Using the lymph fistula rat model to study the potentiation of GIP secretion by the ingestion of fat and glucose

Wendell J Lu¹, Qing Yang², William Sun², Stephen C. Woods¹, David D’Alessio¹, ³, and Patrick Tso¹, ²
Departments of Molecular and Cellular Physiology¹, Patholgy² and Medicine³, University of Cincinnati, Cincinnati, OH.

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Corresponding author:
Patrick Tso, Ph.D.
University of Cincinnati
Department of Pathology and Laboratory Medicine,
Cincinnati, OH 45267
Phone: 513-558-2151
Fax: 513-558-4337
Email: tsopp@email.uc.edu

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) is an important incretin produced in the K cells of the intestine and secreted into the circulating blood following ingestion of carbohydrate and fat containing meals. GIP contributes to the regulation of postprandial insulin secretion and is essential for normal glucose tolerance. We have established a method of assaying GIP in response to nutrients using the intestinal lymph fistula model. Administration of Ensure, a mixed-nutrient liquid meal, stimulated a significant increase in intestinal lymphatic GIP levels that were ~3 fold those of portal plasma. Following the meal, lymph GIP peaked at 60 minutes ($P < 0.001$) and remained elevated for 4 hours. Intraduodenal infusions of isocaloric and isovolumetric lipid emulsions or glucose polymer induced lymph GIP concentrations that were 4 and 7 fold basal levels, respectively. The combination of glucose plus lipid caused an even greater increase of lymph GIP than either nutrient alone. In summary, these findings demonstrated that intestinal lymph contains high concentrations of GIP that respond to both enteral carbohydrate and fat absorption. The change in lymphatic GIP concentration is greater than the change observed in the portal blood. These studies allow the detection of GIP levels at which they exert their local physiological actions. The combination of glucose and lipid has a potentiating effect in the stimulation of GIP secretion. We conclude from these studies that the lymph fistula rat is a novel approach to study in vivo GIP secretion in response to nutrient feeding in conscious rats.
INTRODUCTION

Gastric inhibitory polypeptide, GIP, was discovered in 1973 on the basis of its ability to inhibit acid secretion in gastric pouches (6, 7, 15). Subsequent studies have demonstrated that GIP is a potent insulin secretagogue and has minimal effects on physiologic gastric secretion. Thus the peptide was renamed glucose-dependent insulinotropic polypeptide (27). GIP was the first GI hormone discovered that met the criteria for an incretin (12, 58), i.e. a gut factor released after meals that stimulated insulin secretion at physiologic concentrations. Subsequent studies have shown that GIP is essential for normal glucose tolerance (47, 61).

GIP is secreted by enteroendocrine K cells in the duodenum and jejunum in response to carbohydrate and fat ingestion (35). Carbohydrate-induced GIP secretion is stimulated by the transport of monohexoses such as glucose and galactose released during digestion (59). Fat-stimulated GIP secretion occurs in response to long-chain fatty acids and triglycerides but not to short- or medium-chain fatty acids (44). The release of GIP following meals is proportional to meal size (65) suggesting that it acts as an internal feed-forward signal of the amount of ingested nutrients that will be absorbed.

Studies in obese diabetic ob/ob mouse model point to a clear link among hyperphagia, consumption of a high-fat diet, K-cell hyperplasia and increased circulating GIP concentrations (1). GIP also regulates fat metabolism in adipocytes, including stimulation of lipoprotein lipase activity (19, 42, 49), fatty acid incorporation into adipocytes and fatty acid synthesis (70). These observations suggest that GIP may be a key molecule linking overnutrition to obesity. Evidence from experiments using GIP receptor-deficient (GIPR\textsuperscript{−/−}) mice further support the hypothesis that GIP not only affects glucose homeostasis but also plays a key role in the development of obesity. GIPR\textsuperscript{−/−} mice manifested glucose intolerance and reduced insulin secretion as well as resistance to the development of obesity when chronically fed a high-fat diet (47). GIP does not seem to affect feeding behavior directly (69). As a consequence of its ability to increase insulin secretion during meals, GIP has recently come under increasing investigation as a
potential therapeutic agent for the treatment of type II diabetes, obesity and related metabolic disorders (28, 36).

