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ORIGINAL ARTICLE

# Endogenously released GLP-1 is not sufficient to alter postprandial glucose regulation in the dog

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**Abstract** Glucagon-like peptide-1 (GLP-1) is secreted from the L cell of the gut in response to oral nutrient delivery. To determine if endogenously released GLP-1 contributes to the incretin effect and postprandial glucose regulation, conscious dogs ($n = 8$) underwent an acclimation period ($t = -60$ to $-20$ min), followed by a basal sampling period ($t = -20$ to 0 min) and an experimental period ($t = 0$–320 min). At the beginning of the experimental period, $t = 0$ min, a peripheral infusion of either saline or GLP-1 receptor (GLP-1R) antagonist, exendin (9–39) (Ex-9, 500 pmol/kg/min), was started. At $t = 30$ min, animals consumed a liquid mixed meal, spiked with acetaminophen. All animals were studied twice (± Ex-9) in random fashion, and the experiments were separated by a 1–2-week washout period. Antagonism of the GLP-1R did not have an effect, as indicated by repeated-measures MANOVA analysis of the Δ AUC from $t = 45$–320 min of arterial plasma glucose, GLP-1, insulin, glucagon, and acetaminophen levels. Therefore, endogenous GLP-1 is not sufficient to alter postprandial glucose regulation in the dog.

**Keywords** Incretin effect · Insulin secretion · Gastric emptying

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## Introduction

After a meal, glucagon-like peptide-1 (GLP-1) is secreted from the L cell in the gut [1]. GLP-1, a target of novel diabetes therapies, has many gluco-regulatory effects including its most well documented, enhanced postprandial insulin secretion [1]. In the dog, oral glucose administration induces an insulin response >twofold that observed with an intravenous infusion of glucose, administered to replicate the postprandial glucose profile, indicating that an incretin effect is present in the species [2]. Intraportal infusion of GLP-1 at physiological levels in the dog, during a hyperglycemic clamp, enhances whole body glucose uptake by ~30%, but it does so without elevating insulin levels compared to hyperglycemia alone [3]. In fact, intraportal infusions to create physiological elevations of GLP-1 in the dog have failed to enhance circulating insulin levels in our experiments [3, 4] and those of others [2, 5], suggesting that the peptide does not initiate the incretin effect in the dog.

It remains possible, however, that there are differences in the effects of the peptide when released endogenously versus when it is infused. Half of secreted GLP-1 is degraded prior to reaching the portal vasculature [6]. A physiological elevation of GLP-1 in a region proximal to the site of its secretion may initiate the peptide's postprandial effects, such that elevations in circulating GLP-1 levels are not required. Comparison could be made to cholecystokinin (CCK), which controls gastric emptying and feeding behavior by binding in a paracrine fashion to its receptor on vagal afferents that are proximal to CCK-secreting entero-endocrine cells [7]. It is possible that endogenously released GLP-1 could initiate insulin secretion in a similar manner by binding and activating receptors near GLP-1 secreting L cells. This activation would be

upstream from the portal vein, therefore, not activated by intraportal GLP-1 infusion. Another possibility is that in the dog, GLP-1 contributes to the incretin effect by acting in a synergistic manner with another secretagogue (i.e., glucose-dependent insulinotropic polypeptide, GIP), therefore, explaining why insulin secretion would not be enhanced by infusion of physiological levels of GLP-1 alone.

The incretin effect in the dog [2] cannot be replicated with intraportal infusion of GLP-1 under hyperglycemic conditions [2–5]; therefore, we hypothesize that endogenously secreted GLP-1 is not sufficient to initiate the incretin effect in the dog. To test this hypothesis, mongrel dogs received an intravenous infusion of either saline or exendin (9–39) (Ex-9), an antagonist of the GLP-1 receptor (GLP-1R), in random order, during which an orally delivered liquid meal consisting of carbohydrate, fat, and protein was given to induce endogenous GLP-1 secretion. The meal was spiked with acetaminophen to quantify gastric emptying [8], which could account for differences in circulating glucose levels. A crossover design was employed such that each animal served as its own control. Effects of endogenous GLP-1 secretion on circulating glucose and pancreatic hormone levels, as well as gastric emptying were assessed.

