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Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region

D. Dardevet,^{1,2} M. C. Moore,¹ D. Neal,¹ C. A. DiCostanzo,¹ W. Snead,¹ and A. D. Cherrington¹

¹Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine,

Nashville, TN 37232; and ²Unité de Nutrition et Métabolisme Protéique, Institut National de la

Recherche Agronomique, Centre de Recherches en Nutrition Humaine de Clermont-Fd, 63122 Ceyrat, France

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Dardevet, D., M. C. Moore, D. Neal, C. A. DiCostanzo, W. Snead, and A. D. Cherrington. Insulin-independent effects of GLP-1 on canine liver glucose metabolism: duration of infusion and involvement of hepatoportal region. *Am J Physiol Endocrinol Metab* 287: E75–E81, 2004. First published March 16, 2004; 10.1152/ajpendo.00035.2004.—Whether glucagon-like peptide-1 (GLP-1) has insulin-independent effects on glucose disposal in vivo was assessed in conscious dogs by use of tracer and arteriovenous difference techniques. After a basal period, each experiment consisted of three 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 GLP-1 (7.5 pmol·kg⁻¹·min⁻¹) peripherally (Pe; iv) in P2 and P3, and the PePo group received saline in P1 and GLP-1 peripherally (iv) (P2) and then into the portal vein (Po; P3). Glucose and insulin concentrations increased to two- and fourfold basal, respectively, and glucagon remained basal. GLP-1 levels increased similarly in the PePe and PePo groups during P2 (~200 pM), whereas portal GLP-1 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 GLP-1 was demonstrated (16.61 ± 2.91 and 14.67 ± 2.09 vs. 4.22 ± 1.57 μmol·kg⁻¹·min⁻¹, respectively). In conclusion: GLP-1 increased glucose disposal in the liver independently of insulin secretion; its full action required long-term infusion. The route of infusion did not modify the hepatic response.

glucagon-like peptide-1; glucose uptake; portal infusion; dog

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) 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 (22, 37). The main and active form of GLP-1, GLP-1-(7–36), is rapidly degraded by dipeptidyl peptidase IV (DPP-IV) into GLP-1-(9–36) in the intestinal tissues as well as in the blood (23, 27). The consequence is a rapid elimination of GLP-1 from plasma with a half-life estimated at 1–2 min in several species (22). The earliest biological effect of GLP-1 discovered was its ability to increase glucose-dependent insulin secretion (18, 28) as well as the transcription of the proinsulin gene and biosynthesis of insulin (12). The glucose-dependent effect of GLP-1 on insulin secretion has suggested a potential use of this agent in the treatment of diabetes without deleterious hypoglycemia. Indeed, it lowers postprandial glucose levels in both healthy and human subjects

with type 2 diabetes by stimulating insulin secretion but also by inhibiting glucagon secretion from the α-cell and delaying gastric emptying (1, 17, 40).

GLP-1 receptors are present on β-cells (38), and there are numerous reports of GLP-1 receptors on glucose-consuming tissues such as liver, skeletal muscle and fat (11, 39, 42). Most in vitro studies show that GLP-1 stimulates glucose uptake and metabolism in muscle (24, 41, 44), adipocytes (20, 43), and hepatocytes (2) and that GLP-1 can potentially promote glucose disposal directly, in addition to its effect on insulin secretion. In vivo, GLP-1's insulin secretion-independent effects are much less clear. Some data are in agreement with a GLP-1 effect independent of insulin secretion (8–10, 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 GLP-1 delivery into the systemic circulation.

