Insulin-independent effects of glucagon-like peptide 1 (GLP1) on canine liver glucose metabolism: duration of infusion and involvement of hepatoporal region.

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Short title: GLP1 and non-insulin dependent effects.

Key Words: GLP1, liver, glucose uptake, portal infusion, dog

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Abstract

Whether GLP1 has insulin-independent effects on glucose disposal in vivo was assessed in conscious dogs using tracer and arteriovenous difference techniques. After a basal period, each experiment consisted of 3 periods (P1, P2, P3) during which somatostatin, glucagon, insulin and glucose were infused. The control group (C) received saline in P1, P2 and P3, the PePe group received saline in P1 and GLP1 (7.5 pmol/kg/min) peripherally (i.v) in P2 and P3 and the PePo group received saline in P1 and GLP1 peripherally (i.v) (P2) then into the portal vein (P3). Glucose and insulin concentrations increased to 2-fold and 4-fold basal, respectively and glucagon remained basal. GLP1 levels increased similarly in the PePe and PePo groups during P2 (~200pM) whereas portal GLP1 levels were significantly increased (3-fold) in PePo vs PePe during P3. In all groups, net hepatic glucose uptake (NHGU) occurred during P1. During P2, NHGU increased slightly but not significantly in all groups. During P3, NHGU increased in PePe and PePo groups to a greater extent than in C but no significant effect of the route of infusion of GLP1 was demonstrated (16.61±2.91 and 14.67±2.09 vs 4.22±1.57 μmol/kg/min, respectively). In conclusion: GLP1 increased glucose disposal in the liver independent of insulin secretion; its full action required long term infusion. The route of infusion did not modify the hepatic response.
**Introduction**

Glucagon-like peptide 1 (GLP1) is synthesized from proglucagon in the L cells of the duodenum, distal ileum and colon in response to meal absorption after which it is rapidly released into the portal vein (23, 37). The main and active form of GLP1, GLP1[7-36], is rapidly degraded by dipeptidyl peptidase IV into GLP1[9-36] in the intestinal tissues as well as in the blood (22,27). The consequence is a rapid elimination of GLP1 from plasma with a half-life estimated at 1-2 min in several species (23). The earliest biological effect of GLP1 discovered was its ability to increase glucose-dependent insulin secretion (17, 28) as well as the transcription of the proinsulin gene and biosynthesis of insulin (12). The glucose dependent effect of GLP1 on insulin secretion has suggested a potential use of this agent in the treatment of diabetes without deleterious hypoglycemia. Indeed, it lowers post-prandial glucose levels in both healthy and human subjects with type 2 diabetes by stimulating insulin secretion but also by inhibiting glucagon secretion from the $\alpha$ cell and delaying gastric emptying (1, 18, 40).

GLP1 receptors are present on $\beta$ cells (38) and there are numerous reports of GLP1 receptors on glucose consuming tissues such as liver, skeletal muscle and fat (11, 39, 42). Most *in vitro* studies show that GLP1 stimulates glucose uptake and metabolism in muscle (24, 41, 44), adipocytes (20, 43) and hepatocytes (2) and that GLP1 can potentially promote glucose disposal directly, in addition to its effect on insulin secretion. *In vivo* GLP1’s insulin secretion-independent effects are much less clear. Some data are in agreement with a GLP1 effect independent of insulin secretion (8, 9, 10, 15) whereas others exclude such an action (8, 33, 34). In addition to the species differences, these investigations have been performed with different levels of insulin or glucose and with acute or chronic GLP1 delivery into the systemic circulation.

GLP1 concentrations are physiologically increased during the post-prandial phase and its direct tissue effects may require the presence of insulin and/or hyperglycemia to be fully manifest. Furthermore, GLP1 is secreted into the hepatic portal vein and recent evidence suggests the presence of GLP1 sensors or receptors in the hepato-portal region (5, 30, 32). Because of the rapid degradation of GLP1 in the plasma, the hepato-portal region may play a critical role in the generation of the full effects of GLP1. Because of these
considerations, a recent study was conducted in our laboratory (31) in which the effect of various rates of portal GLP1 infusion were sequentially studied under conditions of controlled hyperinsulinemia and hyperglycemia in the conscious dog. The results showed that GLP1 has an effect independent of its action on insulin secretion. However, despite an apparent dose dependent effect of GLP1, the study design raised the possibility that GLP1 may also have a time dependent effect. We performed the current studies in the conscious dog model in order to assess the time–dependent effect of GLP1 on hepatic and non-hepatic glucose metabolism under conditions which mimic the post-prandial state (hyperglycemia and hyperinsulinemia).

