The regulation of the lymphatic secretion of glucagon-like peptide-1 (GLP-1) by intestinal absorption of fat and carbohydrate

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Lu WJ, Yang Q, Sun W, Woods SC, D’Alessio D, Tso P. The regulation of the lymphatic secretion of glucagon-like peptide-1 (GLP-1) by intestinal absorption of fat and carbohydrate. Am J Physiol Gastrointest Liver Physiol 293: G963–G971, 2007. First published August 30, 2007; doi:10.1152/ajpgi.00146.2007.—Glucagon-like peptide-1 (GLP-1) is an important incretin produced in the L cells of the intestine. It is essential in the regulation of insulin secretion and glucose homeostasis. Systemic GLP-1 concentrations are typically low in rodents, so it can be difficult to assay physiological levels or detect changes in response to nutrients. We have established a method of assaying GLP-1 in response to nutrients using the intestinal lymph fistula model. Intraluminal infusion of Intralipid (4.43 kcal/3 ml) induced a significant increase of lymphatic GLP-1 concentration compared with saline control at the peak of 30 min. (P < 0.001). Isocaloric and isovolumetric treatment with dextrin, a glucose polymer, also caused a significant fourfold increase in peak concentration at 60 min (P = 0.001). These findings indicate that intestinal lymph contains high concentrations of postprandial GLP-1. Second, they reveal that GLP-1 secretion into lymph occurs in response to both enteral carbohydrate and fat, but the response to dextrin occurs later than to Intralipid with peak times at 60 and 30 min, respectively. Third, the combination of Intralipid plus dextrin demonstrated an additive effect in the stimulation of GLP-1 with peak at 30 min. These results indicate that assessment of levels in lymph is a novel and powerful means of studying the secretion of GLP-1 and potentially other gastrointestinal hormones in vivo. Furthermore, the lymph fistula rat model provides insight into the gut hormone concentrations to which the neurons and cells in the lamina propria of the gut are likely exposed.

incretin; gastrointestinal hormone secretion; enteroendocrine cells; intestinal lymph

THE OBSERVATION THAT ORAL glucose results in a greater insulin release than glucose infused intravenously led to the concept of the “incretin effect.” Extensive research during the past three decades has identified two hormones that account for this effect. Glucose-dependent insulinotropic polypeptide (GIP, also known as gastric inhibitory polypeptide) was discovered in the 1970s in extracts of the upper intestine and subsequently found to potentiate glucose-stimulated insulin release. Over a decade later, glucagon-like peptide-1 (GLP-1) was identified with the cloning of the proglucagon gene as an additional cleavage product made in the lower intestine. Incretin stimulation accounts for 30–60% of postprandial insulin release (4), and the incretin effect is abnormal in patients with diabetes (29). Because GLP-1 is intimately involved in the regulation of glucose-dependent insulin secretion and has a number of other effects that also promote glucose tolerance (23), drugs based on GLP-1 receptor signaling have been developed to treat patients with type 2 diabetes (1).

Analyses of the molecular forms of GLP-1 immunoreactivity in rat, human, and porcine intestine revealed that the predominant forms of GLP-1 within intestinal mucosa correspond to GLP-1(7–37) and GLP-1(7–36) amide (27, 32), the biologically active forms of the peptide (36). GLP-1 is secreted by L cells distributed in the distal small intestine and colon in response to carbohydrate- and fat-containing meals, and it stimulates insulin secretion in a glucose-dependent manner (37, 41). GLP-1 is one of the most potent stimulators of insulin secretion (11), with half-maximal effective concentrations affecting β-cells at around 10 pmol/l. Most investigators have found GLP-1 to be equally or more potent than GIP (24, 31, 51), although its circulating level does not rise as high as that of GIP in response to an oral glucose load or test meal.

Besides being an insulin secretagogue, GLP-1 also demonstrates several antidiabetic effects including suppression of glucagon secretion and delay of gastric emptying (23). Furthermore, acute intracerebral injection of GLP-1 or GLP-1 receptor (GLP-1R) agonists reduces food intake (49). Evidence from both human and animal studies has shown that GLP-1R agonists also promote insulin biosynthesis, β-cell proliferation, and survival through activation of antiapoptotic pathways (7). Genetic disruption of GLP-1R expression in mice has produced insights into the physiological importance of GLP-1 action. GLP-1R−/− mice exhibit abnormal glucose tolerance after both an oral and an intraperitoneal glucose challenge and a decrease in insulin secretion (39). Surprisingly, food intake and body weight are not significantly perturbed in GLP-1R−/− mice. The GLP-1R−/− mice also exhibit abnormalities in islet number and size and in adaptive regeneration of β-cells. With the numerous physiological functions associated with GLP-1, we believe it is important to understand the physiological regulation of GLP-1 secretion by nutrients, especially fat and carbohydrates.

