GLP-1 reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein production in rats

Xiaofa Qin, Hui Shen, Min Liu, Qing Yang, Shuqin Zheng, Mary Sabo, David A. D’Alessio, and Patrick Tso

Departments of 1Pathology and 2Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio

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Qin, Xiaofa, Hui Shen, Min Liu, Qing Yang, Shuqin Zheng, Mary Sabo, David A. D’Alessio, and Patrick Tso. GLP-1 reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein production in rats. Am J Physiol Gastrointest Liver Physiol 288: G943–G949, 2005. First published January 27, 2005; doi:10.1152/ajpgi.00303.2004.—Glucagon-like peptide 1 (GLP-1) is a gastrointestinal hormone secreted in response to meal ingestion by enterocortical L cells located predominantly in the lower small intestine and large intestine. GLP-1 inhibits the secretion and motility of the upper gut and has been suggested to play a role in the “ileal brake.” In this study, we investigated the effect of recombinant GLP-1-(7–36) amide (rGLP-1) on lipid absorption in the small intestine in intestinal lymph duct-cannulated rats. In addition, the effects of rGLP-1 on intestinal production of apolipoprotein (apo) B and apo A-IV, two apolipoproteins closely related to lipid absorption, were evaluated. rGLP-1 was infused through the jugular vein, and lipids were infused simultaneously through a duodenal cannula. Our results showed that infusion of rGLP-1 at 20 pmol·kg⁻¹·min⁻¹ caused a dramatic and prompt decrease in lymph flow from 2.22 ± 0.15 (SE) ml/h at baseline (n = 6) to 1.24 ± 0.06 ml/h at 2 h (P < 0.001). In contrast, a significant increase in lymph flow was observed in the saline (control) group: 2.19 ± 0.20 and 3.48 ± 0.09 ml/h at baseline and at 6 h of lipid infusion, respectively (P < 0.001). rGLP-1 also inhibited intestinal triolein absorption (P < 0.05) and lymphatic apo B and apo A-IV output (P < 0.05) but did not affect cholesterol absorption. In conclusion, rGLP-1 dramatically decreases intestinal lymph flow and reduces triglyceride absorption and apo B and apo A-IV production. These findings suggest a novel role for GLP-1 in lipid absorption.

gut hormone; “ileal brake” effect; intestinal lymph duct cannulation

GLUCAGON-LIKE peptide 1 (GLP-1) is a gut hormone synthesized by posttranslational processing of proglucagon in enteroendocrine L cells, predominantly located in the lower jejunum, ileum, and large intestine (12, 14, 24). GLP-1 is released into the circulation after meal ingestion, and secretion is also stimulated by the presence of carbohydrate, fat, or protein in the lower gut (22) and by bile acids and pectin (39). In addition to luminal stimuli, GLP-1 is also secreted in response to other hormones, such as glucose-dependent insulinotropic peptide (23, 40), bombesin, calcitonin gene-related peptide, the β-adrenergic agonist isoproterenol, and the muscarinic cholinergic agonist bethanecol (38). Despite the distal location of the majority of L cells, the GLP-1 response to meal ingestion is rapid, suggesting neural or humoral regulation of secretion (4, 41).

Most research has emphasized the role of GLP-1 in promoting insulin secretion and reducing blood glucose, and these effects have led to potential clinical use in treating patients with diabetes mellitus (11, 13, 28). GLP-1 is one of two known incretins, i.e., gastrointestinal (GI) hormones that potentiate glucose-stimulated insulin secretion (3, 14). GLP-1 has been shown to enhance all steps of insulin biosynthesis (14) and is a potent insulin secretagogue (24, 25). GLP-1 can also suppress the synthesis and release of glucagon (48) and may have some independent effect on glycogen disposition (10). Through these mechanisms, GLP-1 can decrease blood glucose levels without producing severe hypoglycemia (25).