We used the fasted conscious dog as our model because it provides a good reflection of glucose metabolism in humans and because it has the advantage of allowing invasive experimental design, which facilitates the performance of mechanistic studies in vivo (7). Furthermore, it is clear that GLP1 levels in plasma were increased in the dog following an intragastric glucose load to levels similar to those recorded in humans and other species when fed with a mixed meal. The protocol design included a somatostatin-controlled pancreatic clamp to prevent changes in pancreatic hormones and the comparison of a prolonged (180 min) versus short (90 min) duration peripheral GLP1 infusion. The role of the hepatoportal region in the insulin-secretion independent effects of GLP1 was assessed by comparing the effects of peripheral and portal infusions of GLP1 in the same dog.
Materials and Methods

Animals and surgical procedures
Experiments were performed on eighteen 42-h-fasted conscious mongrel dogs (20-25 kg) of either sex that had been fed once daily a standard meat and chow diet (31% protein, 52% carbohydrate, 11% fat, and 6% fiber based on dry weight; Kal Kan, Vernon, CA and Purina Lab Canine Diet no. 5006, Purina Mills, St. Louis, MO). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before experimentation, a laparotomy was performed with animals under general anesthesia. Silastic catheters (Dow Corning, Midland, MI) for blood sampling were placed into the portal vein, a hepatic vein, and a femoral artery, and infusion catheters were inserted into a jejunal vein and a splenic vein, as previously described (29). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the portal vein and the hepatic artery. On the day of the experiment, the catheters were exteriorized under local anesthesia, and intravenous access was established in peripheral veins (cephalic and sapheneous veins). Dogs were used for an experiment only if they met established criteria for good health.

Experimental design
In each of the three groups, the protocol consisted of a tracer equilibration period (0-90 min), a basal period (90-120 min) followed by three test periods of 90 minutes each (P1=120-210 min; P2=210-300 min; P3=300-390 min) during which hyperglycemia and hyperinsulinemia existed (Figure 1). At t=0, a primed (1.2 µCi/kg), continuous (0.17 µCi/min) infusion of [3-3H]glucose (New England Nuclear, Boston, MA) and a continuous infusion of indocyanine green dye (0.08 mg/min; Sigma Chemical, St. Louis, MO) were started. At t=120, a peripheral infusion of somatostatin (0.8 µg·kg⁻¹·min⁻¹) was begun to inhibit endogenous pancreatic insulin and glucagon secretion. Intraportal infusions of insulin (1.2 mU·kg⁻¹·min⁻¹), to achieve hyperinsulinemia (≈3 times the basal arterial levels), and glucagon (0.55 ng·kg⁻¹·min⁻¹), to maintain basal levels, were also started. A dextrose solution was infused peripherally at variable rates starting at
t=120 to clamp the arterial plasma glucose level at 12.5mM. The infusion rate of glucose was adjusted in response to the plasma glucose concentration, which was measured every 5 min. In the two last test periods (210-300 and 300-390 min), the control group (n=6) received a saline infusion via a peripheral vein whereas the two other groups received a GLP1(7-36) amide infusion at 7.5 pmol/kg/min (Bachem Bioscience Inc. PA, USA). In the PePe group (n=6), GLP1 was infused peripherally until the end of the experiment (P2+P3 periods). In the PePo group (n=6), GLP1 was first infused peripherally for 90 min (P2 period) then intraportally (at the same rate) for the next 90 min (P3 period). This infusion rate was chosen to generate mild pharmacological levels of GLP1. Blood was collected on EDTA, and an inhibitor of DPP-IV activity (Linco Research Inc. MO) was immediately added to whole blood to preserve the integrity of the GLP1.

**Analytical procedures**

Plasma glucose and glucose radioactivity (^3H), insulin, glucagon and blood lactate were measured as previously described (31). Plasma GLP1 concentrations were determined by an ELISA method (Linco Research Inc. MO) that specifically quantifies the biologically active form of GLP1.