GLP-1 concentrations are physiologically increased during the postprandial phase, and its direct tissue effects may require the presence of insulin and/or hyperglycemia to be fully manifested. Furthermore, GLP-1 is secreted into the hepatic portal vein, and recent evidence suggests the presence of GLP-1 sensors or receptors in the hepatoportal region (5, 30, 32). Because of the rapid degradation of GLP-1 in the plasma, the hepatoportal region may play a critical role in the generation of the full effects of GLP-1. Because of these considerations, a recent study was conducted in our laboratory (31), in which the effect of various rates of portal GLP-1 infusion was sequentially studied under conditions of controlled hyperinsulinemia and hyperglycemia in the conscious dog. The results showed that GLP-1 has an effect independent of its action on insulin secretion. However, despite an apparent dose-dependent effect of GLP-1, the study design raised the possibility that GLP-1 may also have a time-dependent effect. We performed the present studies in the conscious dog model to assess the time-dependent effect of GLP-1 on hepatic and nonhepatic glucose metabolism under conditions that mimic the postprandial 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 GLP-1 levels in plasma were increased in the dog after an intragastric glucose load to levels similar to those recorded in humans and

Address for reprint requests and other correspondence: D. Dardevet, 702 Light Hall, Vanderbilt Univ. School of Medicine, Nashville, TN 37232-0615 (E-mail: Dominique.P.Dardevet@vanderbilt.edu).

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other species when fed 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) vs. short (90-min) duration peripheral GLP-1 infusion. The role of the hepatoportal region in the insulin secretion-independent effects of GLP-1 was assessed by comparing the effects of peripheral and portal infusions of GLP-1 in the same dog.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on 18 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 saphenous 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 min each (P1 = 120–210 min; P2 = 210–300 min; P3 = 300–390 min) during which hyperglycemia and hyperinsulinemia existed (Fig. 1). At $t = 0$, a primed ($1.2 \mu\text{Ci/kg}$), continuous ($0.17 \mu\text{Ci/min}$) infusion of [$3\text{-}^3\text{H}$]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 $t = 120 \text{ min}$, a peripheral (Pe) infusion of somatostatin ($0.8 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was begun to inhibit endogenous pancreatic insulin and glucagon secretion. Intraportal (Po) infusions of insulin ($1.2 \text{ mU}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), to achieve hyperinsulinemia (≈ 3 times the basal arterial levels), and glucagon ($0.55 \text{ ng}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), to maintain basal levels, were also started. A dextrose solution was infused peripherally at variable rates starting at $t = 120 \text{ min}$ to clamp the arterial plasma glucose level at 12.5 mM . 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 GLP-1-(7–36) amide infusion at $7.5 \text{ pmol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ (Bachem Bioscience, King of Prussia, PA). In the PePe group ($n = 6$), GLP-1 was infused peripherally until the end of the experiment (P2 + P3 periods). In the PePo group ($n = 6$), GLP-1 was first infused peripherally for 90 min (P2 period) and then intraportally (at the same rate) for the next 90 min (P3 period). This infusion rate was chosen to generate mild pharmacological levels of GLP-1. Blood was collected on EDTA, and an inhibitor of DPP-IV activity (Linco Research, St. Charles, MO) was immediately added to whole blood to preserve the integrity of the GLP-1.

Analytical procedures. Plasma glucose and glucose radioactivity (^3H), insulin, glucagon, and blood lactate were measured as previously described (31). Plasma GLP-1 concentrations were determined by an ELISA method (Linco Research) that specifically quantifies the biologically active form of GLP-1.

Calculations. Net hepatic substrate balance (NHB) was calculated using the formula $[\text{H}\cdot\text{Ft} - (\text{A}\cdot\text{Fa}) + (\text{P}\cdot\text{Fp})]$, 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 $(\text{A}\cdot\text{Fa}) + (\text{P}\cdot\text{Fp})$. Net hepatic fractional extraction (FE) was calculated as NHB divided by hepatic load. For all calculated data, plasma glucose concentrations were converted to blood concentrations with factors compiled in our laboratory from extensive data in which plasma and blood glucose values were compared (19). Sinusoidal hormone concentrations were calculated as described previously (31). Nonhepatic 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 period. 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 to assess the specific effect of GLP-1 over the effect achieved by the pancreatic hormones and hyperglycemia per se.

Data are expressed as means $\pm$ 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) was recorded following GLP-1 infusion, indicating that GLP-1 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 GLP-1 did not modify the concentrations of the pancreatic hormones, and the

Fig. 1. Protocol design. P1, P2, and P3, test periods at indicated time intervals. GLP-1, glucagon-like peptide-1; PePe, GLP-1 infused peripherally during P2 and P3; PePo, GLP-1 infused peripherally during P2 and intraportally during P3.