## Materials and methods

### Animals and surgical procedures

Studies were conducted in conscious mongrel dogs of either sex. Eight animals (20–24 kg) were maintained on a diet of meat (Kal Kan, Vernon, CA) and chow (Purina Lab Canine Diet No. 5006, Purina Mills, St. Louis, MO) composed of 34% protein, 14.5% fat, 46% carbohydrate, and 5.5% fiber based on dry weight, with an approximate Calorie consumption of 1500 Cal/day. Water was available for all animals ad libitum. The animals were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocol was approved by the Vanderbilt University Medical Center Animal Care Committee.

Approximately 16 days before experimentation, a laparotomy was performed as previously described [9]. In brief, under general anesthesia, a sampling catheter was inserted into the femoral artery of the dog. The catheter was filled with saline (Baxter Healthcare Corp, Deerfield, IL) containing 200 U/ml heparin (Abbott Laboratories, North Chicago, IL) and knotted. Approximately 2 days before each study, the animals' health was determined by assessment of the leukocyte count ($<18{,}000/mm^3$), hematocrit (>35%), appetite (as evidenced by consumption of daily ration), and stools.

On the morning of the experiment, the catheter was exteriorized from its subcutaneous pocket under local anesthesia. Intravenous access was established in peripheral veins, as needed. Animals were allowed to rest quietly in a Pavlov harness for at least 40 min before the start of the experiment.

Following the first experiment, the arterial catheter and the incision were aseptically cleansed (three times) with betadine/alcohol solution while the animals were under general anesthesia. The catheter was filled with a sterile mixture of heparin and glycerin (1000 U/ml in a 1:1 ratio), the free end was knotted, and it was placed in a subcutaneous pocket. After animals were fully awake and had regained full mobility, they were returned to the kennel.

### Experimental design

Mongrel dogs ($n = 8$) were studied twice in a crossover design, with each animal serving as its own control. Treatment and control studies were conducted in random order, 1–2 weeks apart. After an 18-h fast, the dogs underwent a 40-min acclimation period (−60 to −20 min), a 20-min basal sampling period (−20 to 0 min), and a 320-min experimental period (0–320 min). At $t = 0$, a peripheral infusion of either saline or the GLP-1R antagonist, Ex-9 (500 pmol/kg/min, gift from Amylin Pharmaceuticals, San Diego, CA), was started and continued through the entire experimental period. This rate of Ex-9 infusion has been shown to completely inhibit GLP-1R activation in humans [10]. Partial antagonism of the GLP-1R has been shown to occur at much lower rates in dogs [11] and humans [10] (75 and 100 pmol/kg/min, respectively). At $t = 30$, all dogs received an orally administered liquid mixed meal consisting of 480 Calories [63% carbohydrate (glucose polymer), 17% protein (whey), 20% fat (microlipid)], spiked with acetaminophen (500 mg), to quantify gastric empting [8].

### Sample analysis

Plasma glucose levels were measured using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin and glucagon concentrations were determined by RIA, as previously described [12]. Plasma GLP-1 levels were measured by ELISA (Linco Research, St. Charles, MO), which only recognizes GLP-1 (7–36) amide and GLP-1 (7–37), the active forms of GLP-1, as previously described [3].

Plasma acetaminophen levels were determined using a modified protocol designed for HPLC [13]. A 500 ml aliquot of plasma was spiked with 20 µl of 2-acetaminophenol (40 µg/ml) to serve as an internal standard [14]. Equal volumes of spiked plasma, 0.3 N barium hydroxide, and

0.3 N zinc sulfate were mixed and incubated on ice for 5 min. The sample was then spun at 4°C at 3000 rpm for 10 min. The decanted supernatant was dried using vacuum centrifugation (Speedvac Concentrator, Savant SVC 200H, Thermo Scientific, Waltham, MA). The sample was then reconstituted with 200 µl of a 10% methanol/water solution (v:v). A 50 µl aliquot was injected for delivery into the HPLC column (uBondapak C18 3.9 × 30 w/guard), at a temperature of 45°C. Mobile phases A (5% methanol/water, v:v) and B (15% methanol/water, v:v) were set at a combined flow rate of 0.4 ml/min for the entire duration of assay. With the profile curve indicating the type of transition from one setting to the next, the gradient for the mobile phase was set as follows: initial setting at 100% A, 0 profile curve; $t = 4$ min at 100% A, 11 profile curve; $t = 20$ min at 75% A, 25% B, six profile curve; $t = 30$ min at 100% B, seven profile curve; $t = 40$ min at 100% A, 11 profile curve. Total run time was 64 min, with an 18 min acquisition delay. Fluorescence was measured with variable wavelength UV detector (Waters 481, Millipore, Billerica, MA) set at 240 nm at 0.5 AUFS. Peak area as identified by the ESA 500 Chromatograph and data station are representative of acetaminophen concentration. The peak area increases in a linear fashion, proportional to acetaminophen concentration; therefore, sample concentration is determined as a ratio of the area of the acetaminophen peak in the sample to the area of the internal standard peak. We confirmed this assay to be accurate up to concentrations of 40 µg/ml with an intra-assay CV of 4%.