**Calculations**

Net hepatic substrate balance (NHB) was calculated using the formula \[ \text{[H·Ft}-(A·Fa+P·Fp)] \], where A, P, and H are the arterial, portal vein, and hepatic vein substrate concentrations, and Fa, Fp, and Ft are hepatic arterial, portal vein, and total hepatic blood or plasma flows (as appropriate), respectively. Hepatic substrate load was calculated as A·Fa+P·Fp. Net hepatic fractional extraction (FE) was calculated as \[ \text{NHB+hepatic load} \]. For all calculated data, plasma glucose concentrations were converted to blood concentrations with factors compiled in our lab from extensive data in which plasma and blood glucose values were compared (19). Sinusoidal hormone concentrations were calculated as described previously (31). Non-hepatic glucose uptake represents the difference between the glucose infusion rate (GIR) and net hepatic glucose uptake (NHGU) if the glucose mass remains constant during the study. Net hepatic carbon retention (NHCR), an indicator of the carbon available for glycogen synthesis, was
calculated as NHGU minus net hepatic lactate output. This omitted the contribution of gluconeogenic substrates other than lactate and also of the carbon utilized in hepatic oxidation as discussed previously (31, 36). NHCR should provide a reasonably close estimate of net hepatic glycogen synthesis.

Statistical analysis.

Data are reported as means of the values for the last 30 min of each experimental periods. NHGU, FE and NHCR are expressed as the difference between the mean values observed during the last 30 min of the experimental periods (P2 or P3) and the mean values of the last 30 min of P1 in order to assess the specific effect of GLP1 over the effect achieved by the pancreatic hormones and hyperglycemia per se.

Data are expressed as means ± SE and analyzed by SigmaStat. Two-way repeated-measures analysis of variance was used to compare the time-course data of groups. Classification factors were treatment group and time period. For significant F values, the Student-Newman-Keuls multirange test was employed as a post hoc analysis. Differences were considered significant when P<0.05.
Results

Hormone Concentrations and blood flows.

Arterial and hepatic sinusoidal insulin concentrations increased by a physiological amount during the test periods P1, P2 and P3 (Table 1) with no significant differences among the groups. No increase of insulin levels in either the artery or the portal vein (not shown) were recorded following GLP1 infusion indicating that GLP1 did not overcome the pancreatic clamp initiated by the somatostatin infusion. Arterial and hepatic sinusoidal glucagon concentrations remained basal and similar in all the groups (Table 1). In the present study, the infusion of GLP1 did not modify the concentrations of the pancreatic hormones and the effects recorded will be thus independent of GLP1 effect on insulin and glucagon secretion. Arterial blood flows increased slightly with time but no significant differences were recorded among groups. Portal blood flows tended to decrease after the basal period due to somatostatin infusion. Total hepatic blood flow therefore changed minimally (<10%) in all groups over the course of the study.

With the peripheral (i.v) GLP1 infusion, arterial and portal GLP1 concentrations increased significantly and reached supraphysiological levels (∼200pM) (Table 1). These levels remained constant with time in the PePe group. In the PePo group, arterial and portal GLP1 levels increased to the same extent as those in the PePe group during P2 but portal GLP1 concentrations dramatically increased (∼500pM) with the start of the portal infusion of GLP1 (P3). Interestingly, arterial GLP1 concentrations were only slightly lower with portal vein GLP1 infusion than with peripheral (i.v) GLP1 infusion (Table 1).

Blood glucose levels and hepatic glucose balance.

In response to peripheral glucose infusion, arterial blood glucose increased significantly in all groups from ∼6mM to ∼12.5mM. The hepatic glucose loads (HGL) during the basal period were 150±11, 151±13 and 135±12 µmol/kg/min in Control, PePe and PePo, respectively. In response to the glucose infusion, HGL increased significantly and similarly in all groups and then remained constant over time (P1:261±22, 260±21 and
The combination of hyperinsulinemia and hyperglycemia during the P1 test period switched the liver from net hepatic glucose output to net hepatic glucose uptake (NHGU) in all groups (data not shown). During saline infusion, NHGU increased slightly (over P1) during P2 and P3 ($\Delta=0.16\pm1.53$ and $4.22\pm1.57$, (NS) respectively)(Figure2A). Peripheral infusion of GLP1 did not enhance NHGU during P2 (relative to P1), but it stimulated NHGU ($\approx3$-fold; $16.61\pm2.91 \mu\text{mol/kg/min}$) after more prolonged infusion (P3). The route of GLP1 infusion made no difference in its ability to increase NHGU in P3 ($14.67\pm2.09 \mu\text{mol/kg/min}$ (Figure2A)).