The study of gastrointestinal (GI) hormone secretion has primarily relied on the measurement of circulating levels of GI hormones in the systemic blood or, more rarely, in the hepatic portal blood (34). However, plasma levels of GI hormones in general are quite low, and their half-life is short. In the case of GIP, it is rapidly degraded \textit{in vivo} by the ubiquitous enzyme, dipeptidyl peptidase IV (DPP-IV), forming a metabolite, GIP-(3-42), which has insignificant insulinitropic activity (39). In systemic plasma, the half-life of infused GIP is short, ranging from 7 to 5 min in normal and diabetic human subjects, respectively (14). In addition to the inactivation of GIP by DPP-IV, GIP is also rapidly cleared from the circulation via the kidney. In contrast, intestinal lymph may provide a better site for monitoring the secretion of GIP. The possibility that intestinal lymph contains significant amounts of GIP, and that this pool could be sampled to examine the secretory dynamics of this peptide has not been studied previously. Our preliminary data indicated that the concentration of GIP is significantly higher in intestinal lymph than in hepatic portal blood. We hypothesized that the lymph fistula rat can be an excellent model for studying the secretion of hormones by the GI tract because of the much lower lymph flow rate (2 – 3 ml/h) relative to portal blood flow (8 – 20 ml per min) (33, 57). The difference in flow rate is 160-fold to 400-fold greater in the portal vein versus lymph. Hence, there is much less dilution of the GI hormones secreted by the enteroendocrine cells of the gut in lymph than in portal blood. The purpose of this study was twofold: 1) to validate that the lymph fistula rat model as a paradigm to study GIP secretion \textit{in vivo} in response to the absorption of nutrients; and 2) to determine the effect an isocaloric and isovolumetric meal containing either lipid, or dextrin, or a mixture of lipid and dextrin on the lymphatic GIP secretion. A thorough understanding of the secretory response of GIP to macronutrient absorption is physiologically and clinically relevant and may offer opportunities in GIP therapy for the treatment of type II diabetes.

**MATERIALS AND METHODS**
**Animals and 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. Adult male Sprague-Dawley rats, weighing 240-350 g (Harlan, Indianapolis, IN), were used. Animals were allowed to accommodate to our animal facility on regular rodent chow for two weeks prior to the experiment. Animals were fasted overnight before surgery. For portal vein cannulation, rats had silicone rubber tube (1 mm OD) installed in the portal vein starting just proximal to the confluence of the subportal vessels, or the jugular vein. These cannulas were passed through the abdominal wall and exteriorized at the dorsum of the neck. Following portal vein cannulation surgery, animals were allowed to recover for 5 days and their body weight measured as an indication of their conditions before lymph duct or intraduodenal cannulation was performed. The lymph duct or intraduodenal cannulation was performed as described previously (46). Briefly, 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) (Dural Plastics and Engineering, Dural, Australia) according to the method of Bollman et al. (5) with slight modification. Intraduodenal cannulation was performed 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 for the duodenum and the fundal incision was closed by a purse-string suture. Post-operatively, the animals were infused with 5% glucose in saline and switched to saline infusion alone at 16 hours before the nutrient study as described in previous studies (46). Fasting lymph was collected for 1 hour before nutrient infusion and at 30-min intervals over the subsequent 6 hours. At the time of sample collection, DPP IV inhibitors were added to prevent degradation of GIP in the samples (Sigma, St. Louis, MO).

**Nutrient Infusate Preparation:** In the lymph versus hepatic portal plasma study of GIP, animals were administered intraduodenally a 3 ml (4.43 Kcal) bolus of Ensure (Abbott Laboratories, North Chicago, IL) which is a mixed meal consisting of fat, carbohydrate and protein. In the nutrient study, animals were infused intraduodenally with a single bolus with 3 ml of one of the following four infusates: 1) 3 ml of saline (0.9%, control group); 2) 3 ml of Liposyn consisting of 2.215 ml of Liposyn (20%) and 0.785 ml of
saline; 3) 3 ml of dextrin made of 1.1075 gram dextrin in phosphate buffered saline at pH 6.4; and 4) Liposyn plus dextrin (isocaloric group) composed of equal volumes of half of the above Liposyn and dextrin infusions. Dextrin instead of glucose was used to avoid the potential complication due to the high osmolality in the lumen. With the exception of the saline control group, all nutrient groups received exactly 4.43 Kcal/3 ml. Nutrient infusions were followed by infusion of saline at 3 ml per hour. Liposyn (20%), also known as Liposyn II (20%), (Abbott Laboratories, North Chicago, IL) consisted of equal amounts of safflower and soybean oil with a 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.

**GIP ELISA.** GIP was determined by ELISA assay kit provided by Linco (St. Louis, MO) that detects both intact GIP and its major metabolite GIP<sub>3-42</sub>. Briefly, lymph or plasma samples are added to wells of a microtiter plate pre-coated with anti-GIP monoclonal antibodies. GIP molecules in the samples are captured by the antibodies. A second biotinylated anti-GIP polyclonal antibody is added and bound to the captured GIP. Streptavidin-Horseradish peroxidase is then conjugated to the immobilized biotinylated antibodies. The immobilized antibody-enzyme conjugate is monitored by horseradish peroxidase activities in the presence of the substrate tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by absorbency at 450 nm.