The study of gastrointestinal (GI) hormone secretion has primarily relied on the measurement of circulating levels of GI hormones in the systemic blood or in the portal blood (17, 21). Plasma concentrations of GLP-1 are relatively low compared with those of GIP and other GI hormones such as peptide YY (PYY) and ghrelin (24, 31). Within the blood, GLP-1 is rapidly inactivated by an aminopeptidase, dipeptidyl peptidase IV (DPP IV). The enzyme cleaves the first two amino acids leaving an inactive form. Hence, the half-life of GLP-1 is only 1.5 to 2 min, and the biological effect is even shorter (5, 6, 26). Consequently, less than 10% of the
active peptide that is secreted reaches the arterial blood stream to reach various target organs. A major drawback in studying the mechanisms of GLP-1 secretion is that the sensitivity of assays currently available for its measurement are often at the limits of detection in most settings except when secretion of GLP-1 is driven to high levels in experimental paradigms (5, 34, 35). For example, it has been difficult to determine plasma GLP-1 concentrations in rodent models in their usual settings of fasting and feeding.

We hypothesize the lymph fistula rat can be an excellent model for studying the secretion of hormones by the GI tract, in part, because of the much lower lymph flow rate (2–3 ml/h) relative to portal blood flow (8–20 ml/min) (16, 40). This represents a difference in flow rate that is 160- to 400-fold greater in the portal vein vs. lymph. Hence, in lymph there is much less dilution of the GI hormones secreted by the enteroendocrine cells of the gut. Intestinal lymph, before it drains into the circulation, thus provides an enriched pool of these hormones. The purpose of this study was to investigate the lymph fistula model as a paradigm to study GLP-1 secretion in vivo in response to isocaloric and isovolumetric meals containing either lipid, dextrin, or a mixture of lipid and dextrin. The ability to better understand the secretory response of GLP-1 to macronutrient absorption is physiologically and clinically important and may offer opportunities in GLP-1 therapy for the treatment of type 2 diabetes.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats, weighing 240–350 g (Harlan, Indianapolis, IN), were used. Animals were allowed to acclimate to our animal facility for 2 wk prior to the experiment. During this period, the animals were fed regular rodent chow and housed in a room with a 12-h:12-h light-dark cycle. Both the temperature and the humidity of the room were maintained.

Surgical preparation. All procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and complied with the NIH Guide for the Care and Use of Laboratory Animals. Animals were fasted overnight before surgery. Under halothane anesthesia, the superior mesenteric lymph duct was cannulated with soft vinyl tubing [medical grade, 0.5 mm inner diameter (ID) and 0.8 mm outer diameter (OD); Tyco Electronics Pty, Castle Hill, Australia] by inserting a silicone tube (1.6 mm OD) about 2 cm into the duodenum via a fundal incision of the stomach. The tubing was secured by a transmural suture in the duodenum, and the fundal incision was closed by a purse-string suture. Postoperatively, the animals were intraduodenally infused with 5% glucose in saline (145 mM NaCl, 4 mM KCl, and 0.28 M glucose). Beginning 16 h before the nutrient study (which occurred on the following morning), the 5% glucose in saline was switched to saline alone and was infused continuously at 2 ml/h. Intralipid plus dextrin (isocaloric group) consisted of equal volumes and caloric content (2.215 kcal/1.5 ml) of the Intralipid and dextrin infusates. Nutrient bolus infusions were then followed by infusion of saline at 3 ml/h. Intralipid (20%), also known as Liposyn II (20%; Abbott Laboratories, North Chicago, IL) consisted of equal volumes and caloric content of 2 kcal/ml. Dextrin (Sigma, St. Louis, MO) is a glucose polymer with a molecular weight of 15,000 with a caloric content of 4 kcal/g. GLP-1 radioimmunoassay. GLP-1 concentrations in lymph were determined by a commercially available radioimmunoassay (RIA) kit (LINCO Research, St. Louis, MO). Samples of lymph were measured using GLP-1 (7-36) amide as the standard and <sup>125</sup>I-GLP-1 (7-36) amide as the tracer. Using this method, samples assayed were extracted with ethanol to get optimal results (33). In the RIA, a fixed concentration of labeled tracer antigen was incubated with a constant dilution of antiserum, such that the concentration of antigen-binding sites on the antibody was limited. The antiserum used in this RIA recognizes the COOH terminus of GLP-1, including both amidated and nonamidated forms (LINCO Research); therefore the assay detects all of the major circulating forms of GLP-1 including GLP-1 (7-36), GLP-1 (7-37), GLP-1 (9-36), GLP-1 (9-37), GLP-1 (1-36) and GLP-1 (1-37) amides in biological fluids.