Beyond its role in glucose metabolism, one of the primary functions of GLP-1 may be to regulate the upper GI tract. GLP-1 can inhibit gastric acid secretion (29, 35), gastric emptying (42), motility of the stomach and small intestine (2, 18), and transit of food (9). As a result, GLP-1 has been proposed to be an enterogastrone that mediates the ileal brake phenomenon (19), alone (24) or by its regulation of other hormones such as somatostatin and gastrin (16, 26). The ileal brake effect of GLP-1 on other functions of the intestine, such as the absorption of nutrients, has not been extensively researched. It has been reported that GLP-1 infused intravenously into healthy humans in amounts corresponding to the postprandial release can significantly inhibit pentagastrin-stimulated gastric lipase secretion and lipolytic activity. This inhibitory effect was present even in vagotomized patients (47).

In this study, we investigated the effect of GLP-1 on lipid absorption and apolipoprotein (apo) B and apo A-IV production in the small intestine.

MATERIALS AND METHODS

Materials

Recombinant human/rat GLP-1-(7–36) amide (rGLP-1; rat and human GLP-1 have exactly the same sequence) was kindly provided by BioNebraska (Lincoln, NE) (15). Triolein, cholesterol, egg phosphatidylycholine, and sodium taurocholate were purchased from Sigma-Aldrich (St. Louis, MO), and radioactive [9,10-3H(N)]triolein (53.00 Ci/mmol) and [4-14C]cholesterol (52.90 mCi/mmol) were purchased from New England Nuclear (Boston, MA). All solvents and reagents were of analytic grade.

Animals

Adult male Sprague-Dawley rats weighing 300–350 g were purchased from Harlan Industries (Indianapolis, IN). Animals were quarantined for ≥1 wk before experimentation to allow them to acclimate to their new environment. The animals had free access to standard rodent diet and water. Room lighting was set for a 12:12-h...
light-dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. Animals were randomly divided into two groups: the rGLP-1 group and the control group. The animals in the rGLP-1 group were infused through the jugular vein with rGLP-1 dissolved in saline; animals in the control group were infused through the jugular vein with saline only. Lipid was continuously infused into both groups through the duodenal cannula.

Surgical Preparation

The animals were fasted overnight but allowed free access to water. On the following morning, the animals were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ) administered through a vaporizer. The right atrium was cannulated via the external jugular vein as described elsewhere (27). The atrial cannula was exteriorized at the back of the neck, filled with saline, and sealed. A laparotomy was performed, and the superior mesenteric lymph duct was cannulated with a vinyl tube (medical grade, 0.5 mm ID, 0.8 mm OD; Critchley Products, Silverwater, New South Wales, Australia). The tube was secured with a drop of cyanoacrylate glue (Krazy Glue, Jadow & Sons, New York, NY) and externalized through the right flank. A second cannula (1.02 mm ID, 2.16 mm OD; Silastic medical grade 508-005, Dow Corning Medical Products, Midland, MI) was threaded through a surgical incision of the fundus of the stomach, extended 2 cm into the duodenum, secured with a purse-string suture and a drop of cyanoacrylate glue, and externalized through the right flank. After surgery, the animals were placed in restraining cages in a temperature-regulated chamber at 30°C and allowed to recover overnight. During recovery, the animals received continuous intraduodenal infusion of a glucose-saline solution (145 mM NaCl, 4 mM KCl, and 280 mM glucose) at 3 ml/h.

Infusate Preparation

rGLP-1. Stocks of rGLP-1 were dissolved in saline, aliquoted into small fractions, and frozen at −80°C. An aliquot was freshly thawed and diluted in saline before the experiment was begun. Lipid infusate. The lipid infusate contained 40 μmol of triolein, 7.8 μmol of cholesterol, 7.8 μmol of egg phosphatidylcholine, and 57 μmol of sodium taurocholate per 3 ml with trace amounts of [3H] triolein (1 μCi) and [14C]cholesterol (0.1 μCi). Nonradioactive stock solution of triolein, cholesterol, and phosphatidylcholine, as well as radioactive triolein and cholesterol, was dissolved in chloroform, mixed, and then evaporated under a stream of nitrogen. A solution containing 19 mM sodium taurocholate in PBS (pH 6.4) was then added, and the mixture was emulsified with a sonicator (model 250/450 sonifier, Branson Ultrasonics, Danbury, CT). Aliquots were taken from the top, center, and bottom of the emulsion, and their variation was <2% among the three samples.

