Nutrient-driven incretin secretion into intestinal lymph is different between diabetic Goto-Kakizaki rats and Wistar rats

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Kindel TL, Yang Q, Yoder SM, Tso P. Nutrient-driven incretin secretion into intestinal lymph is different between diabetic Goto-Kakizaki rats and Wistar rats. Am J Physiol Gastrointest Liver Physiol 296: G168–G174, 2009. First published December 4, 2008; doi:10.1152/ajpgi.90506.2008.—The incretin hormones gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) account for ~50–70% of insulin secretion from the pancreas after a meal (28, 43). GIP is secreted from the enteroendocrine K cells that are distributed throughout the entire small bowel but are primarily concentrated in the duodenum (49). GIP secretion from K cells is stimulated by both enteral glucose and lipid (7, 21). GLP-1 is secreted from L cells in the distal small intestine and colon in response to enteral lipid, glucose, and vagal stimulation (12, 15). The specific intracellular mechanisms regulating incretin secretion from enteroendocrine cells after a meal have yet to be elucidated.

In obese patients with impaired glucose tolerance, GIP secretion is significantly higher than in healthy patients (43). With progression to Type 2 diabetes, GIP secretion is normal to high and GLP-1 secretion is low compared with euglycemic individuals after a mixed meal (44, 46). In addition, in both lean and obese diabetic patients, pancreatic GIP receptors show a markedly blunted stimulatory response to endogenous or exogenous GIP resulting in a lack of late-phase insulin secretion (29, 47, 48). There is also a decreased insulinotropic potency of GLP-1 in diabetic patients (19). Augmenting GLP-1 levels via dipeptidyl peptidase IV (DPPIV)-resistant analogs, DPPIV inhibitors, or surgical manipulation, have improved glucose homeostasis in Type 2 diabetic patients (1, 9, 10).

The Goto-Kakizaki (GK) rat is an inbred, polygenic, lean model of Type 2 diabetes mellitus derived from Wistar rats in the 1970s (18). GK rats develop Type 2 diabetes by 12 wk of age. Adult GK rats are characterized by fasting hyperglycemia, hyperinsulinemia, mild insulin resistance, and decreased pancreatic insulin mass and secretory capacity (13, 30). GK rats have been utilized by investigators to study many different aspects of Type 2 diabetes including the incretin changes after metabolic surgery and the effect of incretin modulation on insulin secretion and glucose homeostasis (33, 37, 40, 42, 45). However, baseline incretin secretion profiles in GK rats to various macronutrients have been largely unexplored.

Unfortunately, measuring incretins in systemic plasma has proven difficult because of assay detection sensitivity and the short plasma half-life of both GLP-1 and GIP (less than 2 min in rodents) (2). Our laboratory has shown that lymphatic measurement of incretins offers a sensitive medium for the detection of meal-induced hormone secretion (24, 25). Furthermore, GLP-1 appears to be selectively targeted to the lymphatic circulation (24, 25). It follows that lymphatic collections are a suitable means to assess incretin secretion in response to different macronutrient administration. Appropriately characterizing incretin secretion in these animals will add an essential understanding of their native, physiological secretion pattern that can then be applied to future, therapy-based, incretin studies in GK rats.

MATERIALS AND METHODS

Animals. Fourteen- to sixteen-wk-old male Goto-Kakizaki rats (Taconic, Germantown, NY) and age-matched Wistar rats (Charles River Laboratories, Wilmington, MA) were allowed to acclimate to their environment for 2 wk prior to the beginning of the experiment. Rats had free access to standard rodent chow and water except as noted for the experimental protocol. All procedures were approved by the University of Cincinnati’s Institutional Animal Care and Use Committee.

