against diet-induced atherosclerosis by decreasing the rise in plasma lipid levels following the feeding of high fat; most notably by decreasing total triglyceride and cholesterol levels (70, 97). Since both the overproduction of chylomicrons during chronic disease (such as diabetes)(12, 15) and their subsequent clearance from plasma are both involved in the development of cardiovascular disease, apoA-IV may be indeed be playing multiple important roles. ApoA-IV may be modulating the retention of triglyceride-rich lipoproteins in plasma through its regulation of chylomicron clearance, and may also regulate the production of particles (either by stimulating bulk loading or triglyceride onto apoB-containing chylomicrons, or by modulating regional gene expression to regulate regional lipid absorption)(46, 62, 83, 95). It is unknown how many apoA-IV molecules are secreted from the intestine per chylomicron particle, or whether these changes during chronic disease states, are due to the potential role of apoA-IV in chylomicron clearance and its major role in modulating glucose homeostasis. Another point worth noting is the fact that apoA-IV protects lipoproteins from oxidative processes [72] and so it may be an extremely complicated task to sort out the relative importance of the various roles played by apoA-IV in lipid metabolism and cardiovascular events associated with both the protection and the promotion of vascular diseases.

Role of ApoA-IV in reverse cholesterol transport
ApoA-IV is the largest member of the exchangeable apolipoprotein family. After its synthesis in the enterocytes, it is subsequently secreted on the surface of chylomicrons, which are then rapidly acted upon by lipoprotein lipase in the periphery. As chylomicrons undergo lipolysis in the post-prandial state, apoA-IV dissociates from the chylomicrons as a result of the shrinking surface and is then associated with HDL and also is found in the lipid-free fraction of plasma (24, 69). As such, apoA-IV can promote cellular cholesterol efflux as a potent activator of lecithin-cholesterol acyltransferase (86). In several cell types (including both fibroblasts and adipocytes) apoA-IV containing particles are cholesterol acceptors. This role of apoA-IV as a mediator of cholesterol efflux may partly explain the observation that apoA-IV transgenic mice have significantly reduced atherogenesis than their WT counterparts (16).

Additional physiological functions have been attributed to intestinal apoA-IV expression, including its role as an anti-oxidant and anti-inflammatory factor (69, 78, 85). Although these are potentially important functions of apoA-IV, these functions are also carried out by other apolipoprotein and are not unique to apoA-IV.

### Role of ApoA-IV as a satiety factor

ApoA-IV has been reported to reduce food intake. When rodents receive an intravenous administration of chylous lymph this significantly reduces food intake, whereas infusion of chylous lymph missing apoA-IV does not recapitulate this effect (21). Furthermore, the administration of native apoA-IV inhibits food intake (21, 22). Subsequent studies have determined that central and peripheral administration of recombinant apoA-IV inhibits food intake, and is not toxic (21, 56, 61); whereas administration of apoA-I at comparable doses has no effect on satiety (21). These observations suggest that both intestinal apoA-IV (secreted in response to dietary lipids) and exogenous apoA-IV injected in the periphery can regulate food intake.
**ApoA-IV regulation of gastric emptying and secretion**

Lipid, particularly long-chain triglyceride, inhibits gastric motor and secretory function (38). Free fatty acids of chain length 12 or greater are much more effective in the inhibition of gastric motility than C10 fatty acids (38). Intestinally absorbed long-chain fatty acids are re-synthesized into triglyceride in the enterocytes and are subsequently secreted in chylomicrons (89). Mesenteric lymph enriched with chylomicrons collected from lymph fistula rats reduces gastric motor function (27). In contrast, when chylomicrons are removed from the chylous lymph, gastric motility significantly increases. In subsequent studies, purified recombinant apoA-IV was shown to significantly inhibit gastric motility (26, 27); in contrast, apoA-IV KO mice have significantly faster gastric emptying and greater secretion of gastric acid after an ingested meal (98). It has been demonstrated that this mechanism involves a negative feedback of apoA-IV containing chylomicrons on gastric motility via cholecystokinin-1R (CCK-1R) on capsaicin-sensitive vagal afferent nerve terminals (26).