The net hepatic fractional extraction (FE) of glucose followed the same pattern as NHGU (Figure2B). Short-term peripheral GLP1 infusions did not increase FE significantly whereas prolonged GLP1 infusions did. Nevertheless, peripheral and portal GLP1 infusions resulted in the same increase (Figure2B)($\Delta=1.5\pm1.0$, $6.4\pm1.3$ and $5.1\pm0.9 \%$ for C, PePe and PePo during P3 relative to P1, respectively).

The mean glucose infusion rates (GIR) required to maintain constant hyperglycemia increased significantly over the experimental periods (Figure 3). However, no differences between groups were recorded in any test period. When assessed with the tracer dilution technique, the whole body glucose utilization (Rd) followed the same pattern as the GIR (Figure 3). Rd increased with time in each experimental group but no differences were recorded between groups at any time point. The non-hepatic glucose uptake (non-HGU) was the same in all groups in P1 ($28.5\pm2.9$, $27.6\pm4.3$ and $26.1\pm4.8 \mu\text{mol/kg/min}$ for C, PePe and PePo, respectively). During P2, non-HGU increased significantly in each experimental group ($40.9\pm3.7$, $39.7\pm3.1$ and $43.2\pm8.2 \mu\text{mol/kg/min}$ for C, PePe and PePo, respectively) with no significant difference among groups. During P3, after the prolonged peripheral or intraportal GLP1 infusions, non-HGU was not significantly different from the values recorded in P2.

**Lactate concentrations and net hepatic lactate and glycogen synthesis.**
After initiating the hyperglycemic hyperinsulinemic clamp, arterial blood lactate concentrations increased and remained constant during all the test periods P1, P2 and P3 (from $\approx 0.6$ to 1.0mM) with no significant differences among groups (not shown). Net hepatic lactate balance (NHLB) changed from uptake to output in all groups and after a peak during P1, it declined to a value not significantly different from 0 µmol/kg/min in all groups during P2 and P3 (Figure 4). Concomitantly, P1 was associated with net hepatic carbon retention (NHCR; data not shown). During the peripheral GLP1 infusion (P2), NHCR was significantly stimulated over P1 in PePe and PePo groups when compared to C ($\Delta=1.0\pm1.8$, 6.6±2.2 and 8.6±0.6 µmol/kg/min, for C, PePe and PePo, respectively) (Figure 4). With prolonged GLP1 infusion (P3), NHCR further increased and was significantly higher in the PePe and PePo ($\Delta=5.1\pm1.4$, 16.2±2.7 and 18.5±1.3 µmol/kg/min for C, PePe and PePo, respectively (p<0.05 vs P1 for all groups; p>0.05 for PePe vs PePo)(Figure 4).
Discussion

Previous work performed in our laboratory (31) has shown that GLP1 is able to increase glucose disposal independent of its well recognized effects on insulin and glucagon secretion. Furthermore, the results suggested that the insulin secretion-independent effects of GLP1 were dose dependent but the design of the study left open the possibility of a time dependent effect of GLP1, i.e prolonged infusions may be required to observe the full effects of the hormone. If so, the sensitivity of these processes to GLP1 might be much greater than indicated in that study. The current study was carried out in order to examine this issue. Furthermore, in light of the recent suggestion that GLP1 receptors in the portal vein might play an important physiologic role (5, 30, 32), the potential involvement of the hepato-portal region in the mechanism of action of GLP1 was assessed by comparing peripheral and intraportal infusions of GLP1.

In the current study, GLP1 was infused at a rate of 7.5 picomol/kg/min in order to achieve modest pharmacologic arterial GLP1 concentrations. This rate, based on the study of Nishizawa et al. (31), was chosen to increase GLP1 concentrations enough to observe maximal effects of the peptide. In the present study, GLP1 concentrations reached \( \approx 200 \text{pM} \) in the arterial plasma and went up to \( \approx 500 \text{pM} \) in the portal vein when GLP1 was infused intraportally. Based on the literature (25), the physiological arterial level of GLP1 following an oral glucose load (2g/kg) or mixed meal in dogs is 20pM. Because of the difference between the assays or antibodies used in the determination of GLP1, we have measured (using the same assay as for our present experiment) intact arterial and portal GLP1 concentrations in four overnight fasted dogs under basal conditions and following an intragastric glucose load of 1.5g/kg (Dardevet et al. unpublished data). The basal levels were \( \approx 17 \text{pM} \) in both artery and portal vein. After the glucose bolus, GLP1 levels peaked at \( \approx 40 \text{pM} \) in the artery and \( \approx 65 \text{pM} \) in the portal vein. Based on these data, GLP1 levels in our present experiment were 5-7 times the normal physiological post-prandial concentration.