Experimental Procedure

After an overnight recovery from surgery, lymph was collected for 1 h before lipid and rGLP-1 infusion. Infusion of rGLP-1 (saline in the control group) and lipid was started simultaneously. rGLP-1 was infused via the jugular vein cannula at 3 ml/h. Lymph was collected hourly throughout the 6-h infusion period.

Collection of Luminal and Mucosal Samples

At the end of 6 h of lipid and rGLP-1 infusion, the animals were euthanized with pentobarbital sodium. Both ends of the stomach, small intestine, and cecum were tied off with sutures to prevent leakage of the luminal contents. The small intestine was carefully excised and further divided into four equal-length segments. The luminal contents of the four segments of the small intestine as well as the stomach and cecum were collected in tubes and washed three times with 3 ml of 10 mM sodium taurocholate. The various luminal contents were later homogenized, and aliquots were taken for determination of radioactivity. The four small intestinal segments were cut open longitudinally and placed flat on a glass plate (luminal side up), and the mucosa was scraped with a glass slide. The lipid in the mucosa was extracted according to the methods of Folch et al. (17). Aliquots of lymph, luminal washings, and mucosa were taken for determination of radioactivity by liquid scintillation spectrometry.

TLC Analyses of Luminal Digestion and Mucosal Resynthesis of Infused Triolein

Luminal and mucosal lipids from the intestinal segments were extracted according to the procedure described by Blankenhorn and Ahrens (7). Only the first two quarters of the intestine was used for TLC analyses, because most of the residual lipids were in the upper small intestine. Samples were plated onto activated silica gel G plates, and the lipids were fractionated using a solvent system of petroleum ether-diethyl ether-glacial acetic acid (75:15:1 vol/vol/vol). Iodine vapor was used to visualize the different lipid classes as well as the comigrating lipid standards. Bands corresponding to triglycerides, diglycerides, monoglyceride and phospholipids, fatty acid, and cholesterol ester were scraped into separate scintillation vials, and 1 ml of absolute alcohol was added to help elute the lipids. Radioactivity was determined after addition of an aqueous miscible scintillant (Opti-Fluor, Packard Instrument, Meriden, CT).

Radioactivity Determination, Calculations of Lymphatic, Luminal, and Mucosal Triglyceride and Cholesterol Recoveries, and Assessment of Luminal Digestion and Mucosal Resynthesis of Infused Triolein

Opti-Fluor was used for scintillation counting. Samples were counted for 10 min in a liquid scintillation spectrometer (model TR 1900 Tri-Carb, Packard). Lymphatic triglyceride or cholesterol recovery at a time point was calculated as radiolabeled lipids recovered in the lymph as a percentage of the amount totally infused into the intestine. Radioactivity in triglyceride or cholesterol remaining in the lumen or mucosa of the animals collected by the end of the experiment was calculated as the percentage of the total amount of lipids infused during the 6 h. The luminal digestion and mucosal resynthesis of the infused [9,10-3H(N)]triolein were assessed by the relative distribution of 3H radioactivity in triglyceride, diglyceride, monoglyceride and phospholipid, fatty acid, and cholesterol ester fractions of the luminal or mucosal samples.

GLP-1 Assay

Plasma samples were extracted in 70% ethanol, and GLP-1 was measured using a commercial radioimmunoassay kit (Linco, St. Louis, MO).