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Lymph and duodenal cannulation. Rats were fasted overnight but allowed free access to water. Under isoflurane anesthesia, the peritoneum was entered through a midline incision. The major mesenteric lymphatic duct was identified and cannulated with tubing (medical grade; 0.50 mm ID and 0.80 mm OD; Tyco Electronics, Castle Hill, Australia). The lymphatic tube was secured in place with a drop of cyanoacrylate glue. A small enterotomy was made in the anterior wall of the stomach along the greater curvature, and a second silicone tube (0.04 ID and 0.085 OD; VWR International, West Chester, PA) was placed into the stomach and advanced past the pylorus into the duodenum. The duodenal tube was secured with a purse-string suture. The abdominal wall was closed in two layers. Animals were kept in Bollman restraint cages for the remainder of the study and were housed in temperature-regulated chambers at 25°C (4). Once the rat was fully awake after surgery, an infusion of 5% D-glucose in 0.9% NaCl solution was started through the duodenal tube at 3 ml/h. That evening at 1700, the solution was switched to 0.9% NaCl without glucose at 3 ml/h for the remainder of the experiment, except for administration of the nutrient bolus as described below.

Nutrient administration and sample collection. The following morning after surgery, lymph was continuously collected for 1 h on ice (fasting lymph). The rats were then given a single, intraduodenal bolus of either 3 ml of saline or 3 ml of an isocaloric meal (5–7 rats per group). The nutrient groups included 1) dextrin (Sigma Aldrich, St. Louis, MO) 1.1 g in 3 ml 0.9% NaCl, 2) 2.2 ml of Liposyn II 20% (Hospira, Lake Forest, IL) in 0.8 ml of 0.9% NaCl, or 3) a “mixed meal” of 0.55 g dextrin and 1.1 ml of Liposyn II 20% in 1.9 ml 0.9% NaCl. After 30 min, the rats were resumed on their intraduodenal infusion of 0.9% NaCl for the remainder of the study. Lymph was continuously collected on ice for 30 min, 1 h, 2 h, and 3 h after the nutrient bolus. The collection tube was weighed before and after lymph collection to determine the lymphatic flow rate. A protective reagent was immediately added to the lymph samples to equal 10% total volume. The protective reagent consisted of 0.25 M EDTA (Sigma, St. Louis, MO), 80 U/ml heparin (American Pharmaceutical Partners, Schaumburg, IL), and 0.8 mg/ml aprotinin (Calbiochem, San Diego, CA). Lymph samples were stored at −20°C until use.

Measurement of GIP, GLP-1, triglyceride, and glucose. Intact GLP-1 concentrations were determined by use of a commercially available sandwich ELISA kit (LINCO Research, St. Charles, MO). The GLP-1 monoclonal antibody is specific to the NH2-terminal region of active GLP-1 forms including GLP-1-(7-36) and GLP-1-(7-37). The monoclonal antibody does not cross react with other forms of GLP-1-(1-36, 1-37, 9-36, 9-37), as well as glucagon or GLP-2. As reported by LINCO, the intra-assay coefficient of variation (CV) is 7.4% and the interassay CV is 8%.

Total GIP concentrations were determined using a commercially available rat/mouse sandwich ELISA kit (LINCO Research, St. Charles, MO). The GIP ELISA measures both active GIP-(1-42) and inactive GIP-(3-42) and does not cross react with glucagon, oxyntomodulin, GLP-1, or GLP-2. As reported by LINCO, the intra-assay CV is 2.6% and the interassay CV is 3.7%.

Triglyceride concentrations were determined by use of a triglyceride assay kit from Randox (Oceanside, CA). Glucose concentrations were determined by a modified glucose oxidase-peroxidase method (Diagnostic Chemicals, Charlottetown, PE, Canada).

Analysis and statistics. Total lymphatic flow was calculated for each nutrient as a sum of the hourly lymph flow not including the fasting values (i.e., cumulative lymph flow due to the experimental nutrient only). Area under the curve (AUC) was calculated by the trapezoidal rule. Comparisons of concentrations in lymph between different experimental groups were performed by a two-way analysis of variance assuming normality. All values are presented as means ± SE. Values were determined as statistically significant if $P < 0.05$.