Table 1. Hepatic blood flows and plasma insulin, glucagon, and GLP-1 concentrations in 42-h-fasted dogs under basal conditions and during infusion of somatostatin, intraportal insulin, and glucagon and peripheral glucose

	Basal			P1			P2			P3		
	Control	PePe	PePo	Control	PePe	PePo	Control	PePe	PePo	Control	PePe	PePo
Hepatic arterial flow	6.2 ±0.7	5.6 ±0.4	4.9 ±0.3	7.3* ±0.9	7.8* ±1.1	5.6 ±0.3	8.1* ±1.3	8.7* ±1.3	6.1* ±0.3	9.4* ±1.4	8.9* ±1.1	7.3* ±0.2
Portal vein flow	27.5 ±2.3	28.6 ±3.1	27.3 ±2.8	22.1 ±2.4	23.2 ±2.9	21.1 ±1.7	21.92 ±2.20	22.6 ±3.1	20.5 ±1.7	21.5 ±2.2	22.6 ±3.1	20.7 ±1.7
Total hepatic blood flow	33.7 ±2.4	34.2 ±3.2	32.2 ±2.8	29.5 ±2.6	31.0 ±3.0	26.8 ±1.9	29.9 ±2.6	31.4 ±2.9	26.7 ±1.7	31.0 ±2.7	31.6 ±3.0	28.0 ±1.5
Arterial insulin	9.7 ±1.1	6.7 ±0.9	6.8 ±0.6	20.9* ±1.8	20.4* ±2.5	15.2* ±2.1	21.7* ±1.9	19.8* ±1.9	16.3* ±1.7	22.1* ±1.9	22.1* ±2.2	19.4* ±1.6
Hepatic sinusoidal insulin	19.2 ±3.7	18.0 ±2.3	15.2 ±1.1	70.3* ±5.1	78.1* ±6.7	73.8* ±4.8	76.7* ±6.7	77.9* ±5.3	71.0* ±7.8	76.4* ±7.1	78.1* ±6.6	70.0* ±7.1
Arterial glucagon	47.8 ±3.6	46.7 ±7.7	42.9 ±5.7	41.4 ±5.2	44.8 ±3.4	42.1 ±3.1	38.7 ±2.8	36.7 ±5.3	38.5 ±3.5	40.1 ±3.5	34.7 ±3.5	37.5 ±2.4
Hepatic sinusoidal glucagon	45.2 ±2.3	54.2 ±6.6	50.2 ±4.5	50.3 ±1.4	47.5 ±6.1	55.5 ±3.3	50.5 ±2.7	45.8 ±3.7	52.5 ±2.1	48.3 ±3.4	45.2 ±5.7	48.6 ±3.7
Arterial GLP-1	N.D.	5.4 ±0.8	11.7 ±2.9	N.D.	5.4 ±0.7	9.8 ±3.2	N.D.	210.6* ±27.1	204.6* ±45.6	N.D.	194.8* ±20.2	186.7* ±28.6
Portal GLP-1	N.D.	6.7 ±1.5	12.5 ±4.4	N.D.	4.5 ±0.2	9.8 ±3.8	N.D.	191.1* ±35.4	196.5* ±40.7	N.D.	169.0* ±39.2	539.9* ±71.5

Data are means ± SE; *n* = 6. P1, 120–210 min; P2, 210–300 min; P3, 300–390 min. Glucagon-like peptide-1 (GLP-1) was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). **P* < 0.05 vs. basal period; †*P* < 0.05 vs. PePe; ND, not determined.