### Calculations

Change from baseline area under the curve (Δ AUC) was calculated by determining the area under the curve, using the linear trapezoidal rule, less the area below the average baseline value ($t = -20$ to 0 min) over the duration of $t = 45$–320 min for each parameter. This timeframe encompasses the first sampling after the meal ($t = 45$ min) through the end of the experiment. The Δ AUC for arterial plasma glucose, GLP-1, insulin, glucagon, and acetaminophen levels were calculated for each individual animal, for both the saline and Ex-9 infusion.

### Statistical analysis

To determine if postprandial GLP-1R activation affects postprandial glucose regulation, arterial plasma glucose, GLP-1, insulin, glucagon, and acetaminophen Δ AUC were analyzed by repeated-measures Wilks' λ MANOVA (JMP 8.0, SAS Institute, Cary, NC) using a significance level of 0.05. *Post hoc* univariate analysis was conducted, as necessary.

## Results

### Effect of endogenous GLP-1 activity

The repeated-measures Wilks' λ MANOVA analysis, including the arterial plasma glucose, GLP-1, insulin, glucagon, and acetaminophen Δ AUC, found a whole model effect (Wilks' $\lambda = 0.0000193$, $F$ (40, 16) $= 4.39$, $P = 0.0013$), which can be attributed to variation among animals (Wilks' $\lambda = 0.0000276$, $F$ (35, 15) $= 4.78$, $P = 0.0011$). There was no difference between treatments ($P = 0.21$), indicating that endogenously released GLP-1 is not sufficient for postprandial alterations in arterial plasma glucose, GLP-1, insulin, glucagon, or gastric emptying as measured by acetaminophen absorption.

### Plasma glucose

Basal arterial plasma glucose levels were 119 ± 2 (mean ± standard error, SE) and 118 ± 2 mg/dl prior to saline or Ex-9 infusion, respectively (Fig. 1), and were not affected by either infusion prior to the meal. After administration of the meal, the arterial plasma glucose levels averaged 158 ± 11 mg/dl during saline infusion and 158 ± 8 mg/dl during Ex-9 infusion (Fig. 1). The Δ AUC ($t = 45$–320 min) was 10802 ± 3015 mg/dl*275 min and 11000 ± 2312 mg/dl*275 min, in the presence of saline and Ex-9, respectively. Univariate analysis found a portion of the whole model effect could be attributed to the arterial plasma glucose Δ AUC (Wilks' $\lambda = 11.04$, $F$ (7, 8) $= 9.66$, $P = 0.0036$), due to variation among animals (Wilks' $\lambda = 11.04$, F (7, 7) $= 4.78$, $P = 0.0026$).

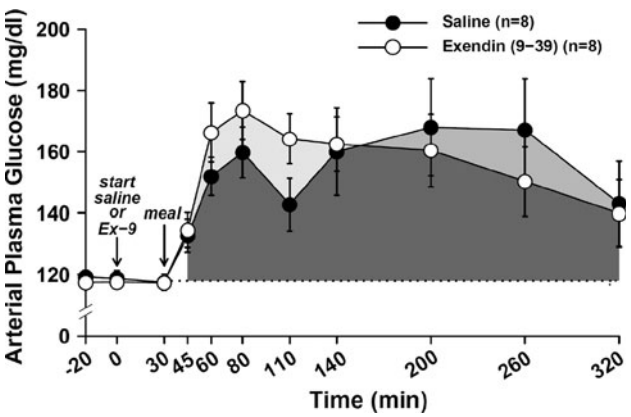


**Fig. 1** Arterial plasma glucose levels during the basal period (−20 to 0 min), the experimental period prior to the meal (0–30 min), and the postprandial experimental period (30–320 min). Data are mean ± SE. *Shaded areas* represents Δ AUC component (*light tone* Ex-9, *medium tone* saline, *dark tone* both treatments) analyzed by repeated-measures MANOVA. *Dotted line* is mean baseline level