**Role of apoA-IV in glucose homeostasis and insulin secretion**

Recently, studies in apoA-IV KO mice have revealed a novel role for apoA-IV in glucose metabolism and insulin secretion (92). ApoA-IV KO mice are glucose intolerant, accompanied with attenuated insulin secretion upon glucose challenge, suggesting that apoA-IV is essential for physiological blood glucose control. Administration of exogenous apoA-IV (in an amount equivalent to the amount secreted in response to a fatty meal) dramatically improved glucose tolerance along with the restoration of insulin secretion (92). Therefore, it is likely that physiologically, the apoA-IV secreted after a fat-rich meal could be rapid and sufficient enough to stimulate insulin secretion.
As an endogenous regulator of insulin secretion, intestinal apoA-IV comprises one of the components of the enteroinsular axis; i.e., the collective signaling pathways between the gut and the pancreatic islets that control nutrient-dependent insulin secretion. ApoA-IV has a half-life of about 7-8 hours, which is much longer than that of typical incretins such as GLP-1 and GIP (44), whose half life is a few minutes. For example, the glucose-lowering effect of a single injection of apoA-IV lasts about 12 hours in KKAy diabetic mice (92).

Experiments in isolated islets have examined the direct effect of apoA-IV on β-cell insulin secretion. ApoA-IV induces a dose-dependent increase in insulin release when the islets are exposed to 20 mM, but not 3 mM glucose. In addition, functional KATP and calcium channels are required for the action of apoA-IV on insulin secretion, but apoA-IV does not act directly to stimulate calcium influx (92). Membrane depolarization by closure of the KATP channels activates voltage-gated Ca\(^{2+}\) channels and lead to Ca\(^{2+}\) influx, which triggers the exocytosis of insulin. Instead, the action of apoA-IV seems to lie downstream of Ca\(^{2+}\) influx and amplify insulin exocytosis (92). While these data suggest apoA-IV acts on the late stages of insulin secretion, the precise molecular mechanism remains to be elucidated. ApoA-IV exhibits high-affinity binding to isolated human hepatocellular plasma membranes, which is saturable, reversible, and specific, supporting the idea that a membrane protein is involved in binding (96). However, a specific receptor for apoA-IV has not been discovered to date. However, apoA-IV is able to enter the hepatocyte and interact with the transcription factor NR1D1 (52). ApoA-IV inhibits gluconeogenesis through NR1D1. Thus through the insulinotropic and the anti-gluconeogenic action of apoA-IV, apoA-IV plays an important role in glucose homeostasis following the ingestion of food.

We have proposed that it is the \textit{change} in circulating apoA-IV levels between fasting and feeding (rather than the absolute level of circulating level of apoA-IV) that stimulates insulin
secretion. This may be another reason why the loss of regulation in apoA-IV that occurs in response to a chronic high fat diet correlates with poor glucose homeostasis (3, 41, 49, 55, 59, 94).

**Actions of apoA-IV in the brain.**

Compelling evidence suggests that central (rather than circulating) apoA-IV is important for the control of food intake and body weight: 1) when $^{125}$I-labeled apoA-IV is injected IV in mice, this apoA-IV does not cross the blood brain barrier (81); 2) intracerebroventricular (ICV) injection of apoA-IV significantly and dose-dependently reduces food intake without eliciting signs of toxicity (22); 3) apoA-IV mRNA and protein are present in the rat hypothalamus neurons (54); using immunohistochemistry, apoA-IV distribution is found in brain areas involved in energy homeostasis, including the arcuate (ARC) and ventromedial hypothalamic (VMH), the paraventricular (PVN), dorsomedial (DMN) nuclei, and the nucleus of the solitary tract (NTS)(81); finally, 4) double-staining immunohistochemistry with a neuronal marker revealed apoA-IV is largely present in neurons.

