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Using the lymphatics to study nutrient absorption and the secretion of gastrointestinal hormones

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Abstract

The lymph fistula rat model has traditionally been used to study the intestinal absorption of nutrients, especially lipids, but recently this model has also been used for studying the secretion of incretin hormones by the small intestine. The small intestine is not only responsible for the digestion and transport of dietary triacylglycerol, through the formation of chylomicrons, but it also secretes the incretin hormones glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) from enteroendocrine cells. Ultimately, both chylomicrons and incretins are found in lymph. Advantages of the lymph fistula rat model in studying chylomicron and incretin secretion are numerous and include: 1) the concentrations of incretin hormones are higher in lymph than in peripheral or portal plasma; 2) there is reduced degradation of incretin hormones by DPP-IV in the lymph compartment; 3) less dilution by the circulating fluid; 4) this model allows the continuous collection of lymph from conscious animals, eliminating any potential side effects on lymph flow and gastrointestinal function due to anesthesia; and finally, and perhaps most importantly, 5) the concentration in the intestinal lymph provides a physiologically accurate representation of the hormonal milieu within the intestinal mucosa where incretins may interact with enteroendocrine and/or dendritic cells and signal through the enteric or autonomic neurons. The importance of GIP and GLP-1 in health and disease is becoming more apparent, especially as the prevalence of type 2 diabetes and other metabolic disorders increases. This review focuses on the use of the lymph fistula rat as a model to study the secretion of incretins, as well as dietary lipid.

Keywords

lymph; lymph fistula rat model; incretin; chylomicron; glucose-dependent insulintropic polypeptide (GIP); glucagon-like peptide-1 (GLP-1)

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1. Introduction

The gastrointestinal tract is the largest endocrine organ in the body. Since the original discovery of 'secretin' by Bayliss and Starling, many other gut hormones have been identified and are now considered to be part of the gastro-entero-pancreatic system [1]. Much attention has been focused on the importance of these gut hormones in mediating obesity, type 2 diabetes, and other metabolic disorders, with specific emphasis on the role of the two incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The incretin hormones are involved in a number of physiologic processes, many centering on the maintenance of whole-body glucose homeostasis. The precise physiological regulators of incretin secretion are only now becoming apparent, and there has been specific interest in understanding the roles of dietary lipids and carbohydrates in stimulating incretin secretion in response to a meal. The past several decades have witnessed increasing interest in the field of incretin biology, and their importance in type 2 diabetes is becoming increasingly apparent.

Rodent models have been indispensable in the study of the incretin hormones. However, low circulating levels of GIP and GLP-1, coupled with the low sensitivity of available immunoassays, have significantly hampered major progress in the incretin field. Recently, the use of the lymph fistula rat model has not only overcome these limitations, but has also allowed for the continuous monitoring of incretin secretion from the gut in response to a meal. In this review, we will explore the physiological regulators of incretin action, as well as the lymph fistula rat as a model to study incretin secretion.

2. The Gastrointestinal System

The gastrointestinal system consists of the gastrointestinal tract and the gastrointestinal glands. The gastrointestinal tract is essentially a tube that extends from the oral cavity, through the esophagus, stomach, small and large intestine, rectum, and the anus. The small intestinal segment of the gastrointestinal tract has many functions, including its ability to digest food, absorb nutrients, and secrete hormones. The small intestine is further divided into three parts: the duodenum, jejunum, and ileum. The small intestine is lined with permanent spiral or circular folds, termed the plicae circulares, which serve to increase surface area, thus promoting nutrient absorption. The mucosal surface area is further increased by fingerlike projections and depressions, known as the villi and crypts, respectively. A continuous sheet of absorptive epithelial cells, called enterocytes, covers the villi and crypts. Enteroendocrine cells, specialized cells within the gastrointestinal system that produce and secrete hormones, can also be found on the intestinal villus, along with other cell types [2].

Enterocytes lining the villi are responsible for nutrient absorption. After absorption by the enterocytes, nutrients enter the circulation via the portal blood (in the case of carbohydrates and proteins) or are secreted into lymph as chylomicron particles (in the case of lipids). The structure of the villi facilitates this divergent nutrient transport. Directly below the epithelial lining of the villi lies the intestinal lamina propria. The lamina propria is composed of loose connective tissue with nerve fibers and immune cells. Additionally, located within each villus is a specialized lymphatic capillary, called a lacteal, which is surrounded by a blood capillary network [3].