Validation of GLP-1 RIA for lymph samples. To determine the linearity of GLP-1 measurement in lymph, lymph samples of 300 μl each were extracted according to the LINCO GLP-1 RIA protocol. Lymph samples collected following dextrin administration were used. Serial dilutions (undiluted, 1:1, and 1:2) of these lymph extracts were then assayed. Nonspecific contributions to the assay were determined by assaying lymph samples treated with or without activated charcoal. Four samples of 500 μl each were incubated overnight with 1 mg of charcoal on a shaker at 4°C. A second group was treated in the same manner but in the absence of charcoal. Following overnight incubation, the samples were centrifuged for 10 min, and 300 μl of lymph were carefully pipetted off the top for extraction and assay.

DPP IV activity assay in lymph and plasma. DPP IV activity was determined using the method as described by Nagatsu et al. (28). Lymph and plasma samples were collected during fasting and following an intraduodenal bolus infusion of 3 ml (4.43 kcal) of Ensure. Briefly, 50-μl samples of lymph or plasma collected from the jugular vein were incubated for 30 min at 37°C with 1 ml of 1.4 mM Gly-Pro-p-nitroanilide (Sigma) as enzyme substrate in 114 mM Tris buffer, pH 8.0. The blank and standard tubes contained water and 150 nmol of p-nitroanilide (Sigma), respectively. The reaction was stopped by adding 3 ml of 1 M acetate buffer, pH 4.2. The enzyme activity was assayed by measuring the increase in specific absorbance at 410 nm and was expressed as nanomoles of dipeptide released from Gly-Pro-p-nitroanilide per milliliter per minute.

Chemical assays of triglycerides and glucose in lymph. Lymph triglycerides were measured by an assay kit supplied by Randox (Crumlin, UK). This enzymatic assay measured the glycerol released from the hydrolysis of triglycerides. Briefly, 5 μl of lymph was added to 200 μl reagent. After 20 min of incubation at 37°C, optical density was read at 500 nm. Triglyceride concentration was calculated from the standard solution provided by Randox. Glucose in lymph samples was determined by an assay employing a modification of the glucose oxidase/peroxidase method described by Trinder (46). A sample to reagent (Diagnostic Chemicals Limited, Charlotte-town, PEI, Canada) ratio of 1:100 was used. The colorimetric assay measured the reaction product, quinonemine, spectrophotometrically at 505 nm. Standard curves were constructed, and sample readings were read from the standard curves.

Statistical analysis. All values are expressed as means ± SE. Two-way repeated-measures ANOVA, with Tukey’s as a posttest...
Validation of GLP-1 RIA in lymph. The GLP-1 (total) RIA was validated according to the criteria given by LINCO Research. Assays are considered acceptable when the readings of all the quality controls fall within the range provided by the company. For quality control of the assay, low concentration and high concentration controls are provided by LINCO. Our low concentration quality control was determined to be 50 pM (data not shown), which fell within the quality control range of 32–66 pM (LINCO Research). The high concentration quality control was 306 pM, which was also within the desired range of 178–370 pM (LINCO Research). The limit of sensitivity for the GLP-1 RIA is between 3 and 333 pM for a 300-μl extracted sample size. The performance of the assay was also validated by the parameters of effective dose (ED) for 20%, 50%, and 80% binding within the given ranges. All effective dose values for ED (20), ED (50), and ED (80) fell within the expected ranges (LINCO Research). All our samples had GLP-1 concentrations fall within the linear portion of the RIA standard curve.

The GLP-1 RIA for the determination of GLP-1 in lymph was further validated by the linearity assay. Serial dilutions of lymph samples collected following dextrin treatment were made such that three concentrations were assayed (undiluted, diluted 1:1, and diluted 1:2). The data for these dilutions had respective averages of 184 ± 20 pM, 95 ± 27 pM, and 51 ± 16 pM (Fig. 1). The GLP-1 concentrations of the samples for the undiluted, diluted 1:1, and diluted 1:2 were therefore 100% (predicted 100%), 51% (predicted 50%) and 28% (predicted 33%) of the undiluted samples, respectively. Thus the GLP-1 radioimmunoassay read the lymph samples linearly.