Measurement of Plasma Apo B and Apo A-IV

Plasma apo B and apo A-IV concentrations were measured by sandwich ELISA, in which 96-well high-binding ELISA plates (Corning, Corning, NY) were coated with 100 μl of rabbit antisem against rat apo B or apo A-IV (1:300 diluted in 0.1 M citrate buffer, pH 3.5) and incubated overnight at 4°C. After the plates were washed in 10 mM PBS containing 0.05% Tween 20 (PBS-Tween 20). One hundred microliters of standard or plasma samples (1:10,000 diluted in PBS-Tween 20 containing 1% BSA) were added in duplicate to the coated wells, and the samples were incubated overnight at 4°C. After the samples were washed, 100 μl of goat polyclonal antibody against rat apo B or apo A-IV (1:3,000

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diluted in PBS-Tween 20 containing 1% BSA) were added, and the samples were incubated for 2 h at 37°C. After the samples were washed, 100 µl of peroxidase-conjugated anti-goat IgG (1:200 diluted in PBS-Tween 20 containing 1% BSA; DAKO) were added, and the samples were incubated for 1 h at 37°C. Color development was initiated by the addition of 200 µl of O-phenylenediamine dihydrochloride peroxidase substrate (Sigma, St. Louis, MO). After 30 min in the dark at room temperature, 50 µl of 3 M HCl were added to stop the reaction, and the absorbance at 492 nm was measured.

Statistics

Values are means ± SE. The difference between the control and rGLP-1 groups in their plasma GLP-1 concentration and luminal and mucosal triglyceride, diglyceride, monoglyceride and phospholipid, fatty acid, and cholesterol ester distributions were analyzed by Student’s t-test. The lymph flow rate, lymphatic, luminal, and mucosal triglyceride and cholesterol recovery, and lymphatic apo B and apo A-IV output were analyzed by two-way repeated-measures ANOVA (1-factor repetition) using SigmaStat version 3.0 (Systat Software, Point Richmond, CA), in which treatment with or without rGLP-1 was used as the first factor and the different time points (for lymph flow rate or for lymphatic triglyceride, cholesterol, apo B, and apo A-IV output) or the different segments of the gut (for luminal or mucosal triglyceride and cholesterol contents) were treated as the second repetition factor. The difference between the rGLP-1 and control groups at each point of the repeated measurements or the differences among the different points of the repeated measurements within the rGLP-1 or control group were determined by Tukey’s post hoc test. P < 0.05 was considered statistically significant.

RESULTS

Effect of rGLP-1 Infusion on Plasma GLP-1 Level

The plasma GLP-1 level of the rats infused with saline or with rGLP-1 at 20 pmol·kg⁻¹·min⁻¹ was measured by ELISA. At the end of the 6-h treatment, the plasma GLP-1 level was 17.5 ± 4.4 pmol/l in the saline group and 80.5 ± 45.7 pmol/l in the rGLP-1-treated group (P < 0.05), which is very close to the reported results [44].

Effect of rGLP-1 Infusion on Intestinal Lymph Flow Rate

In the control group, lipid infusion caused a gradual but significant increase in lymph flow (Fig. 1). Lymph flow in-
mucosal samples collected by the end of the experiments. The percentage of triglyceride was significantly higher \((P < 0.05)\) but the percentage of monoglyceride was significantly lower \((P < 0.01)\) in the luminal contents of the rGLP-1-treated than control animals. However, there was no significant difference in the mucosal triglyceride, diglyceride, monoglyceride and phospholipid, fatty acid, and cholesterol ester composition between the rGLP-1 and control groups.

Effect of rGLP-1 Infusion on Lymphatic Output of Apo B and Apo A-IV

Figure 5 shows lymphatic apo B and apo A-IV outputs at different time points of rGLP-1 infusion. Lymphatic apo B and apo A-IV outputs were significantly lower in the rGLP-1 than in the control group \((P < 0.05)\).

DISCUSSION

The ileal brake phenomenon occurs when nutrients passing into the ileum stimulate the release of GI hormones that exert feedback effects on the upper gut \((19)\). GLP-1 is one of the hormones thought to play a role in the ileal brake effect \((24)\). Some effects of GLP-1 consistent with this role, such as the inhibition of gastric acid secretion and emptying, have been well documented \((2, 29, 35, 42, 45)\). However, GLP-1 actions on the absorption of nutrients in the small intestine have not been thoroughly studied. In this study, we demonstrate effects of exogenous GLP-1 on lymph flow, lipid absorption, and apolipoprotein synthesis in the small intestine with our well-established intestinal lymph duct cannulation model. Because lipid was infused directly into the duodenum, we were able to examine intestinal functions directly without confounding from gastric effects of GLP-1. Our findings suggest that GLP-1 may have a greater role in intestinal function than was previously appreciated.