3. The Lymphatic System

Lymph is formed when fluid and proteins from the interstitial space that are not reabsorbed by blood capillaries enter the lymphatic capillaries [4]. Beyond draining excess fluid and proteins, the lymphatics of the gastrointestinal tract are also responsible for the transport of

lipids (as chylomicrons) and lipophilic compounds into the circulation. After being absorbed by the lacteals in the intestinal villi, these particles are first transported via the intestinal (mesenteric) lymphatic duct and then through the thoracic duct before entering the circulation through the subclavian vein [5].

4. Dietary Lipid Absorption by Enterocytes and Transport in the Lymphatics

Dietary fat is predominantly in the form of triacylglycerol (TAG), comprised of various fatty acids esterified to a glycerol backbone. Less than 10% of dietary fat consists of free fatty acids or other lipids [6, 7]. TAG molecules are water insoluble and must undergo digestion to be absorbed by the enterocytes. Long-chain fatty acids resulting from this digestion are secreted into the circulation as TAGs via the intestinal lymphatics, whereas medium-chain fatty acids resulting from digestion of TAG molecules are absorbed via the hepatic portal vein.

The enzymatic digestion of dietary fat begins in the lumen of the stomach, where both lingual and gastric acid lipases work to hydrolyze dietary TAG to form mostly diacylglycerol (DAG) and free fatty acid (FA). In the intestinal lumen, DAG is further hydrolyzed by pancreatic lipase to form 2-monoacylglycerol (2-MAG) and another FA [8–11]. In neonates, this pancreatic lipase system is immature and gastric acid lipase is particularly important for lipid digestion [12, 13]. Once TAG is completely hydrolyzed into FA and 2-MAG within the intestinal lumen, these components are next emulsified into mixed micelles by bile salts [14–16]. Mixed micelles then converge at the enterocyte surface, where the FA and 2-MAG molecules are absorbed by passive diffusion. Once inside the enterocyte, the 2-MAG and FA migrate to the endoplasmic reticulum (ER), where they are re-esterified to form TAG [17, 18]. It is clear that fatty acid binding proteins are involved in this intracellular transport, but the precise mechanism is still unclear [19–21]. Once re-esterified, the TAG molecule is then incorporated into a lipid droplet coated with cholesterol, phospholipid, and apolipoproteins. The particle is further processed in the Golgi apparatus to form a mature chylomicron particle, characterized by TAG and cholesterol in its core and phospholipids and apolipoprotein B on its surface (Figure 1). Mature chylomicrons are released by the enterocyte into the intestinal lymph through exocytosis.

4.1 Chylomicron secretion from enterocytes to the initial lymphatics (lacteals)

The processes of FA uptake and chylomicron formation and transport are extremely rapid, occurring in as little as 13.6 minutes from apical surface to the lacteal [22]. The journey of chylomicrons from the intercellular space to the lamina propria, however, is hampered by the basement membrane [22, 23]. During active fat absorption, a large number of mature chylomicrons can be found within the intercellular space between enterocytes (Figure 2). Therefore, the intercellular space becomes greatly distended which likely loosens the tight junctions between the enterocytes. These intestinal tight junctions are dynamic structures that are closely controlled by specific tight junction proteins [24]. Interestingly, it has been reported that the integrity of intestinal tight junctions are compromised by the chronic consumption of a high fat diet [25]. How the mature chylomicrons cross the basement membrane in order to enter the lacteals is still unclear. Electron microscopic images suggest that this may involve the breakage of the basement membrane (Figure 3). This hypothesis is further supported by the finding that the cells at the tip of the jejunal villi become disrupted during active fat absorption [26]. Presumably, the villi are then repaired post-absorption. The factors that mediate these breakage and repair events are currently not well understood, but it is tempting to speculate that inflammatory factors, as well as trophic cytokines and gut hormones, may be involved in this repair process.