effects recorded will thus be independent of the GLP-1 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 (iv) GLP-1 infusion, arterial and portal GLP-1 concentrations increased significantly and reached supraphysiological levels (≈200 pM; Table 1). These levels remained constant with time in the PePe group. In the PePo group, arterial and portal GLP-1 levels increased to the same extent as those in the PePe group during P2, but portal GLP-1 concentrations dramatically increased (≈500 pM) with the start of the portal infusion of GLP-1 (P3). Interestingly, arterial GLP-1 concentrations were only slightly lower with portal vein GLP-1 infusion than with peripheral GLP-1 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 ≈6 to ≈12.5 mM. The hepatic glucose loads during the basal period were 150 ± 11 , 151 ± 13 , and $135 \pm 12 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in Control, PePe, and PePo groups, respectively. In response to the glucose infusion, hepatic glucose loads increased significantly and similarly in all groups and then remained constant over time (P1: 261 ± 22 , 260 ± 21 , and 237 ± 14 ; P2: 272 ± 23 , 265 ± 20 , and 244 ± 11 ; and P3: 277 ± 23 , 266 ± 20 , and $251 \pm 10 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in Control, PePe, and PePo groups, respectively). The combination of hyperinsulinemia and hyperglycemia during the P1 test period switched the liver from net hepatic glucose output to NHGU in all groups (data not shown). During saline infusion, NHGU increased slightly (over P1) during P2 and P3 [$\Delta = 0.16 \pm 1.53$ and 4.22 ± 1.57 (not significant, NS), respectively; Fig. 2A]. Peripheral infusion of GLP-1 did not enhance NHGU during P2 (relative to P1), but it stimulated NHGU (≈3-fold: $16.61 \pm 2.91 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) after more prolonged infusion (P3). The route of GLP-1 infusion

made no difference in its ability to increase NHGU in P3 ($14.67 \pm 2.09 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; Fig. 2A).

The FE of glucose followed the same pattern as NHGU (Fig. 2B). Short-term peripheral GLP-1 infusions did not increase FE significantly, whereas prolonged GLP-1 infusions did. Nevertheless, peripheral and portal GLP-1 infusions resulted in the same increase ($\Delta = 1.5 \pm 1.0$, 6.4 ± 1.3 , and $5.1 \pm 0.9\%$ for C, PePe, and PePo during P3 relative to P1, respectively; Fig. 2B).

Fig. 2. GLP-1 effects on net hepatic glucose uptake (NHGU; A) and net hepatic fractional extraction (B) in 42-h-fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP-1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are expressed as means ± SE (*n* = 6) and represent incremental increase of NHGU and FE over insulin period P1. **P* < 0.05 vs. Control group.

The mean GIR required to maintain constant hyperglycemia increased significantly over the experimental periods (Fig. 3). However, no differences between groups were recorded in any test period. When assessed with the tracer dilution technique, the whole body glucose utilization (R_d) followed the same pattern as the GIR (Fig. 3). R_d increased with time in each experimental group, but no differences were recorded between groups at any time point. The nonhepatic glucose uptake (non-HGU) was the same in all groups in P1 (28.5 ± 2.9 , 27.6 ± 4.3 , and $26.1 \pm 4.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for C, PePe, and PePo, respectively). During P2, non-HGU increased significantly in each experimental group (40.9 ± 3.7 , 39.7 ± 3.1 , and $43.2 \pm 8.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for C, PePe, and PePo, respectively), with no significant difference among groups. During P3, after the prolonged peripheral or intraportal GLP-1 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 of the test periods P1, P2 and P3 (from ≈ 0.6 to 1.0 mM), 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 $0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in all groups during P2 and P3 (Fig. 4). Concomitantly, P1 was associated with NHCR (data not shown). During the peripheral GLP-1 infusion (P2), NHCR was significantly stimulated over P1 in PePe and PePo groups compared with C ($\Delta = 1.0 \pm 1.8$, 6.6 ± 2.2 , and $8.6 \pm 0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for C, PePe, and PePo, respectively; Fig. 4). With prolonged GLP-1 infusion (P3), NHCR further increased and was significantly higher in the PePe and PePo groups ($\Delta = 5.1 \pm 1.4$, 16.2 ± 2.7 , and $18.5 \pm 1.3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for C, PePe, and PePo, respectively; $P < 0.05$ vs. P1 for all groups; $P > 0.05$ for PePe vs. PePo; Fig. 4).