The most dramatic of our observations was the decrease of lymph flow in rGLP-1-treated animals. This occurred even in the presence of intestinal lipid absorption, which increased the rate of lymph flow in the control animals. On the basis of our experimental approach, we cannot determine the specific mechanism by which GLP-1 mediates lymph flow, but several possibilities exist. Lymph flow in skeletal muscle is largely dependent on muscular contraction and movement, so one possible explanation for the decreased lymph flow with rGLP-1 administration might be the inhibitory effect of GLP-1 on gut motility \((18, 33)\). If this were the cause for the dramatic decrease in lymph flow, we would have predicted a significant retention of fluid in the mucosa and/or lumen in the rGLP-1-treated animals. During the 6 h of infusion, 18 ml of solution were infused into the duodenum. An average of 18.05 ml was recovered from the lymph of the control group, and only 8.80 ml were recovered from the rGLP-1-treated animals. Despite...
this difference in lymph recovery, we observed neither a significant difference in the amount of liquid remaining in the lumen nor perceptible liquid retention (edema) in the mucosa of the whole intestine of either group (data not shown). Instead, the remaining portion of the infused liquid could be accounted for by increased urine output (data not shown). Indeed, GLP-1 has diuretic and natriuretic properties (32), and another possible explanation for the decrease in intestinal lymph flow is a decrease in plasma volume secondary to diuresis. However, studies by others demonstrated an increase, rather than a decrease, in intestinal and thoracic duct lymph flow in animals treated with diuretic agents such as mannitol, ethacrynic acid, and furosemide (31, 43). Another factor that can affect intestinal lymph flow is an increase in central vein pressure, with a greater pressure gradient for lymphatic emptying. This possibility could be ruled out, because in the animals in our study the intestinal lymph duct was cannulated. We believe the most likely reason for reduced intestinal lymph flow in the rGLP-1-treated animals is an effect of rGLP-1 on the microcirculation. Under normal conditions, the intestinal lymph flow in rats is fairly constant at approximately 3 ml/h, even without liquid infused into the intestine. This rate of lymph flow results from differential amounts of fluid extravasated from the arterial portion of the capillaries and reabsorbed at the venous side of the capillaries. This flow rate is further regulated by factors such as the tone of the arterioles and venules, the permeability of the capillary, the hydrostatic and oncotic pressures of the plasma, and the interstitial fluid. GLP-1 affects blood pressure (5, 8) and endothelial function (34). Therefore, it is possible that the decrease in intestinal lymph flow in the rGLP-1-treated animals may be a result of vascular effects of GLP-1 signaling, a hypothesis that bears further investigation.

A second novel observation from this study was the significant inhibition of triglyceride (triolein) absorption by rGLP-1. This effect seems unlikely to be a result of the effect of rGLP-1 on intestinal lymph flow. Following intestinal lymphatic drainage, triglycerides are transferred to the thoracic duct lymph. This lymph flow is further regulated by factors such as the tone of the arterioles and venules, the permeability of the capillary, the hydrostatic and oncotic pressures of the plasma, and the interstitial fluid. GLP-1 affects blood pressure (5, 8) and endothelial function (34). Therefore, it is possible that the decrease in intestinal lymph flow in the rGLP-1-treated animals may be a result of vascular effects of GLP-1 signaling, a hypothesis that bears further investigation.