RESULTS

Lymph production. Lymph flow rate was monitored continuously for 1 h of fasting and 30 min and 1, 2, and 3 h after the nutrient infusion. Total lymphatic production for the 3 h study was compared for all eight experimental groups (Fig. 1). Lymph production increased with a lipid or mixed-meal bolus in Wistar rats to 9.3 ± 1.1 and 11.1 ± 1.3 ml, respectively, but this did not reach significance compared with the saline Wistar control at 7.0 ± 1.3 ml. Dextrin did not increase total lymph production in either GK or Wistar rats compared with their respective saline control animals. Total lymphatic production was significantly increased in GK rats after a lipid bolus to 14.9 ± 1.2 ml ($P < 0.05$) and with a mixed-meal bolus to 15.8 ± 1.3 ml ($P < 0.05$) compared with GK saline controls at 5.2 ± 1.3 ml. When comparing total lymphatic production between GK and Wistar rats, the diabetic GK rats had a greater total lymphatic production after both a lipid and mixed-meal bolus ($P = 0.002$ and $P = 0.019$, respectively).

Lymphatic triglyceride concentration. As expected, a dextrin bolus did not significantly affect either triglyceride concentration at any time point or the triglyceride concentration AUC for both GK and Wistar rats. A lipid bolus significantly increased GK rat triglyceride concentrations above the GK saline control at 1, 2, and 3 h. A lipid bolus in Wistar rats significantly increased the triglyceride concentration at all time points compared with the Wistar saline control. When comparing GK and Wistar rats, as shown in Fig. 2, Wistar rats had a significantly higher triglyceride concentration AUC after both a lipid and mixed-meal bolus compared with GK rats (lipid: 3,438 ± 204 vs. 1,482 ± 204 mg·dl−1·h, respectively, $P < 0.001$; mixed meal: 3,116 ± 223 vs. 1,315 ± 223 mg·dl−1·h, respectively, $P < 0.001$).

Lymphatic glucose concentration. As expected for their diabetic state, GK control rats tended to have a higher lymphatic glucose concentration AUC 290 ± 42 mg·dl−1·h compared with Wistar control rats, 216 ± 42 mg·dl−1·h, $P = 0.23$ (Fig. 3). There was no difference in lymphatic glucose concentration AUC after a lipid bolus between GK and Wistar rats.
When comparing lymphatic glucose concentration AUC, dextrin significantly increased the glucose AUC in GK rats more than in Wistar rats (602 ± 36 vs. 431 ± 38 mg·dl⁻¹·h⁻¹, respectively, P = 0.002). A mixed-meal bolus also increased GK rat glucose concentration AUC higher than that of Wistar rats, as expected for a Type 2 diabetic rodent (593 ± 42 compared with 513 mg·dl⁻¹·h⁻¹, respectively, P = 0.19), although this did not reach statistical significance. The mixed-meal bolus was a 50:50 combination of Liposyn and dextrin. It is possible that the lymphatic hyperglycemia noted in GK rats after a mixed-meal bolus was not statistically higher than that in Wistar rats because of a similar glucose response in both groups to the Liposyn component.