**Circadian rhythm of hypothalamic apoA-IV.**

To determine the roles of central apoA-IV in the regulation of daily food intake, Liu et al. examined the diurnal patterns of hypothalamic apoA-IV gene and protein expression (58). In freely feeding rats, the hypothalamic apoA-IV mRNA and protein levels were found to peak 3 h after lights on, and with a nadir 3 h after lights off (the normal feeding period of rodents). To make sure that it is not just a coincidence, we restricted the feeding period to four hours during the light cycle. In the food-restricted rats, the daily patterns of the apoA-IV fluctuation were altered with a marked decrease in hypothalamic apoA-IV mRNA and protein levels during the 4 h-feeding period of the light phase. Although corticosterone (CORT) secretion temporally coincided with the decreasing phase of apoA-IV in the hypothalamus, the diurnal expression of hypothalamic apoA-IV
is not regulated by CORT because depletion of CORT by adrenalectomy significantly decreased, rather than increased, hypothalamic apoA-IV mRNA and protein levels (58). The observations that apoA-IV levels in the hypothalamus were inversely related to food intake during the normal diurnal cycle as well as in the period of restricted feeding implies that hypothalamic apoA-IV is involved in the regulation of daily food intake.

Interaction of apoA-IV with other neuro- and endocrine peptides in the regulation of food intake and energy metabolism.

Neuropeptide Y. NPY is a hypothalamic neuropeptide with regulatory action on food intake. Liu, et. al. have demonstrated there is an interaction of apoA-IV with neuropeptide Y (NPY) (57). ICV injection of NPY alone significantly increased hypothalamic apoA-IV expression in a dose-dependent manner (57). While intraduodenal infusion of lipid also increased hypothalamic apoA-IV mRNA levels, there was no further significant increment with the combination of ICV injection of NPY and lipid infusion, indicating that there is a lack of potentiation in the regulation of hypothalamic apoA-IV gene expression by both NPY and lipid. One possibility to explain why administered NPY increases apoA-IV gene expression is to maintain a balance between these two opposing factors thereby regulating food intake, which could be further investigated in apoA-IV KO mice.

Melanocortin system. Hypothalamic melanocortin system plays an important role in the regulation of body weight. α-MSH (α-Melanocyte-stimulating hormone) derived from proopiomelanocortin (POMC) neurons exerts a tonic inhibitory influence over feeding through melanocortin type 3 and 4 receptors (MC3/4-R) in the hypothalamus. Agouti-related protein (AgRP) is a peptide produced in
the ARC, which antagonizes MC3/4-R. Therefore, when administered centrally, AgRP elicits hyperphagia (17). Consistent with this, it has been demonstrated that ICV administration into the third ventricle (i3vt) of metallothionein-II (MT-II), a synthetic MC3/4-R agonist, potently reduces feeding (57), whereas i3vt administration of SHU9119, a synthetic MC3/4-R antagonist, blocks the anorectic effect of MT-II (33). To determine whether apoA-IV exerts its anorectic effect through the melanocortin system, these data suggest that the brain apoA-IV system suppresses food intake by stimulating the ARC POMC system (81). Gotoh et al. also found that ICV administration of MT-II potentiated a subthreshold dose of apoA-IV suppression of 30-min feeding in rats, and the anorectic effect of ICV apoA-IV was almost completely attenuated by a subthreshold dose of SHU9119 (29). These data support a synergistic interaction between apoA-IV and melanocortins that reduces food intake by acting downstream of the ARC.

**Leptin.** Leptin is a peptide synthesized and secreted by adipocytes (9, 20). Like apoA-IV, leptin reduces food intake (35, 80) and it interacts synergistically with apoA-IV in this regard (14, 82). Leptin is an important component of lipid homeostasis and its circulating level is directly correlated with the amount of fat in the body (20). Thus, a high fat diet increases plasma leptin levels in obese humans and rodents (5, 10, 20). Leptin directly acts on leptin receptors in intestinal cells (6, 32, 34, 71) and attenuates the lipid-induced stimulation of apoA-IV synthesis and secretion (14, 66). Consumption of a high fat diet initially increases plasma apoA-IV levels in rodents, however chronic consumption of a high fat diet results in an attenuation of this effect on apoA-IV (41, 59, 94). This suggests that the elevation of circulating leptin induced by high fat feeding might attenuate intestinal apoA-IV response to the consumption of a lipid meal. Animal studies also support this notion. Intestinal apoA-IV levels are markedly increased in ob/ob mice, which lack the ability to make leptin. Further studies are required to determine the molecular mechanism in the regulation
of intestinal apoA-IV by leptin, but it is possible that leptin decreases intestinal apoA-IV gene expression to transport fewer lipids from the small intestine to the circulation.