## Plasma GLP-1 levels

Arterial plasma GLP-1 levels were at a minimum during the basal period and during the infusion of saline or Ex-9 prior to the meal (Fig. 2). Arterial plasma GLP-1 levels increased in response to the meal, peaking at 12.2 ± 4.1 pM at $t = 260$ min in the presence of saline infusion and 24.3 ± 5.8 pM at $t = 200$ min in the presence of Ex-9 infusion (Fig. 2). The Δ AUC ($t = 45$–320 min) was 1251 ± 352 pM*275 min and 3434 ± 1084 pM*275 min, in the presence of saline and Ex-9, respectively, and which did not contribute to the whole model effect (Wilks' $\lambda = 3.89$, $F$ (7, 8) = 3.41, $P = 0.062$).

## Plasma insulin levels

There was no difference in arterial plasma insulin levels between the two groups prior to meal administration (Fig. 3). After the meal was administered, the arterial plasma insulin levels increased in response to postprandial hyperglycemia in the presence of both saline (45 ± 5 μU/ml) and Ex-9 infusion (46 ± 5 μU/ml) (Fig. 3). The Δ AUC for arterial plasma insulin levels from 45 to 320 min was 9989 ± 1402 μU/ml *275 min with saline infusion and 10350 ± 1454 μU/ml *275 min when Ex-9 was infused. Univariate analysis found a portion of the whole model effect could be attributed to the arterial plasma insulin Δ AUC (Wilks' $\lambda = 5.31$, $F$ (7, 8) = 4.64, $P = 0.029$), due to variation among animals (Wilks' $\lambda = 5.29$, $F$ (7, 7) = 5.29, $P = 0.021$).

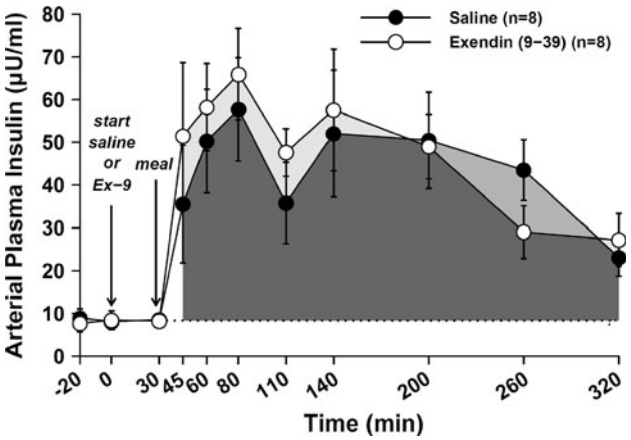


**Fig. 3** Arterial plasma insulin levels during the basal period (−20 to 0 min), the experimental period prior to the meal (0–30 min), and the postprandial experimental period (30–320 min). Data are mean ± SE. *Shaded areas* represents Δ AUC component (*light tone* Ex-9, *medium tone* saline, *dark tone* both treatments) analyzed by repeated-measures MANOVA. *Dotted line* is mean baseline level

## Plasma glucagon levels

Basal plasma glucagon levels were similar between treatments prior to the meal (Fig. 4). After administration of the meal, glucagon levels were 44 ± 6 pg/ml with saline infusion and 48 ± 9 pg/ml with Ex-9 infusion (Fig. 4). The Δ AUC for arterial plasma glucagon levels from 45 to 320 min was −440 ± 298 pg/ml *275 min with saline infusion and 1942 ± 1497 pg/ml *275 min when Ex-9 was infused. Univariate analysis found a portion of the whole model effect could be attributed to the arterial plasma glucagon Δ AUC (Wilks' $\lambda = 8.79$, $F$ (7, 8) = 7.69,