**Validation of GIP ELISA for Lymph Samples.** To test for the linearity of GIP ELISA in lymph, serial dilutions (undiluted, 1:1, and 1:2) of lymph were made and then assayed. A recovery study was performed to assess the sensitivity of the assay by adding known amounts of GIP at varying concentrations to lymph samples. Nonspecific contribution to the ELISA was estimated by assaying lymph samples treated with or without activated charcoal. Following overnight incubation, the samples were centrifuged and supernatant was carefully pipetted off the top for assay.

**Quantitative Assays.** Lymph triglycerides were measured according to the protocol provided by Randox (Randox Laboratories Ltd., Crumlin, UK). This enzymatic assay
measured the released glycerol resulting 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. Glucose in lymph and plasma samples was assayed by the glucose oxidase/peroxidase method as described by Trinder (60) using the kit from Diagnostics Chemicals Limited (Canada). The colorimetric assay measured the reaction product, quinoneimine, spectrophotometrically at 505 nm.

**Statistical Analysis:** All values are expressed as means ± SE. Two-way repeated-measures ANOVA with Tukey’s as a posttest analysis was used to compare all the groups, e.g. dextrin and Liposyn, throughout the 8-hr infusion. The analyses examine the statistical significance between groups as well as among different time points within the group. A difference was considered significant if the $P$ value was <0.05. All statistical analyses were carried out by using the statistics program SigmaStats version 3.1 (SPSS).

**RESULTS**

**Validation of GIP ELISA in Lymph**

Lymph samples collected following dextrin administration were assayed undiluted or after 1:1 or 1:2 dilutions in assay buffer. The GIP concentrations for these dilutions were 199 ± 8 pM, 97 ± 12 pM, and 61 ± 3 pM. Recovery of authentic GIP added to lymph was high. For standards that should read 2000, 666.7, and 222.2 pg/ml in the assay volume recoveries from lymph were 94%, 91%, and 90%, respectively. Nonspecific contributions to the GIP assay from other constituents of lymph were assessed by incubating samples overnight with activated charcoal. Samples treated overnight with charcoal had a mean GIP concentration of 2.2 ± 0.37 pM compared to the untreated samples which measured 181 ± 3.98 pM. These data demonstrate that a conventional assay of GIP performs well in intestinal lymph.
**Effect of Nutrient Stimulation on Lymphatic GIP Concentrations**

The secretion of GIP into lymph and plasma was compared in rats with lymph fistulas and intraportal catheters. Ensure was given in a dose of 3 ml containing 4.43 Kcal and blood and lymph was sampled over 4 hours. Both lymph and plasma exhibited rapid increases in GIP concentration that was apparent at the first sampling at 30 min. GIP concentration in portal plasma reached a peak of 603 ± 244 pg/ml at 60 min (Fig. 1) and then decreased gradually to baseline by 120 min. In lymph, GIP peaked at 1733 ± 257 pg/ml at 60 min, a value that was significantly greater than the contemporaneous level in portal plasma (P < 0.001). GIP in lymph was still significantly higher than fasting at a concentration of 1276 ± 464 pg/ml at 120 min (P = 0.003, Fig. 1) and did not return to fasting levels until 240 min. During the 4-hr study, the mean GIP concentration in lymph was significantly greater than that in portal plasma (P = 0.014). Ensure is a mixed meal containing carbohydrate, fat and protein, next we examined the effect of carbohydrate, fat, and the combination of carbohydrate plus fat.

To test the response to specific nutrients, GIP concentration was assayed in lymph following intraduodenal bolus of Liposyn (20%). Liposyn was administered at the dosage of 4.43 Kcal/3 ml, a caloric amount identical to that used in the study with Ensure. **We like to emphasize that we collected lymph for a period of time and so the concentration of GIP we measured represented the mean GIP concentration of that collection period.** Fasting lymph GIP concentration was 132 ± 19 pM and increased rapidly in response to Liposyn with a significant increase apparent by 30 min compared to the saline control group (P = 0.007) and a peak concentration at 60 min (441 ± 144 pM, P < 0.001; Fig. 2). The mean lymph GIP level remained significantly elevated versus saline control between 60 and 120 min (P = 0.004) and over the entire 6-hr experimental period (P = 0.028). It should be noted that the lymph GIP response observed with intraduodenal infusion of Liposyn is much smaller than that observed with Ensure, despite the fact that the same number of calories were delivered.

In a separate group of animals, dextrin was given as an intraduodenal bolus that was isocaloric and isovolumetric to the Liposyn and Ensure meals (4.43 Kcal/3 ml).
Control animals received an equivalent amount of saline and lymph was sampled for 6 hr. The dextrin, but not the saline, caused lymph GIP concentration to increase by 30 min reaching a peak of 813 ± 118 pg/ml by 60 min (Fig. 2; \( P < 0.001 \) relative to baseline). The increment of GIP was sustained until 120 min (506 ± 145 pg/ml; \( P < 0.001 \)) and then dropped rapidly and was not different than the saline group after 120 min. The mean difference between the two groups was significantly different over the 6-hr assessment following dextrin \( (P = 0.003) \).