Our results clearly show that GLP1 stimulated NHGU but had no effect on non-HGU. These studies created conditions which mimicked postprandial levels of insulin and glucose, conditions during which GLP1 secretion is physiologically enhanced. The
stimulation of glucose disposal was independent of pancreatic hormone secretion since insulin and glucagon levels remained constant during GLP1 infusion. In addition, our study also clearly demonstrated that the effects of GLP1 were time dependent. Indeed, GLP1 effects required over 90 min to be seen and even longer to be maximally effective (150-180 min). These results are in agreement with those of Nishizawa et al. (31), in which the highest dose of GLP1 enhanced NHGU without increasing non-HGU. In that study, the possibility existed that the effect of GLP1 on the liver was a consequence of increased hepatic blood flow which was recorded concomitantly with this high dose of GLP1. In the present study, the hepatic blood flows were not different between groups and thus the possibility of this mechanism explaining an effect of GLP1 on the liver is ruled out.

The insulin secretion independent effect of GLP1 on hepatic glucose uptake that we observed is consistent with the presence of specific GLP1 receptors on the liver cells. Binding of GLP1 to these receptors could initiate intracellular signaling pathways which could target kinases and/or factors involved in glucose uptake and glycogen synthesis. There are numerous reports of the presence of GLP1 receptors in liver (2, 42) and in in vitro studies, GLP1 per se has been shown to increase glycogen synthesis in liver (2). However, the presence of such hepatic receptors remains controversial (2, 4, 6, 42). The utilization of a GLP1 receptor antagonist such as exendin [9-39] could be usefull in future studies to clarify this mechanistic and important physiological issue. The presence of GLP1 receptors in other glucose consuming tissues, such as skeletal muscle and adipose tissue, has also been reported (11, 39, 41) but the mechanism of action of GLP1 in vitro appears to involve an enhancement of insulin’s effects since GLP1 potentiates insulin effects on glucose transport or glycogen synthesis (20, 43, 44). In view of the above data and the established presence of GLP1 receptors on peripheral tissues such as skeletal muscle, it is surprising that we were unable to detect any stimulatory effect of GLP1 on non-HGU in our study. This lack of response in peripheral tissues was not related to a decrease of glucose availability since hyperglycemia was clamped at a fixed value by a peripheral glucose infusion. There are nevertheless several possible explanations: 1) GLP1 has no effect on glucose utilization by peripheral tissues. This hypothesis is in accordance with the few in vitro studies which were unable to detect a GLP1 effect on
glucose metabolism (13, 16), 2) GLP1 indeed increased peripheral glucose utilization but its effect was too small to be detected given the power of our study, 3) the conditions of hyperglycemia and hyperinsulinemia had already maximally stimulated glucose uptake in peripheral tissues and thus no further additional effect could be recorded, or 4) reciprocal cross-talk between liver and non hepatic tissues was such that, when NHGU increased, non hepatic glucose uptake was limited. Hypothesis 3 is unlikely, since McGuinness et al. (26) showed that the half-maximally effective plasma insulin level on glucose utilization by muscle and fat in dog is 80-100 μU/ml, levels which are 5 times higher than the hyperinsulinemia created in our study. Regarding the last hypothesis, Galassetti et al. (14) performed a study in which the non hepatic glucose uptake and hindlimb glucose uptake were measured in the presence or absence of negative arterial-portal glucose gradient (i.e to modulate the NHGU). In order to match the glucose delivery to peripheral tissues whatever the glucose infusion route, a pancreatic clamp was performed to maintain insulin, glucagon and arterial glucose concentrations. This study revealed that the stimulation of NHGU was associated with a decrease in glucose uptake by peripheral tissues by 40%. A similar inhibition was evident upon assessing hindlimb (approximately 2/3 skeletal muscle in the dog) glucose uptake, directly demonstrating that muscle was the main site of this inhibition. Such a mechanism, probably initiated within the hepatoportal region, was observed in the study of Nishizawa et al. (31) because GLP1’s enhancement of non-HGU disappeared when NHGU was stimulated. It remains unclear, however, why in that study we observed a stimulatory effect on non-HGU whereas we did not in the present study. In the first study, GLP1 infusion raised arterial peptide levels to ≈ 400pM which was 2 times the concentrations we achieved in the present study. We cannot exclude the possibility that the short-term stimulation of non-HGU by GLP1 requires high pharmacological levels to be effective. Another possible explanation for this discrepancy could be that, in our study, even with the short-term infusion of GLP1, the hepatic glucose uptake and glycogen synthesis were already slightly enhanced (i.e significant stimulation of glycogen synthesis). We may postulate that this small but already present hepatic stimulation by GLP1 had already initiated a signal toward the peripheral tissues to prevent an increase of peripheral glucose utilization. Whether or not
GLP1 stimulation of non-HGU is dose-dependent and/or directly dependent on the NHGU needs further investigation.