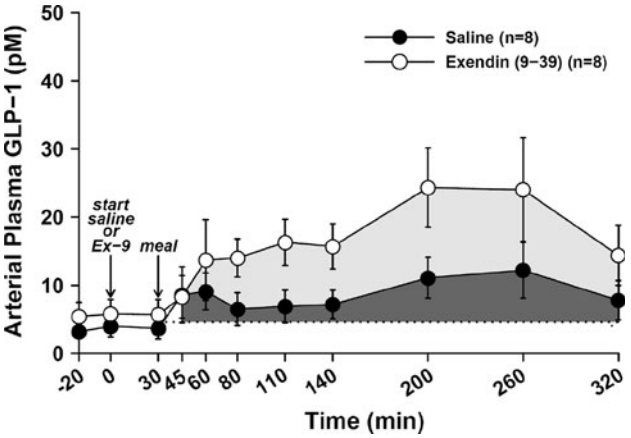


**Fig. 2** Arterial plasma GLP-1 levels during the basal period (−20 to 0 min), the experimental period prior to the meal (0–30 min), and the postprandial experimental period (30–320 min). Data are mean ± SE. *Shaded areas* represents Δ AUC component (*light tone* Ex-9, *medium tone* saline, *dark tone* both treatments) analyzed by repeated-measures MANOVA. *Dotted line* is mean baseline level

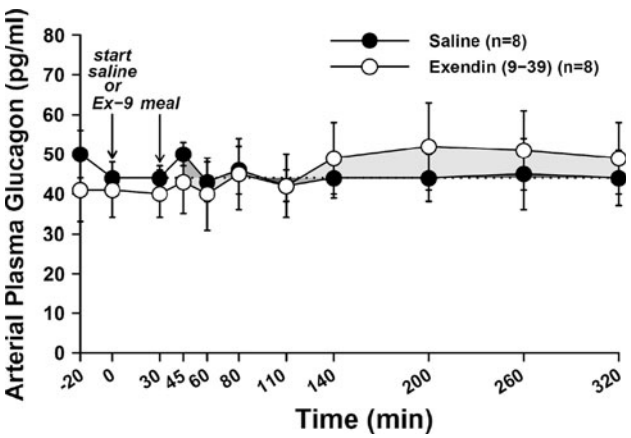


**Fig. 4** Arterial plasma glucagon levels during the basal period (−20 to 0 min), the experimental period prior to the meal (0–30 min), and the postprandial experimental period (30–320 min). Data are mean ± SE. *Shaded areas* represents Δ AUC component (*light tone* Ex-9, *medium tone* saline, *dark tone* both treatments) analyzed by repeated-measures MANOVA. *Dotted line* is mean baseline level

$P = 0.0071$), due to variation among animals (Wilks' $\lambda = 7.24$, $F$ (7, 7) $= 7.24$, $P = 0.0091$).

Plasma acetaminophen levels

To assess gastric emptying, circulating plasma acetaminophen levels were measured. Levels peaked at 7.9 ± 1.1 μg/ml ($t = 200$ min) when saline was infused, compared to 9.3 ± 1.3 μg/ml ($t = 140$ min) when Ex-9 was infused (Fig. 5). The Δ AUC from $t = 45$–320 min during saline infusion was 1794 ± 231 μg/ml*275 min, while during Ex-9 infusion it was 2046 ± 218 μg/ml*275 min (Fig. 5). Univariate analysis found a portion of the whole model effect could be attributed to the arterial plasma acetaminophen Δ AUC (Wilks' $\lambda = 8.12$, $F$ (7, 8) $= 7.11$, $P = 0.0090$), due to variation among animals (Wilks' $\lambda = 7.67$, $F$ (7, 7) $= 7.67$, $P = 0.0077$).

## Discussion

When dogs are given a meal, insulin secretion is significantly greater than when the postprandial glucose profile is matched with a peripheral glucose infusion, indicating the presence of an incretin effect [2]. The purpose of this study was to determine if endogenously secreted GLP-1 was sufficient to induce this incretin effect, therefore, altering postprandial glucose regulation in the dog. To induce endogenous GLP-1 secretion, a liquid mixed meal was administered orally to conscious dogs. Each dog was given the meal 30 min after the start of a constant infusion of saline or a GLP-1R antagonist, Ex-9. The rate at which Ex-9 was infused (500 pmol/kg/min) has been shown to fully block the insulin secretory effect of GLP-1 in humans, and the peptide does not behave as an agonist [10]. The meal successfully increased circulating GLP-1 levels (Fig. 2).