The effect of the mixed nutrient meal, Ensure, on GIP secretion was greater than of the response to equivalent caloric loads of liposyn or dextrin. This suggested that combinations of nutrients could have a potentiating effect on the secretion of GIP. To test this question more precisely, a group of rats was given a mixed nutrient bolus of Liposyn and dextrin that was isocaloric and isovolumetric to the individual nutrient challenges used in the previous experiments (4.43 kcal/3 ml). The nutrient mix was prepared with each nutrient contributing half of the total 4.43 Kcal/3ml dose. In these animals, there was a significant increase in lymph GIP at 30 min. following administration of the nutrients with GIP concentration reaching 731 ± 42 pg/ml \( (P < 0.001, \text{ Fig. 2}) \). Peak lymph GIP concentration was reached by 60 min. \( (1618 ± 82 \text{ pg/ml; } P < 0.001) \) and the lymph GIP concentration decreased gradually to 449 ± 32 pg/ml but was still significantly greater than baseline \( (P < 0.001) \) until 2 hr following the infusion. Over the entire 6-hr interval, the combined Liposyn plus dextrin group had elevated lymph GIP compared to the saline group \( (P < 0.001) \). The combination of Liposyn plus dextrin caused a greater increase of lymph GIP than either nutrient alone \( (\text{Fig. 2}) \). This was true at 30 min as well as 60 min \( (\text{Fig. 2}) \). After 60 min, GIP levels were not different among the nutrient-stimulated groups.

**Effect of Nutrient Infusion on Lymph Flow**

Lymph flow rates in response to intraduodenal infusion of saline, Liposyn, dextrin or the combination are shown in Figure 3. Liposyn induced a peak lymph flow rate of 3.75 ± 0.61 ml/hr at 30 min., but the difference was not significant compared to that of the saline control group \( (P = 0.677, \text{ Fig. 3}) \). Dextrin caused an initial decrease of lymph
flow at 30 min to 1.14 ± 0.17 ml/hr. and this was also not significant relative to saline control group ($P = 0.073$). After 30 min., the lymph flow rate following dextrin started to increase and was similar to that of the saline control group for the remainder of the 6-hr experiment. The combined Liposyn plus dextrin caused a peak stimulation of lymph flow of 4.96 ± 0.58 ml/hr at 60 min. ($P < 0.001$ relative to saline, Fig. 3). Over the entire duration of the 6-hr observation, no significant differences occurred among the lymph flow rates of the three nutrient groups versus the saline group.

**Effect of Nutrient Stimulation on Lymphatic GIP Output**

GIP secretion can be calculated by multiplying the lymph flow rate and mean GIP concentration of the sample collected during that period. The earliest peak appeared in response to Liposyn stimulation at 30 min with a secretion rate of 1159 ± 393 pg/hr (Fig. 4). The increase was sustained throughout 120 min. Dextrin stimulated peak GIP secretion of 2410 ± 566 pg/hr at 60 min and the increase was significant versus control saline with $P < 0.001$. At 120 min, GIP secretion was still elevated at 1647 ± 673 pg/hr, but was not significant relative to saline ($P = 0.07$). After 120 min, the effect of dextrin decreased rapidly reaching baseline level by 180 min. The combination of Liposyn plus dextrin at isocaloric and isovolumetric dosage stimulated GIP secretion as early as 30 min at the rate of 2094 ± 241 pg/hr. A significant increase was observed compared to both the saline control group ($P = 0.005$) and to the dextrin group ($P = 0.004$). Similar to dextrin, the peak GIP secretion for the combined group occurred at 60 min. reaching 8027 ± 1057 pg/hr (Fig. 4). This increase was significantly greater than the other three groups including saline control, dextrin as well as the Liposyn group with $P < 0.001$, respectively. However, the concentration dropped dramatically by 120 min. and returned to baseline for the remainder of the study. Over the duration of the 6-hr study, significant differences in mean GIP secretion rate were observed between the combined Liposyn plus dextrin group and the control saline group ($P < 0.001$) as well as between the combined group and the Liposyn group ($P = 0.029$).

Since most of the GIP secretion occurred during the first three hours following bolus administration of nutrients and secretion has returned to baseline
by 180 min, we examined the cumulative GIP secretion over this time period. The Liposyn group had a cumulative GIP secretion of 4382 ± 132 pg for the 3 hr interval while the dextrin group showed a similar secretion of 5044 ± 398 pg (Fig. 5). The combined Liposyn plus dextrin group displayed a cumulative GIP secretion of 12333 ± 1212 pg which was significantly greater than that of the control saline group of 962 ± 56 pg ($P < 0.001$). The difference between the combined group and the Liposyn or dextrin group was also significant respectively ($P < 0.001$).