**Lymphatic GLP-1 concentration.** Figure 4, A and B, compares GLP-1 concentrations for both GK and Wistar rats after each nutrient. In GK rats, a carbohydrate-containing nutrient bolus (dextrin or a mixed meal) significantly increased GLP-1 concentrations at 30 min compared with a saline bolus (Fig. 4A). Wistar rats had a significantly elevated GLP-1 concentration after a carbohydrate-containing nutrient bolus at 30 min and 1 h compared with a saline bolus. A lipid bolus significantly increased the lymphatic GLP-1 concentration of Wistar rats compared with a saline bolus at 1 h (Fig. 4B). As shown in Fig. 6A, there was a trend, although not statistically significant, for a smaller increase in GLP-1 concentration AUC in GK rats after a lipid bolus compared with Wistar rats (116.1 ± 28.8 vs. 172.1 ± 26.3 pM/h, respectively, P = 0.16). GK rats had a significantly smaller increase in GLP-1 concentration AUC after a dextrin bolus to 82.7 ± 24.3 pM/h compared with 208.3 ± 26.3 pM/h for Wistar rats, P = 0.001. A similar smaller increase in GLP-1 concentration AUC in GK rats was seen after a mixed-meal bolus compared with Wistar rats (149.2 ± 28.8 vs. 416.1 ± 28.8 pM/h, respectively, P = 0.001). The impairment in GLP-1 secretion was so profound in GK rats that the GLP-1 concentration AUC was not significantly increased after any nutrient compared with the GK saline control (42.4 ± 28.8 pM/h).

**Lymphatic GIP concentration.** After all three nutrient boluses, GK rats responded with a significantly elevated GIP bolus (dextrin or a mixed meal) significantly increased GLP-1 concentrations at 30 min compared with a saline bolus (Fig. 4A). Wistar rats had a significantly elevated GLP-1 concentration after a carbohydrate-containing nutrient bolus at 30 min and 1 h compared with a saline bolus. A lipid bolus significantly increased the lymphatic GLP-1 concentration of Wistar rats compared with a saline bolus at 1 h (Fig. 4B). As shown in Fig. 6A, there was a trend, although not statistically significant, for a smaller increase in GLP-1 concentration AUC in GK rats after a lipid bolus compared with Wistar rats (116.1 ± 28.8 vs. 172.1 ± 26.3 pM/h, respectively, P = 0.16). GK rats had a significantly smaller increase in GLP-1 concentration AUC after a dextrin bolus to 82.7 ± 24.3 pM/h compared with 208.3 ± 26.3 pM/h for Wistar rats, P = 0.001. A similar smaller increase in GLP-1 concentration AUC in GK rats was seen after a mixed-meal bolus compared with Wistar rats (149.2 ± 28.8 vs. 416.1 ± 28.8 pM/h, respectively, P = 0.001). The impairment in GLP-1 secretion was so profound in GK rats that the GLP-1 concentration AUC was not significantly increased after any nutrient compared with the GK saline control (42.4 ± 28.8 pM/h).
concentration at 30 min, 1 h, and 2 h compared with the GK saline control (Fig. 5A). Similarly, Wistar rats responded to each nutrient with a significantly elevated GIP concentration at 30 min, 1 h, and 2 h compared with the Wistar saline control (Fig. 5B).

When comparing the GIP concentration AUC between GK and Wistar rats (Fig. 6B), GK control rats receiving a saline bolus had a trend, although not statistically significant, for a lower AUC compared with Wistar control rats. A lipid bolus produced a similar GIP concentration AUC in GK rats (910 pg·ml⁻¹·h ± 114) and Wistar rats (940 pg·ml⁻¹·h ± 114, P = 0.86). Surprisingly following the GLP-1 pattern, a dextrin bolus resulted in a significantly smaller increase in GK rat GIP concentration AUC compared with Wistar rats (707 ± 106 vs. 1,373 ± 114 pg·ml⁻¹·h, respectively, P < 0.001). The increase in GIP concentration AUC after a mixed-meal bolus was also significantly smaller in GK rats compared with Wistar rats (927 ± 125 vs. 1,750 ± 125 pg·ml⁻¹·h, respectively, P < 0.001).

