

Exenatide Sensitizes Insulin-Mediated Whole-Body Glucose Disposal and Promotes Uptake of Exogenous Glucose by the Liver

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OBJECTIVE—Recent progress suggests that exenatide, a mimetic of glucagon-like peptide-1 (GLP-1), might lower glycemia independent of increased β -cell response or reduced gastrointestinal motility. We aimed to investigate whether exenatide stimulates glucose turnover directly in insulin-responsive tissues dependent or independent of insulinemia.

RESEARCH DESIGN AND METHODS—An intraportal glucose infusion clamp was used in dogs to measure glucose turnover to encompass potent activation of the putative glucose/GLP-1 sensor in the porto-hepatic circulation with exenatide. The modified glucose clamp was performed in the presence of postprandial hyperinsulinemia and hyperglycemia with exenatide (20 μ g) or saline injected at 0 min. Furthermore, the role of hyperglycemia versus hyperinsulinemia in exenatide-mediated glucose disposal was studied.

RESULTS—With hyperinsulinemia and hyperglycemia, exenatide produced a significant increase in total glucose turnover by $\sim 30\%$, as indicated by portal glucose infusion rate (saline 15.9 ± 1.6 vs. exenatide 20.4 ± 2.1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.001$), resulting from increased whole-body glucose disposal (R_d , $\sim 20\%$) and increased net hepatic uptake of exogenous glucose ($\sim 80\%$). Reducing systemic hyperglycemia to euglycemia, exenatide still increased total glucose turnover by $\sim 20\%$ (saline 13.2 ± 1.9 vs. exenatide 15.6 ± 2.1 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$) in the presence of hyperinsulinemia, accompanied by smaller increments in R_d (12%) and net hepatic uptake of exogenous glucose (45%). In contrast, reducing hyperinsulinemia to basal levels, exenatide-increased total glucose turnover was completely abolished despite hyperglycemia (saline 2.9 ± 0.6 vs. exenatide 2.3 ± 0.3 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.29$).

CONCLUSIONS—Exenatide directly stimulates glucose turnover by enhancing insulin-mediated whole-body glucose disposal and increasing hepatic uptake of exogenous glucose, contributing to its overall action to lower postprandial glucose excursions.

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Exenatide is the synthetic form of exendin-4 and a long-acting mimetic of the incretin hormone glucagon-like peptide-1 (GLP-1) (1). Originally isolated from salivary secretions of the lizard Gila monster, exendin-4 shows a 53% amino acid sequence identity to GLP-1 (2) and shares many actions with GLP-1 via pancreatic GLP-1 receptor (3). Exenatide stimulates glucose-dependent insulin response (4–6), suppresses glucagon secretion (5), and inhibits gastrointestinal motility (7). Exenatide has also been implicated in regulating food intake (8) and β -cell proliferation (9). Exenatide is resistant to digestion by dipeptidyl peptidase-IV and thus has a longer plasma half-life than GLP-1 (10).

Exenatide is currently used in the treatment of type 2 diabetes because of its effect to lower glycemia and improve glycemic control through multiple mechanisms as mentioned above. Exenatide (13 weeks) in diabetic *db/db* mice led to 50% lower glycosylated hemoglobin (A1C) than in nontreated animals (4). Type 2 diabetic patients who had not attained A1C goals $\leq 8\%$ with sulfonylureas and/or metformin achieved a $\sim 0.9\%$ reduction with exenatide (4 weeks) (11).

However, it appears that exenatide-improved glycemic control cannot be completely explained by its currently established actions. In particular, exenatide may have a direct effect on insulin-responsive tissues, although this latter effect is still under debate. In obese Zucker rats, exenatide (6 weeks) dramatically enhanced insulin sensitivity while preventing the progressive increase in A1C and fasting insulin (12). When compared with pair-fed animals with matching A1C, fasting glucose, insulin, and lipids, insulin sensitivity was still 64% higher with exenatide. In another study, exenatide administered with meals to type 2 diabetic patients resulted in reduced glucose excursions facing significantly lower postprandial insulin levels, not higher, as expected based on the insulinotropic effect of exenatide (5). The reduced postprandial glucose excursion has been attributed to exenatide inhibition of gastric emptying and/or glucagon secretion (5,7). However, by simulating intraportal rate of glucose appearance (R_a) in portal vein after meal with or without exenatide (thus bypassing its gastrointestinal effect), we have shown that exenatide lowered glycemia in the presence of similar insulin and glucagon levels (13). Although some studies have failed to show acute effects of exenatide on insulin action (14,15), our previous result suggests a potential exenatide effect to increase insulin sensitivity. The goal of our current study was to investigate whether exenatide directly stimulates glucose turnover dependent or independent of insulinemia in insulin-responsive tissues.