Central administration of leptin decreases food intake and increases energy expenditure. Shen et al. demonstrated that the hypothalamic apoA-IV mRNA levels is significantly lower in leptin-deficient obese (ob/ob) mice than wild type controls (82). Intragastric infusion of a lipid emulsion significantly stimulated hypothalamic apoA-IV gene expression in lean controls but not in ob/ob mice. When leptin was ip administered daily for 5 d, it significantly stimulated apoA-IV mRNA levels in the hypothalamus of ob/ob mice, compared to the pair-fed controls. Additionally, the fasting-induced reduction of apoA-IV mRNA levels was also restored by centrally administered leptin. Shen et al. also demonstrated that apoA-IV is present in leptin-sensitive phosphorylated signal transducer and activator of transcription-3 (pSTAT3)-positive cells of the ARC as determined by immunohistochemistry (82). The stimulatory effect of leptin on apoA-IV protein expression was significantly attenuated by the suppression of STAT3 expression by small interfering RNA (siRNA) in cultured primary hypothalamic neurons. These observations imply that leptin can regulate apoA-IV gene and protein expression in the hypothalamus, and such effects are at least partially via the STAT3 signaling pathway. More importantly, when subthreshold doses of leptin (1 µg) and apoA-IV (0.5 µg) were ICV co-administered, it leads to a significant reduction of food intake in rats, indicating the existence of a functional synergistic interaction between leptin and apoA-IV, leading to suppression of food intake. The differential effect of leptin on apoA-IV expression in the hypothalamus and the enterocytes is intriguing and deserve to be further investigated.

Cholecystokinin (CCK). The secretion of CCK by the intestinal L cells is stimulated by the consumption of lipid and protein. The fat-induced stimulation is associated with the formation and secretion of chylomicrons (53, 74, 75). CCK stimulates gallbladder contraction and
pancreatic enzyme secretion, modulates intestinal motility, and inhibits food intake (13, 25, 104). Exogenous administration of CCK reduces short-term food intake in experimental rats by reducing meal size (25, 67) and this satiating effect is abolished by deactivation of vagal afferents with capsaicin, vagotomy, or CCK-1R antagonist. Thus, the satiation signals are relayed via CCK-1R on vagal afferent nerves (64, 74, 75, 77, 79). Secretion of CCK and apoA-IV are both induced by fat absorption and this lipid-induced stimulation is dependent on chylomicron formation (30, 37, 74). In addition, both act peripherally (through either intraperitoneal or intravenous administration) as well as centrally to reduce food intake (22, 25, 63, 93).

We conducted a series of experiments to determine if there is interaction between CCK and apoA-IV on the control of food intake and if this is mediated via CCK-1R on vagal afferent fibers. Using sub-threshold doses of either apoA-IV or CCK a combination of both produces a short-term satiation for 1 h and this satiation effect is attenuated by CCK-1R antagonist, lorglumide (61). Increasing the doses of CCK and apoA-IV are increased, the satiating effect is prolonged. Furthermore, we reveal that apoA-IV-elicited satiation is greatly attenuated in CCK-KO mice (60). In contrast, CCK-induced satiating response is greater in apoA-IV KO mice due to increased CCK-1R expression in the nodose ganglia and/or nucleus of the solitary tract (NTS)(102). These findings suggest that endogenous apoA-IV and CCK interact with each other to reduce food intake via CCK-1R and systemic apoA-IV requires an intact CCK system in order for it to work physiologically. This working model was first proposed by Raybould and colleagues that the stimulation of apoA-IV release induced by lipid absorption results in the stimulation of CCK secretion, followed by activation of the vagal nerves via a CCK-1R-dependent pathway.