**Effect of Nutrient Stimulation on Lymph Triglycerides and Glucose**

Lymph triglycerides (TG) levels in response to the various nutrients were examined. No significant change was observed in the saline or dextrin groups and so only the data from the saline group are shown in the figure (Fig. 6). The lymphatic TG output started to increase as early as 30 min in the Liposyn group but the effect was not significant versus saline control until 60 min ($P = 0.041$). By 60 min., the TG level of the combined Liposyn plus dextrin group also increased significantly versus saline control reaching 801 ± 56 mg/dl ($P = 0.007$). The lymphatic TG level of the Liposyn group continued to rise and peaked at 300 min reaching 1409 ± 169 mg/dl. The lymph TG level for the Liposyn group was significant with $P < 0.001$ during the second through the sixth hour with respect to dextrin or control saline (Fig. 6). For the combined Liposyn plus dextrin group, the TG level increased steadily until it reached an apex of 1186 ± 117 mg/dL at 120 min and remained significantly greater compared to the control groups throughout 240 min.

Following the intraduodenal infusion of dextrin there was an increase in lymph glucose with a peak concentration at 60 min. of 217 ± 8 mg/dl. This increase was significant compared to the control saline ($P < 0.001$) and a significant difference was maintained through 120 min ($P < 0.001$, Fig. 7). There was no significant difference in lymph glucose concentration between the Liposyn group and the saline control group (data not shown). The combined Liposyn plus dextrin group also reached by 60 min a mean peak lymph glucose level of 270 ± 19 mg/dl (Fig. 7) that was significant compared to saline control ($P < 0.001$), and remained significant through 120 min. ($P < 0.001$).
Over the entire 6-hr experiment, the mean lymph glucose level of the Liposyn plus dextrin group was significantly greater than that of the control group \( (P = 0.001) \).

**DISCUSSION**

In this study, we used the lymph fistula rat model to study the secretion of GIP in response to intestinal absorption of lipid and carbohydrate. Our findings indicate that the dynamics of GIP release are similar in lymph compared to plasma, although generally magnified. In fact, lymph GIP concentrations are considerably higher than those of plasma from the hepatic portal vein, the region of the blood stream where levels of GIP are highest. These results indicate that the lymph fistula model may have some advantages for studying GIP secretion, and possibly the release of other gut hormones.

This is the first report of the role of intestinal fat and glucose in stimulating the secretion of GIP using lymph fistula rat as a model system. The GIP assay used in these studies performed adequately and intestinal lymph appears to be rich in GIP. The lymph fistula models present two major advantages for studying the secretion of this GIP. First, the high concentrations can provide increased sensitivity for detecting changes in response to nutrients, similar to what we have shown in these studies. Second, sampling of lymph can provide some insight into the local concentrations of peptide that exist in the fluid compartments of the intestinal mucosa, where the peptide might interact with other endocrine cells or with the sensory components of enteric and autonomic neurons.

The caloric dosage of fat and/or glucose we administered was selected because in other experiments it caused a robust increase of lymph glucagon-like peptide-1 (GLP-1) secretion \((13, 46)\). The levels of lymph GLP-1 that we measured in that study are much higher than plasma concentrations of GLP-1 that have been reported previously \((38, 53)\). In our previous studies, we demonstrated that following intraduodenal administration of fat or carbohydrate, GLP-1 levels in lymph are significantly higher than in portal plasma \((13)\). The present findings indicate that GIP is another GI hormone which seems to be
present in intestinal lymph at higher concentration than in portal plasma, rendering the intestinal lymph as a potentially more sensitive site for monitoring GIP secretion than measuring the changes in blood GIP concentration.

The substantially greater level of GIP in lymph than portal plasma suggests that lymph GIP is not derived from blood plasma by filtration, since this would be expected to cause lower levels and a delay in peaks. Instead it is more likely that the GIP measured in lymph represents peptide moving from the K-cells into lacteals. While submucosal blood capillaries are generally thought to be in closer proximity to mucosal cells than lacteals (2), our results are consistent with either spillover of GIP that is not collected in the blood, or targeted secretion to lymph. Further studies will be needed to distinguish between these possibilities.

We tried to minimize the effect of variability in the rate of gastric emptying by administering nutrients intraduodenally. Secretion of GIP is potentially dependent on a number of factors and especially the rate of entry of nutrient into the small intestine (56). The rate of gastric emptying may in turn be modulated by the previous diet (37). For example, the slower rise in plasma GIP following fat ingestion may be related to the slower rate of gastric emptying and absorption of fat relative to carbohydrate (22, 68, 66). Here, we eliminated the effect of variability in the gastric emptying rate by introducing the nutrients directly into the duodenum. This is a notable methodologic advantage of our study since we compared the effects of liquid meals of lipid and carbohydrate that would be expected to have differing entry rates into the intestine if administered through the stomach. Because we tested the effects of lipid and/or glucose infused directly into the duodenum we were able to determine that at the level of the intestinal mucosa glucose elicits a more rapid release of GIP than does lipid. This novel observation suggests that the two nutrients activate K-cells by distinct mechanisms and is consistent with their potentiating effect when administered in combination.