4.2 Secretion of incretins by the endocrine cells of the gastrointestinal tract

Incretins, GIP and GLP-1, are gastrointestinal hormones secreted by the endocrine cells of the small intestine to promote glucose-stimulated insulin secretion by the endocrine pancreas [27, 28]. GIP is synthesized and secreted from the enteroendocrine K cells, located in the duodenum and the proximal jejunum, primarily in response to the absorption of dietary fat and carbohydrate [27]. GIP has many anabolic functions throughout the body, including roles in 1) enhancing glucose-dependent insulin secretion; 2) up-regulating insulin gene transcription and biosynthesis in the pancreas; 3) stimulating β -cell proliferation and reducing apoptosis; and 4) promoting lipogenesis in adipose tissue [29–31]. Diverse tissues, including liver, muscle, and adipose, express GIP receptors; interestingly, mice lacking the GIP receptor are resistant to diet-induced obesity [32]. Therefore, GIP receptor antagonists and inhibitors have become attractive targets for potential anti-obesity therapies [33, 34].

GLP-1 is primarily secreted from the enteroendocrine L cells, located mainly in the distal jejunum, ileum, and proximal colon. Through tissue-specific proteolytic cleavage of the proglucagon gene, GLP-1 is produced and is secreted in response to nutrient, neural, and endocrine factors [35]. Similar to GIP, GLP-1 functions in a trophic manner on the pancreas to enhance glucose-dependent insulin secretion, β -cell proliferation, and to inhibit β -cell apoptosis. GLP-1 is also involved in the regulation of blood glucose levels, both by decreasing gastric emptying via the ileal brake reflex (thereby reducing the delivery of absorbed glucose to the circulation following a meal) [36–39], but also by reducing the total glucose load by reducing food intake [40–43].

Once secreted into the circulation, the incretin hormones are rapidly degraded by the ubiquitously expressed enzyme dipeptidyl peptidase-IV (DPP-IV) [44]. DPP-IV can be found in numerous tissues and cell types, such as on the surface of endothelial cells. Unfortunately for GIP and GLP-1, an abundance of DPP-IV is found on the cells lining the blood vessels of the intestinal mucosa. Because of this, the incretin hormones have a short half-life, lasting only 2–3 minutes [35, 44–46].

Given their role in glucose homeostasis, it is not surprising that impairments in incretin secretion and action have been reported in patients with type 2 diabetes. Several studies have reported decreased postprandial GLP-1 secretion [47–49]. In contrast, GIP secretion from enteroendocrine cells in type 2 diabetic patients is normal or slightly elevated. These patients do, however, present with impaired insulinotropic effects of GIP at the pancreatic β -cells [47, 50, 51]. The attenuated GLP-1 secretion and compromised GIP function contribute to the pathology of type 2 diabetes. Because the glucoregulatory properties of GLP-1 are still functional in insulin resistant individuals, unlike that of GIP, therapeutic strategies have focused on the development of GLP-1 receptor agonists. Exenatide, the only approved GLP-1 receptor agonist, is a synthetic form of exendin-4, a 39-amino acid peptide originally isolated from the venom of the *Heloderma suspectum* lizard (Gila monster) [52]. Exendin-4 is a potent agonist of the GLP-1 receptor and is not susceptible to DPP-IV degradation.

5. The Lymph Fistula Model

As described above, both lipids (and lipophilic compounds) and hormones are secreted into the intestinal lymph from the gastrointestinal system. Therefore, direct sampling of the lymph through lymph cannulation has been remarkably important in studying the physiology of intestinal nutrient absorption. Two general lymph cannulation techniques have been described in the literature: either cannulation of the intestinal lymphatic duct or cannulation of the thoracic duct. Intestinal lymphatic cannulation allows for the collection of lymph from the stomach, intestine, pancreas, spleen, and portions of the liver, while cannulation of the thoracic duct allows for the collection of lymph not only from the intestinal lymphatic duct,

but also from the remainder of the body (excluding lymph drained from the upper right quadrant). While numerous lymph fistula animal models have been developed, including several large animal models used to study lymphatic transport of lipids and lipophilic compounds, the most widely used model is the lymph fistula rat. We will therefore focus on the lymph fistula rat model for the remainder of this article.