Fig. 3. GLP-1 effects on glucose infusion rate (A) and whole body glucose utilization (R_d ; B) in 42-h-fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP-1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are expressed as means $\pm$ SE ($n = 6$) for each test period. * $P < 0.05$ vs. P1; † $P < 0.05$ vs. P2.

Fig. 4. Net hepatic lactate balance (A) and net hepatic carbon retention (B) in 42-h-fasted dogs in the presence of somatostatin, intraportal insulin and glucagon, and peripheral glucose infusions. GLP-1 was infused peripherally during P2 and P3 (PePe group) and peripherally during P2 and intraportally during P3 (PePo group). Data are expressed as means $\pm$ SE ($n = 6$) for each test period. GE, glucose equivalent. * $P < 0.05$ vs. Basal period; ** $P < 0.05$ vs. Control group.

DISCUSSION

Previous work performed in our laboratory (31) had shown that GLP-1 is able to increase glucose disposal independently of its well-recognized effects on insulin and glucagon secretion. Furthermore, the results had suggested that the insulin secretion-independent effects of GLP-1 were dose dependent, but the design of the study had left open the possibility of a time-dependent effect of GLP-1, i.e., that prolonged infusions might be required to observe the full effects of the hormone. If so, the sensitivity of these processes to GLP-1 might be much greater than what was indicated in that study. The present study was carried out to examine this issue. Furthermore, in light of the recent suggestion that GLP-1 receptors in the portal vein might play an important physiological role (5, 30, 32), the potential involvement of the hepatoportal region in the mechanism of action of GLP-1 was assessed by comparing peripheral and intraportal infusions of GLP-1.

In the current study, GLP-1 was infused at a rate of $7.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ to achieve modest pharmacological arterial GLP-1 concentrations. This rate, based on the study of Nishizawa et al. (31), was chosen to increase GLP-1 concentrations enough to observe maximal effects of the peptide. In the present study, GLP-1 concentrations reached $\approx 200 \text{ pM}$ in the arterial plasma and went up to $\approx 500 \text{ pM}$ in the portal vein when GLP-1 was infused intraportally. Based on the literature

(25), the physiological arterial level of GLP-1 following an oral glucose load (2 g/kg) or mixed meal in dogs is 20 pM. Because of the difference between the assays or antibodies used in the determination of GLP-1, we measured (using the same assay as for our present experiment) intact arterial and portal GLP-1 concentrations in four overnight-fasted dogs under basal conditions and following an intragastric glucose load of 1.5 g/kg (unpublished data). The basal levels were ≈ 17 pM in both artery and portal vein. After the glucose bolus, GLP-1 levels peaked at ≈ 40 pM in the artery and ≈ 65 pM in the portal vein. On the basis of these data, GLP-1 levels in our present experiment were five to seven times the normal physiological postprandial concentration.

Our results clearly show that GLP-1 stimulated NHGU but had no effect on non-HGU. These studies created conditions that mimicked postprandial levels of insulin and glucose, conditions during which GLP-1 secretion is physiologically enhanced. The stimulation of glucose disposal was independent of pancreatic hormone secretion, as insulin and glucagon levels remained constant during GLP-1 infusion. In addition, our study also clearly demonstrated that the effects of GLP-1 were time dependent. Indeed, GLP-1 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 GLP-1 enhanced NHGU without increasing non-HGU. In that study, the possibility existed that the effect of GLP-1 on the liver was a consequence of increased hepatic blood flow, which was recorded concomitantly with this high dose of GLP-1. In the present study, the hepatic blood flows were not different between groups, and thus the possibility of this mechanism explaining an effect of GLP-1 on the liver is ruled out.