Intraduodenal administration of Liposyn at dosage of 4.43 Kcal/3 ml caused a significant increase in lymph GIP concentration compared to the saline control consistent
with reports of GIP stimulation in plasma in response to fat in humans reaching a peak between 60-120 min (24, 49). The timing of the GIP response is consistent with its acting as an incretin and stimulating insulin secretion. GIP was shown to potentiate glucose-dependent insulin secretion through mechanisms involving activation of cAMP/Rap1, cAMP-GEFII-Rim2, and phospholipase A2 (21, 40, 62). GIP stimulation of insulin secretion or proliferation of insulin secreting cell line via its G protein-coupled receptor has also been reported to involve activation of the mitogen-activated protein kinases (MAPK) (4, 21).

Likewise, an isocaloric and isovolumetric bolus of intraduodenal dextrin caused a similar pattern of lymph GIP as did Liposyn. Dextrin, a complex glucose polymer, was used instead of glucose monomer to minimize any effects of osmolarity on GIP secretion. The particular dextrin used has a molecular weight which is 83-fold greater than glucose. Similar to Liposyn, dextrin induced a peak GIP concentration at 60 min. The stimulation following dextrin was relatively greater than with Liposyn. The combined administration of fat and carbohydrate proved to be an even more potent stimulus for lymph GIP levels than the administration of the individual nutrients. The combination of Liposyn plus dextrin induced a 16-fold increase in peak GIP lymph concentration. This suggests that the products of lipid and carbohydrate digestion are synergistic in stimulating GIP secretion by the K cells. This result is supported by the GIP response to Ensure, a mixed nutrient solution. The results of the present study are consistent with previous reports where co-ingestion of fat with carbohydrate increased the GIP response in plasma beyond that produced by either nutrient alone (9, 11) in humans. A similar level of potentiation was observed in those previous studies. In the presence of both fat and carbohydrates, 19-fold and 14-fold stimulation of plasma GIP concentration was achieved using different carbohydrates (9), while only 5-fold and 3-fold increase was observed in the presence of either nutrient alone. There are a number of possibilities which could explain this potentiation by lipid and carbohydrate together. One possibility is that the combination of liposyn and dextrin caused increased absorption of nutrients and that this increased GIP secretion. Although there was no significant difference in the lymph TG levels between animals that were given Liposyn only versus those that were given Liposyn plus dextrin,
the amount of lipid infused into the gut was half as large in the latter experiment. One interpretation of this finding of equal lymph TG with a lower enteral lipid load is that lipid absorption was faster in the mixed nutrient experiment. A similar argument can be made about glucose absorption since lymph glucose was similar with and without the addition of Liposyn despite the 2 fold difference in glucose loads. Previous studies have suggested that digestion of carbohydrates and absorption of glucose and other hexoses by enterocytes is necessary for GIP secretion \textit{in vivo} (3, 17, 26). In addition, inhibition of carbohydrate digestion (25, 29, 52) or interference with intestinal glucose transport (59) significantly attenuates GIP release in response to nutrients. Similarly, digestion of triglyceride seems to be critical for lipid stimulated GIP release, and the response is selective with long-chain, but not medium-chain, fatty acids causing secretion (55). If rates of GIP release are driven by rates of nutrient absorption, changes in lipid/glucose uptake by the combined Liposyn/dextrin load could account for increased lymph GIP.

The potentiation by combined nutrient administration could also be due to different and complementary stimulation of the enteroendocrine K cells by fatty acids and glucose that result in synergy when both are present. The mechanism of nutrient activation of K-cells is not completely understood. GIP secretion in response to nutrients has been examined using gut K cell lines engineered to express human insulin gene (GIP/Ins cells). Studies with GIP/Ins cells indicate that the mechanism of GIP release is independent of $K_{ATP}$ channel (67). Release of GIP in response to nutritional stimuli was shown to be regulated by PKC and AMPK-related kinases (45). However, not much is known about the interaction of lipid and carbohydrate stimulus to the K-cell. Whether the nutrients interact directly with the K cells or through mediators released by the enterocytes remain to be elucidated. Our current results suggest that the lymph fistula model may be useful in studying the process of nutrient stimulated GIP in that it provides a means of monitoring intestinal substrate fluxes, while providing a sensitive measure of the outcome since GIP concentrations in lymph are so high.