As part of the GLP-1 RIA validation in lymph, we also assayed for the nonspecific reading of the GLP-1 antibody used. The samples were incubated overnight with or without activated charcoal. Charcoal strips the lymph of all peptides (e.g., GLP-1, GIP, or albumin). Samples treated overnight with charcoal had a mean GLP-1 concentration of 6.6 ± 0.67 pM compared with the untreated samples, which measured 184 ± 20 pM (Fig. 2). Thus the contribution of nonspecific binding to GLP-1 measurements in lymph was only a minute fraction of the total immunoreactivity.

DPP IV activity in lymph and plasma. DPP IV activity was measured in lymph, and plasma samples were collected during fasting and following intraduodenal administration of Ensure (a mixed meal). During fasting, DPP IV activity in plasma was much higher than that in lymph with values of 140.12 ± 15.81 nmol·min⁻¹·ml⁻¹ and 11.61 ± 1.91 nmol·min⁻¹·ml⁻¹, respectively (P < 0.001, Fig. 3). At 60 min after the infusion of Ensure, plasma DPP IV activity (113.60 ± 11.60) was still significantly greater than that in lymph (71.34 ± 4.10 nmol·min⁻¹·ml⁻¹; P= 0.008). DPP IV was still significantly greater in plasma (177.93 ± 12.60) than in lymph (106.61 ± 10.99 nmol·min⁻¹·ml⁻¹; P < 0.001) at 120 min following nutrient infusion as well as at 240 min (122.48 ± 12.66 vs. 50.33 ± 5.49 nmol·min⁻¹·ml⁻¹; P < 0.001).

Effect of nutrient stimulation on lymphatic GLP-1 concentrations. GLP-1 concentrations in the fasting lymph from animals receiving Intralipid or the saline control were not significantly different (Fig. 2). In the lymph collected after the nutrient bolus there was a significant increase in GLP-1 concentration. The GLP-1 concentrations in the lymph collected after the nutrient bolus were significantly higher than those collected during fasting and those collected after the intraduodenal administration of Ensure.
indicate significant differences between each nutrient group and the saline control values at that time. **$P < 0.01$; ***$P < 0.001$.

statistically different ($P = 0.689$). There was a significant increase in GLP-1 in lymph after the 3-ml intraduodenal bolus of Intralipid (4.43 kcal), whereas animals that received enteral saline had only minimal GLP-1 responses (Fig. 4). Lymph GLP-1 of the Intralipid group peaked at 30 min, reaching a concentration of 154 ± 23 pM, and gradually decreased to baseline level after 6 h. Compared with the baseline concentration of 31 ± 8 pM, Intralipid induced a fivefold increase in GLP-1 ($P < 0.001$), and it remained significantly elevated from 1 to 4 h. No significant increase was observed in the saline group throughout the entire 6 h. Over the entire 6-h period, the increase in mean GLP-1 concentrations in response to Intralipid was statistically significantly relative to those of the controls ($P < 0.001$).

Intraduodenal infusion of an isocaloric and isovolumetric dose (4.43 kcal/3 ml) of dextrin also increased lymphatic GLP-1. The increase was apparent by 30 min after the bolus infusion, reaching 96 ± 29 pM ($P = 0.019$). Dextrin induced a peak stimulation of GLP-1 concentration of 179 ± 20 pM at 60 min (Fig. 4). The rise in GLP-1 concentration at 60 min relative to fasting was also statistically significant ($P < 0.001$). It is notable that the peak of lymphatic GLP-1 concentration caused by the infusion of dextrin lagged behind that of Intralipid. GLP-1 concentrations in response to dextrin remained significantly elevated at 2 h and 3 h ($P < 0.001$ and $P = 0.021$, respectively). The mean GLP-1 concentration following dextrin was significantly higher than that of the control group over the 6-h interval.

To determine if there is an additive or a potentiating effect of the two macronutrients in stimulating lymphatic GLP-1, Intralipid and dextrin were injected together. It is important to note that we did not change the calories or the volume of the bolus dose of nutrients infused to maintain these variables constant with the previous experiments. The combined bolus infusion maintained the same volume (3 ml) and had the same total caloric content (4.43 kcal) as the Intralipid or dextrin infusions alone. Equal volumes (1.5 ml) and equal caloric contents (2.215 kcal) of Intralipid and dextrin were used to make the infusate. The mixed infusion induced a peak of GLP-1 of 237 ± 24 pM at 30 min (Fig. 4). This was sevenfold higher than fasting levels ($P < 0.001$). This increase remained significant throughout 240 min ($P < 0.026$) before decreasing to background level.