Circulating apoA-IV does not cross the blood-brain barrier (81), but is able to increase CCK-elicited activity in vagal afferent fibers, which discharge via a CCK-1R-dependent pathway in vitro (26). Blockade of CCK-1R using lorglumide significantly reduces apoA-IV-induced satiation,
suggesting that CCK-1R on vagal afferent nerves plays an important role in relaying apoA-IV-induced anorectic signals to the brain (60). Subdiaphragmatic vagal deafferentation (SDA) is a surgical procedure which eliminates all neuronal signals mediated via vagal afferent fibers from the upper gut, including the liver, while leaving half of the vagal efferent fibers intact (4, 68). We have demonstrated that Long-Evan rats with SDA have attenuated apoA-IV-elicited satiation effect (60). These findings support the notion that systemic apoA-IV and CCK work co-dependently to suppress food intake and peripheral apoA-IV requires an intact CCK system and CCK-1R on vagal afferent nerves to exert its satiety effects in the brain.

**Regulation of apoA-IV synthesis and secretion**

ApoA-IV synthesis and secretion is stimulated by active lipid absorption and chylomicron formation in the small intestine (36, 37). Interestingly, neither protein nor carbohydrate absorption by the small intestine effects apoA-IV secretion. The stimulation of apoA-IV secretion occurs rapidly (within half an hour following the beginning of active lipid absorption) (21). ApoA-IV synthesis and secretion relies on chylomicron formation. This effect is abolished when chylomicron formation is inhibited (37), and in studies in which short chain fatty acids are infused directly into the intestine (which are directly secreted into the portal vein), there is no stimulation of apoA-IV synthesis and secretion (42).

Fasting and refeeding modulates both circulating and central apoA-IV levels (54). Feeding overnight (the normal feeding periods for rodents) significantly decreases apoA-IV gene expression in both the jejunum and hypothalamus. Refeeding with low-fat chow in fasting rats for 4 h evokes a pronounced increase of apoA-IV gene expression in jejunum, but not in the hypothalamus. However, refeeding with lipid restores apoA-IV mRNA levels in both jejunum and hypothalamus in fasted animals.
Effects of obesity and chronic high fat diet on apoA-IV

Plasma apoA-IV is known to rise after a fatty meal. However, in obese humans, fasting plasma apoA-IV levels are higher than in normal weight individuals (18, 19, 91). This increase in fasting plasma apoA-IV also correlates with higher plasma triglyceride levels both at fasting and in response to a lipid meal (18, 19, 91). This increase in plasma apoA-IV levels may be due to a loss of regulation of apoA-IV in the intestine during obesity. Hints at a mechanism come from studies in rodents, where apoA-IV is *acutely* down-regulated in response to a high fat meal (103), but is not down-regulated in the face of a *chronic* high fat diet (3, 41, 49, 55, 59, 94). This loss of responsiveness may be an important factor in the effectiveness of apoA-IV in regulating food intake and insulin secretion, both of which are disturbed in obese individuals.

ApoA-IV gene expression in the hypothalamus is affected by the obesity induced by high fat diet (HFD). In Long-Evans rats fed a HFD (20%), low fat diet (LFD) (4%), or standard chow (CHOW) for 2, 4, 6, 8, or 10 weeks, Liu, et al. found that the body weights in HFD-fed rats are significantly greater than that in rats fed LF and CHOW diets (59). LF and CHOW rats had comparable hypothalamic apoA-IV mRNA, which is consistent with the observations that intestinal and plasma apoA-IV protein levels were comparable across dietary groups and time. However, a slow and progressive diminution in hypothalamic apoA-IV mRNA over time was observed in HFD fed rats, and the apoA-IV mRNA levels became significantly lower than that of LFD or CHOW rats by 10 weeks. Additionally, intragastric infusion of lipid emulsion to overnight-fasted animals significantly increased hypothalamic apoA-IV gene expression only in LF and CHOW rats, but not in HFD-fed rats. These results suggest that chronic consumption of a HFD significantly attenuates hypothalamic apoA-IV gene expression, as well the response of apoA-IV gene expression to dietary lipids (59).
These observations raise the possibility that impaired apoA-IV gene expression in the hypothalamus could contribute to HFD-induced obesity.