**DISCUSSION**

Studies of the lean type-2 diabetic GK rat have previously focused on the defects in pancreatic β-cell mass and insulin secretory capacity. However, GK rats have recently been used to study the response of various incretin-based therapies on glucose homeostasis (37, 40, 42, 45). Furthermore, the GK rat has been utilized to study the improvement of diabetes after differing metabolic surgeries including duodenal-jejunal exclusion and ileal transposition, by mechanisms that augment meal-induced incretin secretion (31–33, 36). However, only a few authors have examined the specific changes in incretin secretion to a specific nutrient in GK rats, and of these cases, only one incretin was assessed or no comparison was made to nondiabetic Wistar animals, from which the GK rats were originally derived (31–33). In this study, we examined the differences in lymphatic incretin secretion of GK rats compared with nondiabetic Wistar rats and how different nutrients affect incretin secretion.

Incretin secretion from the gastrointestinal tract is traditionally measured in the systemic circulation. However, plasma GIP-(1-42) and GLP-1-(7-36) are quickly degraded by DPPIV to GIP-(3-42) and GLP-1-(9-36) (2). Plasma may not be the ideal place to characterize incretin secretion in rodents because of the short half-life, portal dilution, renal clearance, and assay sensitivity. Our laboratory has utilized lymph as a novel alternative medium to measure incretin hormone levels in rodents. This technique involves the cannulation of the superior mesenteric lymphatic duct to allow for continuous lymphatic sampling of the gastrointestinal tract in conscious rats.

![Fig. 5. A: changes in gastric inhibitory protein (GIP) concentration with time after various nutrient boluses for GK rats compared with the saline GK control. B: changes in GIP concentration after various nutrient boluses for Wistar rats compared with the saline Wistar control. *Significant differences between the experimental nutrient group compared with saline controls. Statistical significance determined at P < 0.05. Data are presented as means ± SE.](http://ajpgi.physiology.org/)
Data from our laboratory and collaborators have shown that incretin concentrations are significantly higher in lymph than portal or systemic blood (8, 24, 25). Furthermore, GLP-1 appears to be specifically targeted to intestinal lymph, compared with other gut hormones (like peptide YY), making lymph an ideal medium to characterize meal-induced incretin secretion in GK rats (8).

This study supports the previous finding from our laboratory that in healthy animals, such as Sprague-Dawley rats, a mixed meal of carbohydrate and lipid increases incretin secretion greater than an isocaloric challenge with either solitary nutrient (24, 25). In this study we found that in Wistar rats, the delivery of a mixed meal increased lymphatic GLP-1 and GIP concentrations to a greater extent than the delivery of either nutrient alone, despite a similar caloric content. Contrastingly, this augmented incretin response to a meal of mixed nutrients was not as apparent in GK rats, suggesting that any potentiating effect of a mixed meal on incretin secretion is greatly diminished in this rodent model of diabetes. This observation creates an ideal study end point for future incretin-centered therapies in GK rats, i.e., the return of mixed-meal augmentation of incretin secretion.

We were surprised to find that the GLP-1 secretion defect in GK rats did not affect all of the tested nutrients equally and was most prominent in dextrin-containing meals only. Clinical studies have shown that GLP-1 concentrations in Type 2 diabetic patients are lower than in healthy subjects after a mixed meal (44, 46). The responsible mechanisms that alter incretin secretion in diabetic patients are unknown although recent data suggest that these changes are a consequence, rather than a cause, of diabetes (20, 26, 48). When comparing Type 2 diabetic patients to patients with diabetes secondary to chronic pancreatitis, both groups exhibit a similar loss of incretin effect (20). We therefore expected that GLP-1 in GK rats would also be decreased for all nutrients because of the diabetic state of these animals. To our knowledge, it is not known whether the smaller increase in meal-induced GLP-1 secretion in Type 2 diabetic patients applies to all types of nutrients equally or to glucose-based nutrients only as seen in this study.

In addition, this study found the surprising result that the dextrin-mediated incretin defect in GK rats applies similarly to both GLP-1 and GIP. Most clinical studies have found that GIP secretion is increased or unchanged in Type 2 diabetic patients compared with euglycemic patients, although these findings may reflect the obese nature of the study population rather than diabetes (17, 43). It is also possible that the decrease in GIP secretion is a finding unique to this rodent model or, in contrast, reflects a phenomenon of the lean diabetic state, since this is an understudied human population.