5.1 The Lymph Fistula Rat Model

Bollman and colleagues (1948) provided one of the first reports of thoracic and intestinal lymphatic duct cannulation in the anesthetized rat [53]. Porter and Charman described an updated version of this model in 1996, which differs from the Bollman protocol in the triple cannulation of the intestinal lymphatic duct, jugular vein, and duodenum, but retains the anesthetization of the rat post-surgery [54]. Prior to the operation and every 2 hours thereafter, rats are kept anesthetized with sodium pentobarbitone. Unlike the Bollman procedure, the animals remain anesthetized during the entirety of the study, with lymph continuously collected from the intestinal lymphatic duct cannula, plasma sampled via the jugular vein cannula, and nutrients and saline (for rehydration) are provided through a duodenal cannula. While this model eliminates problems associated with the animals' movement during lymph collection, a major drawback is that the lymph flow rate is greatly reduced by the use of continuous anesthesia. In conscious animals, the fasting lymph flow rate is approximately 2–3 ml/h; however, in anesthetized rats the fasting lymph flow rate is only 0.1–0.6 ml/h [55–57].

Due to the negative effects of anesthesia on lymph flow rate, the use of the conscious lymph fistula model in rats has become the preferred model in which to study the transport of lipids and lipophilic compounds. Tso and Simmonds (1984) provide a detailed description of the lymph fistula procedure and collection in unanesthetized animals [58]. Similar to the procedure described by Bollman and colleagues (1948), once the lymph duct has been cannulated, the cannula is ligated into place at two locations. Unique to this model, however, is that the cannula is further secured using a drop of methyl cyanoacrylate glue. Following surgery, animals are allowed to recover from anesthesia and are then placed in restraint cages. Rehydration generally occurs via a duodenal cannula, although a jugular vein cannula can also be used. This modified Bollman procedure described by Tso and Simmonds is economical and has a high success rate (approximately 80 – 90%, when performed by a trained surgical technician). This model has been extensively used in lipid absorption and transport studies [22, 59–64].

An additional advantage of the method of Tso and Simmonds is that a second cannula can be placed in the hepatic portal after the lymph cannula has been placed. This offers a second site in which to sample nutrients, hormones, and lipids that are absorbed via the portal vein. Real-time comparisons between the appearance of these molecules in the portal circulation versus the lymphatics are at the cutting edge of research into the intestinal absorption and secretion of lipids, incretin hormones, and lipophilic toxins [65].

6. Using the lymph fistula rat model to study apolipoproteins during active fat absorption

The conscious lymph fistula rat model has been indispensable in studying the secretion of apolipoproteins associated with chylomicrons by the small intestine. The apolipoproteins include apo A-I, apo A-IV, and apo B. Using this model, we have shown that there is only a slight increase in apo A-I during active TAG absorption, whereas there is a marked increase in the synthesis and secretion of apo A-IV by the small intestine during active fat absorption [66]. We have also studied the effect of blocking chylomicron formation within the

enterocyte (using Pluronic L-81) on the secretion of TAG, apo A-I, and apo A-IV [67–69]. Pluronic L-81 treatment inhibited both the rise in lymphatic TAG and apo A-IV output in experimental rats, and upon the cessation of the Pluronic L-81 infusion, the accumulated lipid was rapidly cleared into lymph as CM. This was associated with a marked increase in apo A-IV output [68]. Thus, we concluded that the increase in apo A-IV synthesis and secretion is dependent upon the formation and secretion of CM. In contrast, the secretion of apo A-I does not rely on the formation and secretion of CM.

CM produced by the human small intestine contains apo B48, whereas the liver expresses the apo B100 isoform for the production of very-low-density lipoproteins (VLDL). Using the lymph fistula rat model in apobec-1 KO mice, which lack the editing enzyme apobec-1 and are thus only able to produce apo B100[70], we found that the apobec-1 KO mice transported TAG as efficiently as wild-type mice when infused with the lower lipid dose; however, the apobec-1 KO mice transported significantly less TAG to lymph than WT mice when given the high lipid dose [60, 70]. This led to the accumulation of mucosal TAG. When we examined the size and number of CM particles secreted by the apobec-1 KO and WT intestine into lymph, there was not a significant difference in the size of the CM particles produced, but there were significantly fewer CM particles secreted by the apobec-1 KO intestine [60]. This was not due to a decrease in TAG metabolism but instead due to a lack of apo B48. This study was the first to suggest that apo B48 is the preferred protein of the intestine to coat the surface of intestinal chylomicrons, thus ensuring efficient CM formation and lipid absorption. Without the availability of the lymph fistula mouse model, it would not be possible to address this interesting question.