The insulin secretion-independent effect of GLP-1 on hepatic glucose uptake that we observed is consistent with the presence of specific GLP-1 receptors on the liver cells. Binding of GLP-1 to these receptors could initiate intracellular signaling pathways that could target kinases and/or factors involved in glucose uptake and glycogen synthesis. There are numerous reports of the presence of GLP-1 receptors in liver (2, 42), and in *in vitro* studies GLP-1 *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 GLP-1 receptor antagonist such as exendin-(9–39) could be useful in future studies to clarify this mechanistic and important physiological issue. The presence of GLP-1 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 GLP-1 *in vitro* appears to involve an enhancement of insulin's effects, since GLP-1 potentiates insulin effects on glucose transport or glycogen synthesis (20, 43, 44). In view of the aforementioned data and the established presence of GLP-1 receptors on peripheral tissues such as skeletal muscle, it is surprising that we were unable to detect any stimulatory effect of GLP-1 on non-HGU in our study. This lack of response in peripheral tissues was not related to a decrease of glucose availability, as hyperglycemia was clamped at a fixed value by a peripheral glucose infusion. There are, nevertheless, several possible explanations. 1) GLP-1 has no effect on glucose utilization by peripheral tissues, this hypothesis being in accord with the few *in vitro* studies that were unable to detect a GLP-1 effect on glucose

metabolism (13, 16); 2) GLP-1 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 nonhepatic tissues was such that, when NHGU increased, non-HGU was limited. The third hypothesis is unlikely, because 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 μ U/ml, levels that are five times higher than the hyperinsulinemia created in our study. Regarding the last hypothesis, Galassetti et al. (14) performed a study in which the non-HGU and hindlimb glucose uptake were measured in the presence or absence of negative arterial-portal glucose gradient (i.e., to modulate the NHGU). 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 (14) 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 two-thirds of 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 hepatportal region, was observed in the study of Nishizawa et al. (31) because GLP-1's enhancement of non-HGU disappeared when NHGU was stimulated. It remains unclear, however, why we observed in that study a stimulatory effect on non-HGU whereas we did not in the present study. In the first study (31), GLP-1 infusion raised arterial peptide levels to ≈ 400 pM, which was two times the concentrations we achieved in the present study. We cannot exclude the possibility that the short-term stimulation of non-HGU by GLP-1 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 GLP-1, 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 GLP-1 had already initiated a signal toward the peripheral tissues to prevent an increase of peripheral glucose utilization. Whether or not GLP-1 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 GLP-1 on glucose utilization independent of its effect on insulin secretion. The results, however, are divergent and conclusions hard to draw. Most of the clamp studies showing no insulin secretion-independent effect of GLP-1 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 GLP-1 effects. The glucose-dependent effect of GLP-1 has largely been studied with regard to its effect on pancreatic β -cells. Indeed, it is now well established that GLP-1 enhances insulin secretion only in the presence of increased ambient glucose concentrations (1). Such a mechanism might also be postulated for the insulin secretion-independent effect of GLP-1 on glucose utilization. In addition, the present study

showed that GLP-1 may have tissue-specific effects. We recorded an effect of GLP-1 on liver but not on nonhepatic tissues. Because of the limited contribution of the liver to whole body glucose utilization, whole body glucose R_d and GIRs were not significantly increased during the GLP-1 infusion in the present study. The controversial results recorded in vivo regarding GLP-1's effect on glucose metabolism may arise from this tissue specificity, since a small increase in NHGU caused by GLP-1 may simply not be detected if only whole body glucose R_d and GIRs 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 also be involved in the insulin secretion-independent effect of GLP-1. Our study clearly showed that GLP-1 infused intraportally did not further increase glucose utilization by the liver or peripheral tissues compared with a peripheral infusion of GLP-1. However, the dose of GLP-1 used may not have been optimal to identify the hepatoportal component of GLP-1 action. Indeed, the infusion rates used substantially increased portal GLP-1 concentrations even when infused peripherally. If the hepatoportal region is involved in the insulin secretion-independent effect of GLP-1, this rise in portal GLP-1 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 GLP-1 on the liver or peripheral tissues.

In conclusion, GLP-1, 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 GLP-1 and plasma levels normally observed during a normal postprandial state. Nevertheless, our findings support a role for GLP-1 as a tool for the reduction of postprandial hyperglycemia in individuals with diabetes.

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