There were no differences in lymph flow rates during fasting among animals in the various conditions. The steady and similar lymph flow rates reflect the healthy condition of the animals following surgery. Lymph triglyceride increased only in the two
groups containing Liposyn, the combined and the Liposyn groups. Lymph TG concentrations gradually increased and peaked at 300 min before plateauing indicating that it requires 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 (31, 63). As shown in the lymphatic TG and lymphatic GIP output, while fat absorption stimulates GIP secretion, it is not the transport of lipid per se that was responsible for the stimulation of GIP secretion. If it is the amount of lipid transported by the gut that is responsible for the stimulation of GIP secretion, we would expect a smaller GIP secretion in animals infused with Liposyn plus dextrin than the Liposyn only group. But the data suggested the opposite, and we would also expect the GIP secretion to peak later but in fact lymphatic GIP peaked much earlier. The rapid GIP response to the Liposyn infusion implies that lipid absorption, but not lipid transport, is associated with the stimulation of GIP secretion.

The lymph fistula model showed that there were higher levels of incretins in lymph compared to the blood. This is important in studying the physiological role and significance of incretins. GIP was demonstrated to inhibit glucagon-stimulated glucose production at physiological hormone concentration of 500 pg/ml or 0.1 nmol/l (32). The levels of GIP present in lymph exceeded 500 pg/ml during the period from 60 min through 240 min following the administration of a mixed meal (Fig. 1). However, in the portal plasma this concentration was reached only at the peak time point of 60 min and rapidly declined thereafter. This demonstrates the importance of the incretin concentration in lymph. The concentrations of incretins to which the enteric cells and neurons in the lamina propria and submucosal spaces of the gut are exposed are more accurately reflected by concentrations detected in lymph than in the blood plasma. The higher levels of GIP measured in lymph are also the concentrations which can elicit physiological responses as in the case of glucose production. Compared to systemic circulation, the incretin levels observed in portal plasma is greater since it is the first site where incretins are transported following their release in the gut. Due to the rapid degradation of GLP-1 and GIP
by DPP-IV, studies have reported incretin concentrations in the following decreasing order of portal vein, inferior vena cava, and tail vein (38) consistent with the short half-lives of incretins. The majority of GLP-1 and GIP are degraded by the time they reach the arterial blood stream to target the organs. Since there is evidence that GI hormones mediate some of their effects locally and incretins are rapidly degraded, it is important to be able to detect the concentrations at which they exert these actions. Therefore, the levels of incretins detected in lymph more accurately reflect the concentrations which are of physiological importance relative to those in the portal or systemic circulation.

It is interesting to compare the secretory behavior of lymph GIP to that of GLP-1 in our previous studies as both incretins are released in response to fat and carbohydrate. Both GLP-1 and GIP had higher levels in the gastrointestinal lymph compared to those in hepatic portal plasma following administration of Ensure, a mixed meal of fat, glucose, and protein (13). However, there were a number of differences in the profiles of the two hormones. First, stimulation of GLP-1 was more rapid and peaked by 30 min compared to that of GIP peaking at 60 min (46). This interesting observation makes one wonder about the physiological importance of GIP stimulated GLP-1 secretion (54). Second, the level of increase between the peak lymph and peak portal plasma was greater for GLP-1 concentration at six-fold relative to three-fold for GIP concentration. Third, lymph GIP levels were significantly greater than in portal plasma through 120 min while GLP-1 levels were greater only during the initial 60 min. Therefore, in response to a mixed meal, GLP-1 demonstrated a more rapid, robust and short-lived response than GIP. This is similar to what has been described previously in the plasma of humans and consistent with GLP-1 release being driven by high rates of nutrient appearance in the gut, while GIP secretion is a function of nutrient uptake (56).

Stimulation of incretins by individual nutrients revealed some of the similarities they share. Both GLP-1 and GIP secretion peaked by 30 min following Liposyn administration (46). Dextrin induced peak secretion of both incretins by 60
min. However, there were also some differences in their responses to fat or glucose. In spite of the same peak time for the two hormones, GLP-1 peak stimulation by Liposyn was ten-fold greater than fasting versus four-fold for GIP. In contrast, with dextrin the GIP peak response was nine-fold higher than fasting compared to five-fold for GLP-1. Hence, peak stimulation of incretins was induced more rapidly by Liposyn than dextrin, and Liposyn was a stronger stimulant of GLP-1 while dextrin was a stronger stimulant of GIP.

We also reported the differential effects that combined administration of fat and carbohydrate has on the secretion of these incretins. GLP-1 exhibited an additive effect at its peak concentration and secretion compared to the effect of individual nutrient whereas peak GIP concentration and secretion had a marked potentiating effect which was triple that of the individual nutrients.