The mechanism by which intestinal apoA-IV becomes desensitized to dietary fat, and whether this process is reversible, are important frontiers in the biology of apoA-IV since this insensitivity may be a contributing factor to problems with plasma lipid levels and glucose homeostasis during obesity.

Conclusion and future directions

Increasing evidence suggests that apoA-IV plays important roles in the integrated control of metabolic diseases including obesity, cardiovascular disease, and diabetes. The rapid rise of circulating apoA-IV after fat ingestion is also consistent with its involvement in the short-term regulation of satiety and glucose homeostasis. Interestingly, both rodent and human studies suggest that intestinal apoA-IV synthesis and secretion were initially increased under high fat feeding, but become less responsive to fat after chronic consumption (3, 41, 49, 55, 59, 94). This stimulation in the face of a fatty meal may be integral to the ability of apoA-IV to regulate food intake and bodyweight. Therefore, strategies (such as bariatric surgery) that can restore or increase the normal response of apoA-IV to lipid feeding may have a beneficial effect on weight control.

Roux-en-Y gastric bypass (RYGB) results in significant and lasting weight loss in morbidly obese patients. In addition to this weight loss, greater than 80% of obese patients with RYGB exhibit an accompanying amelioration of type 2 diabetes (87). The mechanism for this effect is still unknown. Growing evidence suggest that bypass of the foregut alters the hormonal output of the intestine which regulate appetite, satiety, glucose homeostasis, and energy metabolism (7).

An intriguing study in obese patients first demonstrated a potential role for apoA-IV in ameliorating diabetes after gastric bypass. Initially, plasma apoA-IV decreases markedly in obese
subjects in a short time after gastric surgery or undergoing weight loss, but plasma apoA-IV levels rise significantly 17 months after RYGB (11). In agreement with other studies, body weight, diabetes, obesity-related co-morbidities and medication used were decreased in these obese patients following RYGB. It is noted that mortality is decreased by 40% in obese subjects after RYGB, especially cardiovascular-related deaths (2).

As mentioned earlier in this review, apoA-IV also possesses anti-inflammatory and anti-atherogenic properties. ApoA-IV has been reported to reduce the host susceptibility to atherogenesis by decreasing the secretion of pro-inflammatory cytokines and diminishing atherosclerotic lesions in mice(76). It is tempting to speculate that an increase of circulating apoA-IV may contribute to weight loss as well as the improvements in inflammation and cardiovascular disease after RYGB.

This idea is supported by another recent human study, which investigated the effect of RYGB on body weight and cardiovascular risk factors after one year (73). HDL-cholesterol and apoA-IV protein were found significantly increased after RYGB. More importantly, there is a strict linear relationship between weight loss and apoA-IV levels. Although this is not a cause-effect study, the significant association between changes in weight and apoA-IV levels suggests a possible effect of apoA-IV on food intake reduction and weight loss in humans. ApoA-IV KO mice could provide a powerful tool to further demonstrate the importance of apoA-IV in mediating the effect of RYGB, including determining whether the improvement of obesity and diabetes is blunted by a deficiency of apoA-IV.

Evidence in the literature adds apoA-IV to the list of gastrointestinal hormones whose plasma concentrations rise after RYGB, including peptide tyrosine-tyrosine (PYY) and GLP-1(65). Notably, changes in GI hormones following RYGB typically occur within days to weeks (65). Further studies are needed to examine the time course of changes in apoA-IV levels after surgery
and to see if apoA-IV participated in the early amelioration in metabolic abnormalities of obese patients. Whether the response of apoA-IV to fasting and feeding (which is lost after a chronic high-fat diet) is recovered after RYGB is another question that warrants further investigation.