7. Using the lymph fistula rat model to study incretin secretion

Traditionally, in vivo study of incretin hormones involves the measurement of circulating levels in peripheral blood. This requires a tremendous volume of fluid, as concentrations of incretin hormones are quite low in plasma due to their rapid degradation by DPP-IV. In addition, investigators have also been constrained by low-sensitivity immunoassays for the detection of incretin hormones in plasma. Investigators studying GIP and GLP-1 secretion in rodent models are particularly limited in the number and size of blood samples that can be taken from a single animal during the course of a study. Because of these limitations, continuous monitoring of GIP and GLP-1 secretion from the intestine in rodents has been difficult, despite the intense interest in the outcome of these kinds of studies. Fortunately, the conscious lymph fistula rat model has recently provided an excellent system for studying the secretion of incretin hormones from the enteroendocrine cells.

We published the first study showing that intestinal lymph contains measurable quantities of insulin, glucose, GLP-1, and PYY and that the lymph profile of these factors mirrors plasma levels during dietary nutrient absorption [71]. There are, however, major differences in the relative concentrations of insulin, PYY, and GLP-1 between the lymph and plasma compartments. Our data suggests that insulin, secreted primarily into the bloodstream from the pancreas, enters the lymph compartment by filtration from the capillaries, moving down its concentration gradient from the bloodstream into the lymph fluid compartment [71].

Conversely, we have observed nearly 10-fold higher GLP-1 concentrations in lymph versus portal plasma, when we compare lymphatic GLP-1 concentration with the portal plasma GLP-1 concentration following the ingestion of an Ensure® meal (as shown in Figure 4). The higher lymph-to-plasma ratio of GLP-1 suggests that GLP-1 is concentrated within the lymph compartment. This is in part due to the lower concentrations of DPP-IV, within lymph versus plasma (Figure 5) [71]. Therefore, lymph may represent a protective environment for GLP-1 in which bioactive GLP-1-(7–36) amide is protected from rapid

degradation by DPP-IV to the inactive peptide GLP-1-(9–36) amide [44, 72]. In support of this hypothesis, we have found that the amounts of active versus total GLP-1 are not significantly different in lymph.

Although insulin in the lymph likely originates from the plasma compartment, it is likely that GLP-1 is secreted directly into the lymph from intestinal L cells [71, 73, 74]. This is supported by the finding that GLP-1 concentrations in lymph are significantly greater than in plasma, with a 5- to 10-fold higher concentration postprandially in the intestinal lymph versus portal blood [71, 75]. This finding is consistent with the idea that GLP-1 is directly released into the intestinal lymphatics, rather than the filtration of GLP-1 from the plasma into lymph. Immunocytochemical studies show that most gastrointestinal endocrine cells (including the GLP-1 secreting L cells) are polar. These cells are organized such that their apical end contact the intestinal lumen, while their basal ends lie near the submucosal capillaries and lacteals [76, 77]. This intestinal morphology suggests that the first compartment exposed the basal side of the mucosal cells is the submucosal capillaries of the intestinal villus, followed by the initial lacteals [73].

Therefore, the lymph fistula rat model not only yields more physiologically accurate data regarding incretin hormone secretion, but also does so with greater experimental ease. In addition to these advantages, the lymphatic concentration of incretin hormones most likely reflects concentrations of GIP and GLP-1 within the lamina propria. Through these studies, we have established that the lymph fistula rat model is well suited for the study of incretin secretion by the gastrointestinal tract.

8. Incretin secretion is stimulated by macronutrients

Since the concentrations of both GIP and GLP-1 are significantly higher in the intestinal lymph compared to the portal or peripheral plasma, the lymph fistula rat model provides an ideal system to study the dynamic secretion of the incretin hormones in response to various stimuli, such as nutrients.