In summary, we have demonstrated that 1) intestinal lymph is enriched with GIP and offers the potential for continuous monitoring of GIP secretion and other gut hormones in vivo; 2) the difference in GIP secretion pattern between lymph and plasma may be due to the administration of nutrient intraduodenally versus orally or that lymph may be more reflective of the actual levels of GIP in the GI tract; and 3) the combination of Liposyn and dextrin has a huge potentiating effect on each other in the stimulation of GIP. The present study raises the possibility that in addition to GIP, there may be other gastrointestinal hormones whose secretion can also be studied using the lymph fistula rat model. The fact that the concentration of GIP, may be other GI hormones as well, is so much higher in lymph would suggest to us that the concentration of GI peptides that the intestinal neurons and cells in the lamina propria are exposed to are higher than appreciated previously.
REFERENCES


FIGURE LEGEND

1 Concenetrations of GIP in samples of portal plasma (■) and intestinal lymph (♦) taken before and after an intraduodenal Ensure bolus. Data are presented as mean ± SEM. Asterisks denote significant differences in lymph versus portal plasma GIP at specific times: ** \( P < 0.01 \); *** \( P < 0.001 \).

2 Comparison of stimulation of GIP concentration in response to isocaloric and isovolumetric treatment of Liposyn (■), dextrin (▲), Liposyn plus dextrin combined (♦) or saline (♦). Data are mean ± SEM. Asterisks indicate significant differences between each nutrient group and the saline control values at specific times: * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \).

3 The lymph flow rate during the 6-hour period following administration of intraduodenal infusion of Liposyn (■), dextrin (▲), Liposyn plus dextrin combined (♦) or saline (♦). No significant difference among groups was found over the 6-hour study. Data are presented as mean ± SEM. Asterisks indicate significant differences between each nutrient group and the saline control values at specific times: * \( P < 0.05 \).

4 Lymphatic GIP secretion rate in response to isocaloric and isovolumetric intraduodenal infusion of Liposyn (■), dextrin (▲), Liposyn plus dextrin combined (♦) or saline (♦). Data are means ± SEM. Asterisks indicate significant differences between each nutrient group and the saline control values at that time: ** \( P < 0.01 \); *** \( P < 0.001 \).

5 Cumulative lymphatic GIP output following bolus intraduodenal infusion of Liposyn (gray bar), dextrin (white bar), Liposyn plus dextrin combined (hatched bar) or saline (black bar). Values are sum of GIP secretion over the first 3-hr time course. Data are means ± SEM. Asterisks indicate significant differences between each nutrient group and the saline control values at that time or significant
difference in cumulative GIP output between the combined nutrient group and Liposyn or dextrin alone: ** \( P < 0.01; \) *** \( P < 0.001. \)

6 Triglycerides content in lymph collected hourly following isocaloric and isovolumetric intraduodenal infusion of Liposyn (■), Liposyn plus dextrin combined (●) or saline (♦). Data are means ± SEM. Asterisks indicate significant elevations of nutrient group over the saline control values at that time: * \( P < 0.05; \) ** \( P < 0.01; \) *** \( P < 0.001. \)

7 Glucose concentrations in lymph collected hourly following isocaloric and isovolumetric intraduodenal infusion of dextrin (▲), Liposyn plus dextrin (●) or saline (♦). Data are means ± SEM. Asterisks indicate significant differences between each nutrient group and the saline control values at that time: * \( P < 0.05; \) *** \( P < 0.001. \)
Figure 1

Time (min)

GIP (pg/ml)

Lymph (n=4)

Portal Plasma (n=4)

** P<0.01 *** P<0.001
Figure 2

- Liposyn (n=6)
- Dextrin (n=6)
- Liposyn + Dextrin (Isocal, n=6)
- Saline (n=6)

* P<0.05  ** P<0.01  *** P<0.001
Figure 3

- **Liposyn (n=6)**
- **Dextrin (n=6)**
- **Liposyn + Dextrin (Isocal, n=6)**
- **Saline (n=6)**

* $P < 0.05$
Figure 4

- Liposyn (n=6)
- Dextrin (n=6)
- Liposyn + Dextrin (Isocal, n=6)
- Saline (n=6)

** P<0.01  *** P<0.001
Figure 5

** Figure showing the comparison of GIP (pg) levels among different treatments:

- Saline
- Liposyn
- Dextrin
- Liposyn + Dextrin (Isocaloric)

Statistical significances:

- ** P<0.01
- *** P<0.001
Figure 6

- Liposyn (n=6)
- Liposyn + Dextrin (Isocal, n=6)
- Saline (n=6)

TG (mg/dL) vs. Time (min)

* P<0.05  ** P<0.01  *** P<0.001
Figure 7

- Dextrin (n=6)
- Liposyn + Dextrin (Isocal, n=6)
- Saline (n=6)

* P<0.05  ** P<0.01  *** P<0.001