Comparing all of the nutrient groups, when given alone, dextrin and Intralipid each elicited a similar fourfold increase in peak levels of GLP-1 concentration at 179 ± 20 and 154 ± 23 pM, respectively (Fig. 4). However, these groups had different peak times at 60 min and 30 min, respectively. The combination of dextrin and Intralipid stimulated the greatest peak GLP-1 concentration of 237 ± 24 pM in lymph, which represented a sevenfold increase from the fasting level. Lymph GLP-1 of all groups returned to baseline by 6 h. The combined infusion also induced a significant increase in GLP-1 concentrations compared with either dextrin or Intralipid alone at 30 min ($P < 0.001$ and $P = 0.003$, respectively) as well as at 60 min vs. Intralipid ($P = 0.004$).

Effect of nutrient infusion on lymph flow. Intralipid increased flow by approximately twofold by 30 min (Fig. 5). Dextrin, on the other hand, caused an initial decrease in lymph flow at 30 min, which then increased by 1.5-fold of basal from 60 through 120 min. Intralipid plus dextrin stimulated an increase in lymph flow by about twofold, peaking at 60 min. Saline infusion did not result in any significant change in lymph flow. Lymph flow rates fell within the range of 2 to 4.5 ml/h most of the time for all of the nutrient treatment groups. Over the entire 6-h period, there were no significant differences in the overall mean lymph flow rates among the saline control, the Intralipid group, the dextrin group, or the combined group.

Effect of nutrient stimulation on lymphatic GLP-1 output. To compare the rate of intestinal GLP-1 secretion stimulated by different nutrients, we calculated the product of GLP-1 concentration and lymph flow rate. In general, GLP-1 secretion followed a similar pattern as GLP-1 concentration. No significant differences were observed during fasting among any of the nutrient groups. Intralipid induced a greater than ninefold increase in GLP-1 secretion, reaching a peak output of 691 ± 118 fmol/h from a baseline of 73 ± 16 fmol/h at 30 min ($P < 0.001$, Fig. 6). Dextrin elicited a comparable increase that peaked later at 60 min ($P < 0.001$). The combination of...
Intralipid plus dextrin caused a 14-fold surge reaching 1,045 ± 218 fmol/h at 60 min (P < 0.001). The level of GLP-1 secretion following the mixed infusion was greater than that following dextrin alone at 30 min (P < 0.001) and greater than that following either dextrin or Intralipid alone after 60 min (P < 0.001 and P < 0.001, respectively). GLP-1 secretion for all groups had returned to baseline by 4 h. The difference in the mean GLP-1 secretion rate calculated over the 6-h period was significant between the Intralipid and control saline groups (P = 0.005), between the dextrin and control (P = 0.022), and between the Intralipid plus dextrin and control groups (P < 0.001).

Cumulative GLP-1 output was calculated by summing the hourly GLP-1 secretions values over the entire 6-h period. Animals in the Intralipid group had a 3.6-fold increase compared with the saline control group (P = 0.005, Fig. 7). The dextrin-treated group also had a comparable 3.1-fold increase, which was also significantly different from the saline control (P = 0.022). The greatest increase occurred after the infusion of both Intralipid plus dextrin, which stimulated a 4.5-fold increase over the control group (P < 0.001).

**Effect of nutrient stimulation on lymph triglycerides and glucose.** Lymph triglyceride levels, in response to the various nutrients, were examined. No significant change was observed in the saline or dextrin groups, so only the data from the saline group are shown in Fig. 8. Intralipid-infused animals had the greatest increase in lymph triglycerides, reaching a peak of 2,239 ± 292 mg/dl at 30 min. The isocaloric-combined Intralipid plus dextrin group had a similar steady increase as the group infused with Intralipid only during the first 3 h, peaking at 1,198 ± 124 mg/dl at 2 h before gradually declining. Even at the end of 6 h, the lymph triglyceride levels of the Intralipid and the combined groups remained significantly elevated at 2,056 ± 237 and 657 ± 88 mg/dl, respectively. Both the Intralipid alone and the Intralipid plus dextrin combined groups had elevated triglycerides compared with the saline or dextrin groups from 60 min on. (P < 0.001, Fig. 8). The difference in the mean lymph triglyceride levels over the 6-h time course was significant between the Intralipid and control groups (P < 0.001), as well as between the Intralipid plus dextrin and control groups (P < 0.001).

The saline group had stable lymph glucose averaging around 117 ± 4 mg/dl (Fig. 9). Both the dextrin and the combined groups had increased lymph glucose, reaching peaks of 246 ± 16 and 269 ± 21 mg/dl, respectively, at 60 min. There was no significant difference in the glucose concentration in lymph between the Intralipid group and the saline control group (data not shown). The difference in the mean lymph glucose concentration over the entire 6-h period was statistically significant between the dextrin and control groups (P = 0.005) and between the combined and saline control groups (P < 0.001).