Using the lymph fistula rat model, we confirmed that indeed a mixed meals, as well as individual meals of carbohydrate or lipid, are potent stimulators of GIP and GLP-1 secretion. From our initial studies, using only one caloric dose (4.4 kcal) of lipid (Intralipid) or carbohydrate (dextrin), it appears that both macronutrients are equally potent GLP-1 secretagogues, whereas carbohydrate is more effective than lipid at stimulating GIP secretion [75, 78]. More detailed, subsequent reports confirmed these findings.

In our follow-up studies, we evaluated the effects of increasing caloric doses of the three macronutrients (carbohydrate, lipid, and protein) on GIP and GLP-1 secretion using the lymph fistula rat model. Both the GIP-secreting K cells and the GLP-1-secreting L cells respond dose dependently to increasing caloric amounts of lipid and carbohydrate; however, the sensitivities of the hormones to each macronutrient is different, validating the results from our initial studies. We found that for all doses (ranging from 0.275 to 4.4 kcal) carbohydrate is the more potent GIP secretagogue (compared to lipid); in contrast, both carbohydrate and lipid are equally effective at stimulating GLP-1 secretion [61, 79].

Although further investigations are needed, we propose that the similar response of GLP-1 to both lipid and carbohydrate support that hormone's role in the ileal brake reflex, whereas the much larger effect of carbohydrate on GIP secretion reflects the insulinotropic potential of the infused nutrient. When consuming a predominantly carbohydrate-based meal, regulating glucose homeostasis is necessary; thus, the enhancement of glucose-stimulated insulin secretion via GIP signaling is advantageous. On the other hand, the GLP-1-secreting L cells are more responsive to the amount of nutrient present rather than the type of nutrient

(carbohydrate versus lipid). Calorie for calorie, regardless of nutrient, the same output of GLP-1 is secreted. We speculate that both carbohydrate and lipid are contributing equally to the ileal brake reflex via a GLP-1-based mechanism. As nutrients reach the distal portion of the gut, GLP-1 is secreted in a dose-dependent manner to reduce intestinal transit and enhance proximal nutrient absorption. Unlike carbohydrate and lipid, protein does not appear to be a potent incretin secretagogue [61, 79]. Although the majority of the GLP-1-secreting L cells are located in the distal small intestine (distal jejunum and ileum) and proximal colon, a few reports have identified small subsets of GLP-1-secreting cells in the duodenum [80–82]. Regarding our proposed ileal brake model, the function of these proximally-located L cells may be two-fold: 1) The GLP-1 released from these cells may still be participating in the ileal brake response; however, as it would be disadvantageous to have a robust braking signal from the proximal small intestine, these cells are thus sparsely distributed throughout the duodenum. 2) On the other hand, the GLP-1 secreted from these cells may be playing a larger insulinotropic role, in conjunction with the proximally-secreted GIP. Our results suggest that the GLP-1-secreting cells are equally responsive to both carbohydrate and protein [61, 79]. If, indeed, the proximally-located L cells are playing a larger insulinotropic role, it would be intriguing to replicate our studies in isolated segments of the small intestine to determine if these L cells are more responsive to carbohydrate, compared to their distally-localized counterparts.

Without the aid of the lymph fistula rat model, the above described studies on incretin secretion in response to different types and amount of macronutrients could not have been accomplished. Of interest, for future studies, is to investigate non-nutrient secretagogues using the lymph fistula model. Beyond direct nutrient stimuli, others have reported on a variety of neural and endocrine factors that stimulate the release of GLP-1 [35]. For example, signaling via the vagus nerve appears to be an important source of GLP-1 release, as bilateral subdiaphragmatic vagotomy abolished fat-induced GLP-1 secretion and stimulation of the celiac branch of the vagus nerve triggered the release of GLP-1 [83]. Although not shown to have an effect on human GLP-1 secretion, in rodents, GIP has been implicated in regulation GLP-1 secretion via the vagus nerve, as well [83, 84]. Additionally, leptin, bile acids, hyperglycemia, and hyperinsulinemia are all documented secretagogues for GLP-1 [85–87]. All of these mediators have been established via traditional blood sampling or with cell culture systems, it would be intriguing to investigate how these factors stimulate the release of GLP-1 in the intestinal lymph.