How apoA-IV is regulated by RYGB is unclear. ApoA-IV is most highly expressed in the duodenum and the first part of the jejunum, the parts of the GI tract bypassed by the RYGB procedure (83). It is possible that the fast gastric empting with passage of nutrients directly into the jejunum might stimulate apoA-IV secretion in the lower gut. However, intestinal secretion of apoA-IV is also stimulated by an ileal factor, probably peptide tyrosine-tyrosine (PYY), which is increased following RYGB (7). Intravenous infusion of physiological doses of PYY elicits significant increases in both apoA-IV synthesis and lymphatic transport through translation in rats which probably acts centrally via the vagus nerve to send a signal to the gut to stimulate apoA-IV synthesis (39).

Current data are unable to ascertain whether the observed increases in apoA-IV after bypass surgery arise from the action of PYY. This interesting hypothesis deserves further study.

**Conclusion**

ApoA-IV plays a variety of important physiological roles and seems to link dietary lipid absorption with cardiovascular disease risk, diabetes, and obesity. This underscores the potential for apoA-IV to serve as a therapeutic target for the treatment of these diseases, and emphasizes the importance of exploring the precise molecular details of apoA-IV.

**GRANTS**

We are extremely grateful to the National Institutes of Health (NIH) for the support of this study. This work was supported by NIH Grants DK-076928 and DK-059630 (University of Cincinnati
MMPC) to Dr. Tso, F32-091173 and DK101663 to Dr. Kohan, DK92779 to Dr. Liu, and DK83550 and DK97436 to Dr. Lo.

DISCLOSURES

The authors have no disclosures or conflicts of interest to report.


Figure 1. Structure of the intestinal villus. The surface of the small intestine is comprised of fingerlike projections, called villi and crypts. A continuous sheet of epithelial cells, which are the primary absorptive cells of the intestine, covers the villi and crypts. Below the absorptive epithelium is the intestinal lamina propria and a lymphatic capillary (lacteal), which is surrounded by a blood capillary network. Dietary triglyceride and lipophilic compounds are transported from the absorptive enterocyte into the lacteal and eventually into the mesenteric lymph duct (© 2010 Marcia Hartsock, MA, CMI)(101).

Figure 2. Lymph Fistula Mouse Model. After opening the abdominal cavity of the rat, the stomach, small intestine, and colon are retracted and gently pushed under the abdominal muscle walls. The surgeon’s fingers can be used to keep the liver out of the surgical plane. The inferior vena cava and left renal vein will now be exposed and the intestinal lymphatic duct can be seen as a clear vessel running parallel to and lying directly above the superior mesenteric artery. The inset shows the site of the cannula placement. The incision should be made proximal to the vena cava, allowing insertion of the cannula and advancement approximately 3-5 mm. Once secured by a drop of cyanoacrylate glue, the cannula is positioned beneath the liver and exteriorized through the right flank of the animal (© 2010 Marcia Hartsock, MA, CMI)(46, 101).

Figure 3. ApoA-IV KO Chylomicrons Exhibit Delayed Clearance Rates From Plasma. Radiolabeled chylomicrons (containing $^3$H-triglyceride) were freshly isolated from either A-IV KO or WT donor mice and then given IV to either (A) A-IV KO or (B) WT mice (n=6). Blood was collected 0 to 20
min post-injection. Values are means ± SE. AUC analysis was performed. *$P < 0.05$ versus mice receiving WT donor chylomicrons (46).

**Figure 4. ApoA-IV KO Mice Secrete Larger Chylomicrons.** (A) Size distribution of lymph lipoproteins in A-IV KO and WT mice (n=6). Lymph samples were stained with 2% phosphotungstic acid prior to transmission electron microscopy, and 800 particles were counted from each group (46).
Figure 2

- Lymphatic duct
- Portal v.
- Superior mesenteric a.
- Left renal v.

Inset:
- Cannula
- Lymphatic duct
- Vena cava
- Liver
- Artery
Figure 3

A. Chylomicron clearance in A-IV KO mice

B. Chylomicron clearance in WT mice
Figure 4

A.

% Total Lymph Lipoproteins

Particle Size (nm)

- WT
- AIV KO