Table 1. Relative distribution of 3H radioactivity in lipids in lumen and mucosa of upper small intestine of rats treated with or without rGLP-1

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Luminal</th>
<th>Mucosal</th>
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<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>rGLP-1</td>
</tr>
<tr>
<td>TG</td>
<td>11.08±1.32</td>
<td>15.79±2.55*</td>
</tr>
<tr>
<td>DG</td>
<td>7.57±0.56</td>
<td>9.60±0.26</td>
</tr>
<tr>
<td>MG + PL</td>
<td>32.08±0.79</td>
<td>28.39±1.49†</td>
</tr>
<tr>
<td>FA</td>
<td>49.27±2.02</td>
<td>46.22±1.31</td>
</tr>
<tr>
<td>CE</td>
<td>2.02±0.13</td>
<td>1.81±0.15</td>
</tr>
</tbody>
</table>

Values are means±SE; expressed as percentage. Triglycerides (TG), diglycerides (DG), monoglyceride (MG), phospholipids (PL), fatty acid (FA), and cholesterol ester (CE) were extracted from luminal or mucosal samples and separated by TLC. 3H radioactivity was originally labeled on the 3 FAs of the infused triolein. rGLP-1, recombinant glucagon-like peptide 1-(7–36) amide. *P<0.05; †P<0.01 vs. saline (control) group (Student’s t-test).
to decrease lymph flow, because this should have reduced lymphatic cholesterol absorption as well, and this was not observed. One potential explanation for the selective inhibition of triglyceride absorption by rGLP-1 is its effect to inhibit lipase (47), because triglyceride, but not cholesterol, requires hydrolysis by lipase for absorption (36). This notion is supported by our analysis of the luminal lipid composition, which showed a significantly higher percentage of triglyceride but less monoglyceride in the lumen of rGLP-1-treated animals at the conclusion of the experiment.

Other factors may also have contributed to the decreased lymphatic trefoilin output in rGLP-1-treated animals. In these rats, the amount of triolein was increased not only in the lumen but also in the mucosa. However, there was no difference in the mucosal triglyceride, diglyceride, monoglyceride, or fatty acid composition between the rGLP-1 and control groups, suggesting that resynthesis of triglyceride in the enterocytes is normal. We did observe decreases in lymphatic apo B and apo A-IV output, and because these apolipoproteins are structural elements of chylomicrons, it is possible that the accumulation of triglyceride in the mucosa and the decreased secretion into the lymph were caused by their decreased availability. Although there was no significant difference in lymphatic cholesterol absorption, we did observe more cholesterol in the small intestine and less cholesterol in the cecum of the rGLP-1-treated animals. This may have been caused by inhibited intestinal motility in these animals. We infused the radiola beled trefoilin and cholesterol as one emulsion. The discrepancies between triglyceride and cholesterol in their lymphatic output and luminal and mucosal distribution in response to GLP-1 suggest that the uptake, processing, and secretion of triglyceride and cholesterol by enterocytes in the intestine are not always proportional and linked.

We found a significant inhibition of lymphatic apo A-IV output by rGLP-1. However, analysis of the mucosal apo A-IV mRNA by real-time PCR showed no significant difference between the rGLP-1 and control groups (data not shown). This suggests that the inhibition of apo A-IV production by rGLP-1 is at the posttranslational level. Interestingly, our previous study showed that peptide YY (PYY), another gut hormone also produced by intestinal L cells in response to nutrient digestion, stimulated intestinal apo A-IV synthesis without a change in apo A-IV mRNA level (27). GLP-1 and PYY share several functions, such as inhibition of gastric acid secretion (46), upper GI motility (2, 30), and food intake (6, 21). However, they also have some differences. For instance, synthesis of PYY, similar to apo A-IV, is more vigorously regulated by lipids (1, 37), whereas GLP-1, as an incretin, is more closely related to the metabolism of glucose and carbohydrates (11, 20). The active but opposite regulation of apo A-IV production by GLP-1 and PYY suggests that apo A-IV may play a role in further fine tuning or ultimately carrying out the functions of GLP-1 and PYY, not only on food intake, but also on carbohydrate or lipid metabolism.

In summary, these studies demonstrate that rGLP-1 reduces intestinal lymph flow, triglyceride absorption, and apolipoprotein synthesis. Together, these effects, along with the actions of GLP-1 on the stomach, tend to limit the release of triglyceride into the circulation after lipid-containing meals. It is unclear how these novel effects of GLP-1 on intestinal function fit in the context of its other physiological actions. However, it is interesting that these findings suggest parallel effects of GLP-1 to limit the excursion of lipids and glucose after meals.

GRANTS

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REFERENCES