9. Conclusions and Future Directions

Defects in incretin secretion have been implicated in the pathophysiology of type 2 diabetes and obesity. In addition to finding the specific roles of incretins in mediating these diseases, the precise mechanism by which enterocytes are translating nutrient signals into incretin hormone secretion and the potential factors involved are currently unclear and quite intriguing. The lymph fistula rat model is a valuable model in which to study the regulation and the secretion of GIP and GLP-1 hormones.

Advantages of the lymph fistula rat model are numerous: 1) the concentrations of incretin hormones are higher in lymph than in peripheral or portal plasma; 2) there is reduced degradation of incretin hormones by DPP-IV in the lymph compartment; 3) less dilution by the circulating fluid; 4) this model allows the continuous collection of lymph from conscious animals, eliminating any potential side effects on lymph flow and gastrointestinal function due to anesthesia; finally, and perhaps most importantly, 5) the concentration in the intestinal lymph provides a physiologically accurate representation of the hormonal milieu within the intestinal mucosa where incretins may interact with enteroendocrine and/or dendritic cells and signal through the enteric or autonomic neurons.

Many questions remained unanswered, however. As suggested by D'Alessio and colleagues, are incretin hormones specifically targeted for secretion into intestinal lymph [71]? Does this differential transport of incretins into lymph play a physiological role in their action in the periphery? Do other gastrointestinal hormones, such as gastrin, secretin, and xenin, have elevated concentrations in lymph versus plasma? It has been recently shown that the lymphatic concentration of ghrelin is also higher than in plasma [88]. Regarding the mechanisms of nutrient-induced incretin secretion, what is the role of nutrient absorption in regulating incretin release? Is nutrient uptake sufficient to stimulate secretion or are there secondary paracrine factors that stimulate incretin secretion? How is the innervation of the intestinal mucosa involved in incretin action? The use of the lymph fistula rat model, in combination with other *in vivo* and *in vitro* approaches, will certainly continue to yield valuable data regarding incretin biology, not only for the amelioration of metabolic disease, but also in the development of potential pharmaceutical targets.

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Figure 1. Formation of mature chylomicron particles by the enterocyte

After the ingestion of lipid, dietary triacylglycerol is incorporated into multiple lipid droplets located within vesiculated channels of smooth endoplasmic reticulum (long arrows). The lipid droplet is further processed in the Golgi apparatus to form mature chylomicrons, characterized by triacylglycerol and cholesterol in their core and phospholipids and apolipoprotein B on their surface. Here, a Golgi zone (G) contains many vesicles filled with nascent chylomicrons measuring 600–3500 Å (magnification X 11,420)[89].

Figure 2. Transport of chylomicrons from the enterocyte

During active lipid absorption, a large number of mature chylomicrons can be found within the intercellular space (IS) between enterocytes. Chylomicrons must move from the intracellular space to the lamina propria, crossing the basement membrane (BM). Note the chylomicrons contained within the large intracellular spaces between absorptive cells. The basement membrane lies contiguous with the basal plasma membrane of absorptive cells (magnification X 27,600)[90].

Figure 3. Chylomicrons must cross the basement membrane to enter the lamina propria
This electron micrograph demonstrates a break at the basement membrane (arrows), and chylomicrons can be seen moving from the intercellular space (IS) to lamina propria (LP) (magnification X 27,600)[90].

Figure 4. GLP-1 concentrations are higher in lymph than in plasma

A. Concentrations of glucagon-like peptide 1 (GLP-1) in samples of portal plasma (closed circles) and intestinal lymph (open circles) taken before and after intragastric Ensure® infusion. **B.** Area under the curve for GLP-1 in plasma (filled bar) and lymph (open bar). * $P < 0.05$ vs. lymph [71].

Figure 5. The concentration of DPP-IV is lower in lymph than in plasma

Lymph may represent a protective environment for GLP-1, since the concentration of the GLP-1 degrading enzyme DPP-IV is much lower in lymph than in plasma. DPP-IV activity was determined by incubating plasma or lymph with substrate (1.4 mM Gly-Pro-p-nireoanilide) at 37°C. The enzyme activity is expressed as nanomoles of product formation per milliliter per minute [91].