Several studies have been performed in vivo to assess the role of GLP1 on glucose utilization independent of its effect on insulin secretion. The results, however, are divergent and conclusions are hard to draw. Most of the clamp studies showing no insulin secretion-independent effect of GLP1 were conducted under euglycemic conditions (33, 34) whereas, when a positive effect was recorded, the studies were performed in the presence of hyperglycemia (35, 40). These results suggest that the presence of hyperglycemia is required to fully express GLP1 effects. The glucose dependent effect of GLP1 has largely been studied with regard to its effect on pancreatic β cells. Indeed, it is now well established that GLP1 enhances insulin secretion only in the presence of increased ambient glucose concentrations (1). Such a mechanism might be also postulated for the insulin–secretion independent effect of GLP1 on glucose utilization. In addition, the present study showed that GLP1 may have tissue specific effects. We recorded an effect of GLP1 on liver but not on non-hepatic tissues. Because of the limited contribution of the liver to whole body glucose utilization, whole body glucose utilization (Rd) and glucose infusion rates were not significantly increased during the GLP1 infusion in the present study. The controversial results recorded in vivo regarding the GLP1’s effect on glucose metabolism may arise from this tissue specificity, since a small increase in NHGU caused by GLP1 may simply not be detected if only whole body glucose utilization and glucose infusion rates are assessed.

Recent reports highlight the importance of the hepatoportal area in the physiological activity of GLP-1 (3, 5, 21). For example, Nakabayashi et al. (30) reported that intraportal GLP-1 infusion at a physiological dose stimulated afferent vagal nerve activity in rats. This activation, in turn, stimulated efferent signaling in the pancreatic branch of the vagus nerve, suggesting a neural component of GLP-1’s stimulation of insulin secretion. We hypothesized that the hepatoportal region may be also involved in the insulin secretion independent effect of GLP1. Our study clearly showed that GLP1 infused intraportally did not further increase glucose utilization by the liver or peripheral tissues when compared to a peripheral infusion of GLP1. However, the dose of GLP1 used may not
have been optimal to identify the hepato-portal component of GLP1 action. Indeed, the infusion rates used substantially increased portal GLP1 concentrations even when infused peripherally. If the hepatoportal region is involved in the insulin secretion independent effect of GLP1, this rise in portal GLP1 may have already been too great to allow us to distinguish portal from peripheral delivery. Further studies are thus needed to address the involvement of the hepatoportal region in the mechanism of action of GLP1 on the liver or peripheral tissues.

In conclusion, GLP1, at low pharmacological levels, increased NHGU and hepatic glycogen synthesis. This effect was time-dependent and independent of insulin or glucagon secretion. The physiological relevance of our observation needs to be verified with lower doses of GLP1 and plasma levels normally observed during a normal post-prandial state. Nevertheless, our findings support a role for GLP1 as a tool for the reduction of postprandial hyperglycemia in individuals with diabetes.
Acknowledgements

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References


Figure Legends

**Figure 1:** Protocol design.

**Figure 2:** GLP1 effects on Net Hepatic Glucose uptake (NHGU) and Net Hepatic Fractional Extraction of Glucose (FE) in 42h fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are expressed as mean ± SE (n=6) and represent the incremental increase of NHGU and FE over the insulin period P1. * p<0.05 versus Control group.