**DISCUSSION**

We used the lymph fistula rat as an experimental model to study the secretion of GLP-1 (an incretin) by the intestine when stimulated by infusion of lipid and/or carbohydrate into the duodenum. We believe that the lymph fistula model is well-suited for studying gastrointestinal hormone secretion because 1) there is less dilution of what is released from intestinal cells.
since the blood flow in the portal vein varies between 8–20 ml/min in the rat, whereas the lymph flow varies between 2 and 3 ml/h representing a dilution factor that is 160- to 400-fold greater in blood (16, 40); 2) there is less degradation of GLP-1 by DPP IV in lymph than in plasma; and 3) the concentration in lymph reflects better the actual concentration that the neurons and dendritic cells are exposed to in the lamina propria of the gut. We believe there is less DPP IV activity in lymph than in plasma because when we measured either the total or the active GLP-1 levels in lymph using respective RIA kits (both from LINCO Research), the lymph GLP-1 values yielded by the two assays were very close to each other, suggesting very little degradation. This is not surprising because the circulating human soluble DPP IV (identical to CD26) has a molecular weight of about 110 kDa (8) and a radius of 45 Å (personal communication with Dr. Neil Granger). A protein with that radius will have lymph-to-plasma ratio of less than 0.1 (13). Indeed, when we analyzed the DPP IV activity in lymph and plasma, we observed the following. DPP IV activity was 12-fold higher in plasma than in lymph under fasting conditions. Throughout the 4-h study, DPP IV remained twofold higher in plasma than lymph.

This is the first report investigating the role of intestinal fat and glucose in stimulating the secretion of GLP-1, using lymph as an assay model. The lymph fistula model offers a sensitive measurement of GLP-1 in lymph samples collected from the gastrointestinal tract. In addition to validating the GLP-1 RIA in terms of quality control values and assay performance according to LINCO’s criteria, we also validated the GLP-1 RIA for studying lymph samples by use of linearity assay. In a further validation assay for nonspecific binding, samples treated with activated charcoal had very low GLP-1 concentrations, demonstrating that the GLP-1 RIA is specific to GLP-1 in lymph with very low nonspecific immunoreactivity. The dose of fat or glucose administered was selected to provide a robust nutrient stimulus for GLP-1 secretion. In studies done in human subjects, it was demonstrated that a threshold of caloric delivery exceeding 1.4 kcal/min was necessary to stimulate measurable secretion of GLP-1 into the circulation (38). The results of the present study demonstrate that GLP-1 concentration and output following administration of dextrin, Intralipid, or a combination of both displayed similar patterns of stimulation in lymph as reported in plasma. These findings provide novel evidence that GLP-1 increases in response to lipid and glucose and that the lymph fistula model can be utilized as a more sensitive and accurate paradigm for detecting GLP-1 secretion.

The secretion of GLP-1 has been proposed to be regulated by the rate of entry of nutrients into the small intestine (38, 52). We therefore minimized the effect of variability in the rate of gastric emptying by administering nutrients intraduodenally. Previous reports on outcome of repeated oral glucose tolerance tests yielded remarkable intrapatient variability in normal subjects (25). This was explained by the hypothesis that such variation can be produced by ingestion of the glucose solution during different phases of the normal fasting activity cycle of the upper gut (42). The velocity of the initial phase of glucose emptying from the stomach may also vary with the antral motor activity at the time of ingestion. Gastric emptying showed similar variation with the activity cycle. In contrast, such variation was not seen when glucose was administered intraduodenally (42) since plasma glucose levels were not affected by the preceding interdigestive phase. Direct administration of nutrients into the duodenum bypasses any potential influence of the rate of emptying of nutrients from the stomach on GLP-1 secretion. This is a notable methodological aspect of our study since we compared the effects of liquid meals of lipid and carbohydrate that would be expected to have vastly differing entry rates into the intestine if given through the stomach. Because we tested the effects of lipid and glucose directly in the duodenum, we were able to determine that at the level of the intestinal mucosa, lipid elicits a more rapid release of GLP-1 than glucose. This novel observation suggests that the two nutrients activate L cells by distinct mechanisms and is consistent with their additive effect.