The exact mechanisms of nutrient-based incretin secretion from enteroendocrine cells are unknown. In vitro studies of L and K cells have found that multiple intracellular signaling pathway are involved and may differ depending on the nutrient stimulus. Activation of cAMP pathways and increases in intracellular calcium stimulate GIP and GLP-1 release from both K and L cells (16, 41). However, there also appear to be many differences in the ways that K and L cells sense enteral nutrients and signal incretin secretion. Several studies have identified an important role of vagal cholinergic innervation in the secretion of GLP-1, whereas GIP secretion appears to be independent of vagal innervation (2, 3, 5, 6). GLP-1 secretion from L cells has been linked to KATP channels, whereas recent evidence suggests that the secretion of GIP may be KATP independent (14, 35, 50). GLP-1 secretion may depend on the rate of delivery of nutrients to the intestine determined by gastric emptying (39), and GIP secretion may be dependent on not just the presence of nutrients but the rate of absorption and nutrient processing (11, 15).

Because we found a relatively well-preserved secretion of both incretins in GK rats to lipid but with a diminished secretion to carbohydrate-containing meals, we speculate that the secretion defect is most likely of an incretin-signaling or regulatory mechanism shared by both K and L cells, rather than a defect of different mechanisms unique to each enteroendocrine cell population. There are data that support an effect of insulin resistance on mixed-meal-induced incretin secretion. In a mixed-meal study by Rask et al. (34) on nondiabetic patients, a decreased secretion of both GLP-1 and GIP was found, which correlated with insulin resistance but not with obesity. Furthermore, a recent article by Lim et al. (23) demonstrated that the L cell is insulin responsive, and insulin resistance in vitro and in vivo results in decreased meal-stimulated GLP-1 release. Although rat enteroendocrine cells have not been found to express the insulin receptor (23), the impaired secretion of GIP and GLP-1 by enteroendocrine cells in the GK rat found in this study parallel the findings of Rask and suggest a defective mechanism that applies to both K and L cells during enteral glucose stimulation, such as insulin resistance or hyperglycemia.

In vivo and in vitro studies of GK rats have shown a markedly decreased insulinotropic action of glucose on the pancreas (13). Whereas some studies have shown impairment of several genes within the pancreas affecting insulin secretion as well as decreased β-cell neogenesis (27, 30), this study offers the possibility that diminished incretin secretion to oral glucose may also contribute to in vivo hyperglycemia in GK rats because of a reduced incretin effect and impaired insulin secretion from the pancreas.

This study also provides the interesting finding that GK rats have reduced lymphatic lipid absorption in the first 3 h after a lipid-containing bolus compared with Wistar rats (as reflected by the lymphatic triglyceride concentration). This observation implies that, in addition to the reduced incretin response to glucose, there are other intestinal defects present in GK rats of lipid processing or transport that might extend beyond the enteroendocrine cell to include the enterocytes. This conclusion counters recent evidence documenting overproduction of postprandial lipids in the insulin-resistant state (22, 38). Interestingly, despite this defect in lymphatic lipid absorption, the incretin response to the lipid meal was preserved in GK rats.

There is still a great deal to be learned regarding enteroendocrine signaling pathways and the similarities and differences between the mechanisms used by K and L cells to secrete incretins after a meal. On the basis of our present findings, we propose the possibility of a shared enteral glucose-mediated incretin pathway defect that applies to both K and L cells in GK rats. Determination of the exact mechanisms involved and the relevance to lean Type 2 diabetic patients is of significant clinical interest, with the potential for the advancement of pharmaceutical agents aimed at enhancing native incretin secretion by a glucose-mediated pathway.
LYMPHATIC INCRETIN SECRETION

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