**Figure 3:** GLP1 effects on glucose infusion rate and whole body glucose utilization (Rd) in 42h fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are mean ± SE (n=6) for each test period: P1=120-210 min; P2= 210-300 min and P3= 300-390 min; * p<0.05 versus P1 period, † P<0.05 versus P2 period.

**Figure 4:** Net hepatic lactate balance and net hepatic carbon retention (NHCR) in 42h fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are mean ± SE (n=6) for each test period: Basal= 90-120 min; P1=120-210 min; P2= 210-300 min and P3= 300-390 min; * p<0.05 versus Basal period. GE= glucose equivalent; * p<0.05 versus Control group.
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**[3-3H] Glucose**

- Somatostatin peripherally (0.8 μg/kg/min)
- Insulin intraportally (1.2 mU/kg/min)
- Glucagon intraportally (0.55 ng/kg/min)
- Glucose peripherally (variable to clamp at 12.5 mM)

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**GLP1 Infusion**

- 7.5 pmol/kg/min peripherally

**PePe Group**

**PePo Group**

- 7.5 pmol/kg/min peripherally
- 7.5 pmol/kg/min intraportally

**Figure 1**
GLP1 Effect: Delta over P1

Figure 2

A

NHGU (μmol/kg/min)

Control  PePe  PePo

P2  P3

B

Net Hepatic Fractional Extraction of Glucose

P2  P3
Figure 3
Figure 4

- Control
- PePe
- PePo

**Net Hepatic Carbon Retention (µmol GE/kg/min)**

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**Net Hepatic Lactate Balance (µmol/kg/min)**

- Uptake
- Output

GLP1 Effect: Delta over P1

- Control
- PePe
- PePo

**Net Hepatic Carbon Retention (µmol GE/kg/min)**

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Table 1: Hepatic blood flows and plasma insulin, glucagon and GLP1 concentrations in 42h fasted dogs under basal conditions and during infusion of somatostatin, intraportal insulin and glucagon, and peripheral glucose. GLP1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group).

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<tr>
<td></td>
<td>Control</td>
<td>PePe</td>
<td>PePo</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Hepatic Arterial Flow</strong></td>
<td>6.2 ± 0.7</td>
<td>5.6 ± 0.4</td>
<td>4.9 ± 0.3</td>
<td>7.3 ± 0.9*</td>
</tr>
<tr>
<td><strong>Portal Vein Flow</strong></td>
<td>27.5 ± 2.3</td>
<td>28.6 ± 3.1</td>
<td>27.3 ± 2.8</td>
<td>22.1 ± 2.4</td>
</tr>
<tr>
<td><strong>Total Hepatic Blood Flow</strong></td>
<td>33.7 ± 2.4</td>
<td>34.2 ±3.2</td>
<td>32.2 ± 2.8</td>
<td>29.5 ± 2.6</td>
</tr>
<tr>
<td><strong>Arterial Insulin</strong></td>
<td>9.7 ± 1.1</td>
<td>6.7 ± 0.9</td>
<td>6.8 ± 0.6</td>
<td>20.9 ± 1.8*</td>
</tr>
<tr>
<td><strong>Hepatic Sinusoidal Insulin</strong></td>
<td>19.2 ± 3.7</td>
<td>18.0 ± 2.3</td>
<td>15.2 ± 1.1</td>
<td>70.3 ± 5.1*</td>
</tr>
<tr>
<td><strong>Arterial Glucagon</strong></td>
<td>47.8 ± 3.6</td>
<td>46.7 ± 7.7</td>
<td>42.9 ± 5.7</td>
<td>41.4 ± 5.2</td>
</tr>
<tr>
<td><strong>Hepatic Sinusoidal Glucagon</strong></td>
<td>45.2 ± 2.3</td>
<td>54.2 ± 6.6</td>
<td>50.2 ± 4.5</td>
<td>50.3 ± 1.4</td>
</tr>
<tr>
<td><strong>Arterial GLP1</strong></td>
<td>N.D</td>
<td>5.4 ± 0.8</td>
<td>11.7 ± 2.9</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Portal GLP1</strong></td>
<td>N.D</td>
<td>6.7 ± 1.5</td>
<td>12.5 ± 4.4</td>
<td>N.D</td>
</tr>
</tbody>
</table>

Data are mean ± SE, n=6. P1=120-210 min; P2= 210-300 min and P3= 300-390 min
*: p<0.05 versus basal period; †: p<0.05 vs PePe; N.D=not determined