The present study reveals the potential of the lymph fistula model as a more sensitive paradigm to detect GLP-1. A similar stimulatory response of GLP-1 to fat and carbohydrate, as we have observed in lymph, was also observed in plasma; however, the levels detected in lymph were much higher. For example, the peak levels of lymph GLP-1 in response to dextrin was 179 pM. In contrast, Wistar rats given oral glucose have peak GLP-1 concentration of 85 pM in plasma (21). Overall, the sixfold increase in lymph GLP-1 concentration relative to fasting that we observed is greater than the 2.4-, 1.8-, and 1.2-fold increases in plasma seen in respective studies in rats and humans (12, 21, 24). Following fat administration in our lymph fistula model, a 4.5-fold peak elevation was detected in lymph vs. a 1.5- to 3-fold elevation in plasma reported in previous studies (10, 12, 24, 30). The rapid increase in lymph GLP-1 with substantial elevations at the 30-min sampling time is consistent with an incretin role. Certainly, some of the differences may be due to factors such as subjects and nutrient dosage tested; however, it demonstrates the use of the lymph as a sensitive model.

Compared to Intralipid, an isocaloric and isovolumetric dose of dextrin elicited a different pattern of GLP-1 stimulation. Dextrin, a glucose polymer, was used to minimize any effects of high osmolality on GI function. The particular dextrin employed in our studies has a molecular weight that is 83-fold
greater than that of glucose. Dextrin induced a peak GLP-1 concentration at 60 min compared with that of Intralipid at 30 min. The slight delay in the peak stimulation of GLP-1 by dextrin may be due to the physical form of the nutrient. Both the chemical and physical forms of nutrients are important in the stimulation of insulin secretion, which, in turn, is released in part in response to incretin secretion. It has been shown that complex carbohydrates are less stimulatory of insulin secretion than monosaccharides (3, 44). Studies on diabetic treatment by Jenkins have demonstrated that with the addition of dietary fiber to increase the viscosity of food, there is a positive correlation between a significant decrease in blood glucose and increased viscosity (22). A delay in mouth-to-cecum transit time also occurred. This was consistent with our observations of delayed peak GLP-1 stimulation by dextrin vs. Intralipid. In our study, the caloric content of the commercially prepared Intralipid was 10 kcal/g, whereas that of dextrin was 4 kcal/g. Therefore, 2.5-fold more dextrin than Intralipid was required in the preparation of the isocaloric and isovolumetric nutrient infusion mixture. The dextrin infusion mixture was thicker and more viscous than the Intralipid mixture and therefore may have resulted in a slower rate of digestion and absorption by the intestine. Even though the caloric contents of the two types of nutrients were the same, their physical properties were different. Therefore, we cannot exclude the possibility that the delay in the peak stimulation of GLP-1 by dextrin was due to the physical form of the nutrient, which in turn affects its hydrolysis to form glucose.

The mixed infusion used in the present study proved to be a potent stimulus for GLP-1. Interaction of the two types of nutrient induced a peak concentration that indicated an additive or greater effect. That is, the combination of Intralipid and dextrin, in half the amounts given alone, caused a significantly greater GLP-1 response than what would have been expected from the administration of either of the individual nutrients. This additive effect was seen only at the peak time point. Whereas the cumulative GLP-1 output over the entire 6 h also was greatest in the combined group, the relative elevation was not as great as the additive effect observed with the peak GLP-1 secretion. In agreement with our lymph studies, previous studies in plasma have reported that the combination of lipid and carbohydrate significantly stimulated GLP-1 concentration (21). The sevenfold increase we observed in the lymph study was certainly more prominent than the twofold increase in the plasma (21), supporting our proposal that the lymph fistula model is a more sensitive model for studying GLP-1 secretion by the gut in conscious animals. The findings from this study strongly imply that a mixed infusion enhances the L cell response.

Lymph flow rates during fasting were comparable among animals in the four nutrient groups as shown in Fig. 5. These steady and similar lymph flow rates were an indication of the excellent and stable conditions of the animals following surgery. Intralipid stimulated the earliest rise in lymph flow at 30 min, whereas dextrin induced an initial drop followed by an increase at 60 through 120 min. The combined nutrient group displayed the highest flow rate at 60 min. The pattern of lymph flow rates provides further support that the dextrin mixture required a longer time for digestion and absorption relative to Intralipid. The initial decrease in lymph flow rate of the dextrin-treated animals may be attributed to the presence of undigested dextrin in the gut lumen, causing an increased retention of fluid in the lumen due to osmolarity. This may lead to decreased movement of water from the gut lumen to the interstitial fluid of the villus, thus resulting in reduced lymph formation. The rise in lymph flow rate of the combined group may be delayed compared with Intralipid alone due to the presence of dextrin.