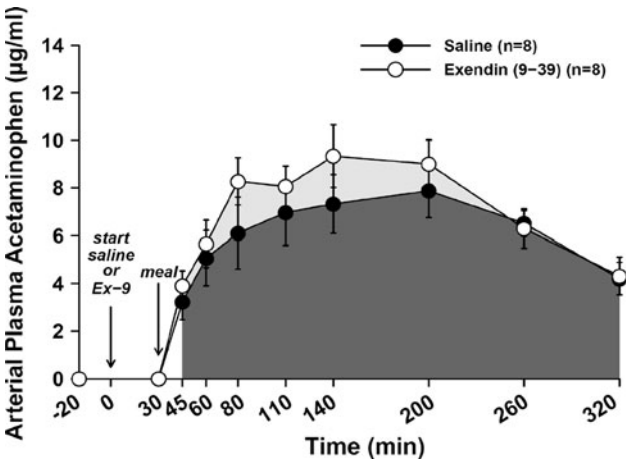


**Fig. 5** Arterial plasma acetaminophen levels following the meal. Data are mean ± SE. *Shaded areas* represents Δ AUC component (*light tone* Ex-9, *medium tone* saline, *dark tone* both treatments) analyzed by repeated-measures MANOVA

Inhibiting the action of endogenously secreted GLP-1 had no effect on postprandial glucose regulation. This conflicts with a recent study indicating that when humans consume a meal while circulating glucose levels are maintained at a constant level with a variable rate hyperglycemic clamp, inhibiting GLP-1R activity with Ex-9 significantly reduces postprandial insulin secretion in both healthy humans and those with diabetes [15]. Our current findings show that in the dog, postprandial inhibition of the GLP-1R with Ex-9 does not alter arterial plasma insulin or glucagon levels. These findings are consistent with our earlier data [3, 4], and the study of others [2], in which GLP-1 was elevated by a physiologic amount and failed to alter α- or β-cell secretion. Therefore, the findings of this study and previous data provide strong evidence that the role of endogenously secreted GLP-1 in postprandial glucose regulation in the dog does not include alterations in endocrine pancreatic function or the induction of the incretin effect, which may be different from the contribution of endogenous GLP-1 to postprandial glucose regulation humans. However, we have previously shown that intraportal infusion of exendin-4, a potent GLP-1R agonist, to create circulating levels of ~215 pM, directly stimulated insulin secretion [16]. In addition, isolated canine pancreata increase insulin release in the presence of 1 nM GLP-1 levels [17]. These results indicate that canine β-cells respond to GLP-1, but require high levels of the peptide to initiate a significant effect.

The question thus arises as to what causes the incretin effect observed in the dog [2]. The canine pancreatic islet is known to increase insulin secretion in response to the other recognized incretin hormone, GIP [18]. It is possible, therefore, that GLP-1 and GIP work in a synergistic manner, which is not replicated with GLP-1 infusion alone [2]; however, our data provide evidence against this possibility because the inhibition of GLP-1 had no effect. It is also possible that GIP is the primary stimulatory hormone for the incretin effect in the dog. At present, the exact mechanism of the incretin effect in this species remains unclear.

Endogenous GLP-1 significantly decreases the rate of gastric emptying in humans [19]. Transit of a meal, spiked with acetaminophen, from the stomach to the small intestine can be quantified by the appearance of acetaminophen in circulating plasma. Earlier studies indicate that pharmaceutical concentrations of a GLP-1R agonist dramatically reduce the rate of gastric emptying in the dog [20], but in the current study postprandial antagonism of the GLP-1R did not change arterial plasma acetaminophen levels. This suggests that, although activation of the GLP-1R

can decrease the rate of gastric emptying in the dog, endogenously secreted GLP-1 does not provide sufficient activation for such an effect.

Endogenous GLP-1 is not sufficient to alter postprandial glucose regulation in the dog, as blocking the GLP-1R did not change arterial plasma glucose or insulin levels or the rate of gastric emptying as indicated by plasma acetaminophen levels. The acute role of endogenous GLP-1 secretion in canines remains unclear, but it is possible that chronic effects, such as those found with the activation of the receptor in the central nervous system [21], may have physiological relevance.

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