Lymph triglyceride concentration in response to various nutrient treatments revealed that the dextrin or saline groups had no increase in lymph triglyceride (TG) levels as anticipated. For simplicity of the graph, only the saline control data are included in Fig. 8. The greatest elevation in lymph TG concentration was observed in the Intralipid-treated group, which also contained the highest lipid dose in the infusion. The lymph TG level in the combined nutrient group exhibited an increase about half the magnitude observed in the Intralipid-treated group, consistent with the reduced dose of lipid given in this experiment. Lymph TG concentrations gradually increased and peaked at 300 min before reaching a plateau, indicating that it took some time for lipid to get transported into the lymphatic circulation. This profile is typical of normal lipid digestion, absorption, and processing by mucosal cells; the major delay between administration and appearance of lymph is likely due to the time for chylomicron formation (14, 18, 48). Based on the lymphatic GLP-1 output and lipid output, we speculate that although lipid absorption is associated with the stimulation of GLP-1 secretion, it is not the transport of lipid per se that was responsible for the stimulation but rather something associated with lipid absorption. If it is the amount of lipid transported by the gut that is responsible for the stimulation of GLP-1 secretion, we would expect the GLP-1 secretion to also peak later, but it peaked much earlier. Also, if it is the amount of lipid transported that acts as a signal for stimulating GLP-1 secretion, we would expect a smaller GLP-1 secretion with the animals infused with Intralipid plus dextrin than the Intralipid group, yet the data were exactly opposite, i.e., a bigger increase in GLP-1 secretion with the mixed infusion than occurred with the Intralipid or dextrin alone. Exactly how lipid absorption is responsible for stimulating GLP-1 secretion by the enteroendocrine cells is unclear at the moment but is a topic of active research in our laboratory.

As expected, the concentration of glucose in lymph increased in both of the dextrin-containing groups, the dextrin and the combined groups. Both groups had increases in the range between 2- and 2.5-fold. The timing of the peak elevation in lymph glucose at 60 min coincided with that of GLP-1 secretion in both the dextrin and the combined groups. The transport of glucose into the portal circulation and lymph after absorption from the intestine is rapid, and our findings are consistent with dextrin-induced GLP-1 release being linked to intracellular uptake or metabolism of glucose. Thus, unlike the lymphatic lipid transport phenomenon, lymphatic glucose transport and lymphatic GLP-1 secretion seem to go hand in hand and peak at the same time. These findings are consistent with previous reports of glucose functioning as a secretagogue of GLP-1 (12, 24, 45).

The present findings indicate that the lymph fistula model is a sensitive and useful model for studying GLP-1 secretion in vivo. As aforementioned, the levels of GLP-1 that we measured in lymph are considerably higher than plasma GLP-1.

The physical form of the nutrient, which in turn affects its hydrolysis to form glucose.
concentrations that have been reported previously. The peak levels of lymph GLP-1 varied from a five- to a sevenfold increase over baseline depending on the nutrient stimuli. In contrast, GLP-1 in plasma had increases of two- to threefold following fat and/or glucose administration in rats and humans (10, 12, 21, 30, 41). Some of the differences may undoubtedly be explained by variations in dosage or subjects tested. These findings nevertheless reveal the sensitivity and usefulness of the lymph-fistula model in studying GLP-1 secretion in response to different macronutrients. Studies have reported that more than 50% of the newly secreted GLP-1 is metabolized to its primary metabolites when passing from the intestinal stroma into the capillary bed (15), and about 90% of GLP-1 is degraded before it reaches the systemic circulation (20). Because of the time lapse between blood sampling and the secretion of GLP-1 in the gut, a significant amount of the newly secreted GLP-1 may have been degraded. Therefore, plasma concentration of GLP-1 may not reflect the actual concentrations that act on the target organs. Due to factors such as dilution and the much higher levels of degradation enzymes in plasma than in lymph, we believe the levels of most, if not all, gastrointestinal hormones in lymph are going to be much higher than those found in systemic blood. In addition, the lymph fistula model is a conscious model, such that animals are not subjected to the complications of anesthesia.

In summary, we have demonstrated that the lymph fistula model is an excellent model for studying the secretion of GLP-1 by the enteroendocrine cells in the gut in response to the absorption of different macronutrients. This model allows the continuous monitoring of GLP-1 secretion by the endocrine cells, and it will also allow us to determine the level of GLP-1 exposure of the enteric neurons and dendritic cells. This information will be of use to gut physiologists for both in vitro as well as in vivo experiments. It would be very important in the future to see whether this method can be applied to the study of other gastrointestinal hormones.

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