RESEARCH DESIGN AND METHODS

Experiments were conducted in male mongrel dogs (Harlan, Indianapolis, IN), housed under controlled conditions and fed once daily (Labdiet; PMI Nutrition International, Richmond IN). Animals were used for experiments only if they were judged to be in good health as determined by body weight, food intake and stools, temperature, hematocrit, and direct observation. All surgical and experimental procedures were approved by USC Institutional Animal Care and Use Committee.

Surgical procedures. At least 1 week before the first experiments, chronic catheters (Tygon, inner diameter = 0.05 inch; Norton Plastics, Akron, OH) were implanted under general anesthesia. One catheter was placed in jugular vein (with the tip advanced into right atrium) and the other in portal vein (4-cm upstream from porta hepatis). All catheters, filled with heparinized saline (10 units/ml), were led subcutaneously to the back of the neck, exteriorized, and secured in place.

Experimental design. Experiments were performed on animals in a conscious relaxed state. Animals were fasted for 14–16 h with free access to water. For all experiments, animals were brought to the laboratory at ~6:00 A.M. and placed into a Pavlov sling. Three types of experiments were performed in paired experiments with subcutaneous injection of either exenatide or saline to examine the effect of exenatide on glucose turnover. In all studies, we used a novel glucose clamp technique, the intraportal glucose infusion–glucose clamp. Unlike the classical clamp where exogenous glucose is given systemically, in these experiments, we infused exogenous glucose directly into portal vein to maintain the desired systemic glycemia. Previous evidence has suggested the presence of glucose sensors in the porto-hepatic circulation that may interact with GLP-1 to affect glucose clearance (16–22). Intraportal glucose infusion was used to stimulate any extant portal/hepatic sensors, as would happen during postprandial nutritional entry of carbohydrate into the systemic circulation. The intraportal glucose infusion–glucose clamp was performed under the following conditions: study 1, in the presence of hyperinsulinemia and hyperglycemia; study 2, in the presence of hyperinsulinemia but systemic euglycemia; and study 3, in the presence of hyperglycemia but basal insulin levels.

Study 1: Effect of exenatide on glucose turnover in the presence of postprandial hyperinsulinemia and hyperglycemia. Paired experiments with or without a single subcutaneous injection of exenatide were performed in nine animals (eight with tracer infusions), 28.8 ± 0.5 kg body wt. The modified glucose clamp was composed of two periods: a 2-h equilibrium period (–120 to 0 min), followed by injection of saline or exenatide, and a 4-h experimental period (0–240 min; Fig. 1A). At –120 min, immediately after a systemic injection of 25- μ Ci bolus of [3 -H]-D-glucose (“tracer”; DuPont-NEN, Boston, MA), a continuous infusion of tracer at 0.25 μ Ci/min was initiated via a peripheral venous catheter. Ninety minutes later, blood samples for the basal period were taken every 10 min from –30 to 0 min. At 0 min, peripheral infusions of somatostatin (Bachem, Torrance, CA), at 1 μ g · kg^{–1} · min^{–1}, and porcine insulin (Eli Lilly, Indianapolis, IN), at 0.75 mU · kg^{–1} · min^{–1}, were initiated to obtain systemic hyperinsulinemia of ~250 pmol/l. An intraportal infusion of porcine glucagon (Sigma, St. Louis, MO), at 0.65 ng · kg^{–1} · min^{–1}, was simultaneously started to maintain plasma glucagon at basal levels. At 0 min, a single subcutaneous injection of either exenatide (20 μ g Byetta; Amylin, San Diego, CA) or saline of equal volume was administered in paired experiments. During the experimental phase (0–240 min), blood samples were taken from another peripheral venous catheter every 10 min; plasma glucose were measured immediately. Exogenous glucose (50% dextrose, 454.5 mg/ml; B Braun, Irvine, CA) was given via the portal vein catheter at variable rates to maintain systemic glucose at 150 mg/dl. An additional tracer infusion was given at variable rates via the jugular vein catheter to minimize fluctuations in systemic plasma specific activity; the ratio between the rate of the additional systemic tracer infusion and that of intraportal glucose infusion was kept at 1.8 μ Ci/g. The average of the four samples taken from 150–180 min was defined as the clamp steady state when plasma glucose, insulin, and exogenous glucose infusion rate were least variable.

Study 2: Effect of exenatide on glucose turnover in the presence of hyperinsulinemia but systemic euglycemia. Paired experiments with or without a single subcutaneous injection of exenatide were conducted in eight animals (six with tracer infusions), 28.2 ± 0.7 kg body wt. The modified glucose clamp was used to measure glucose turnover as discussed above, except that intraportal glucose infusion was given to maintain systemic glucose at basal levels (Fig. 1A).

Study 3: Effect of exenatide on glucose turnover in the presence of hyperglycemia but basal insulin levels. Paired experiments with or without a single subcutaneous injection of exenatide were carried out in six animals (six with tracer infusions), 29.5 ± 0.8 kg body wt. The modified

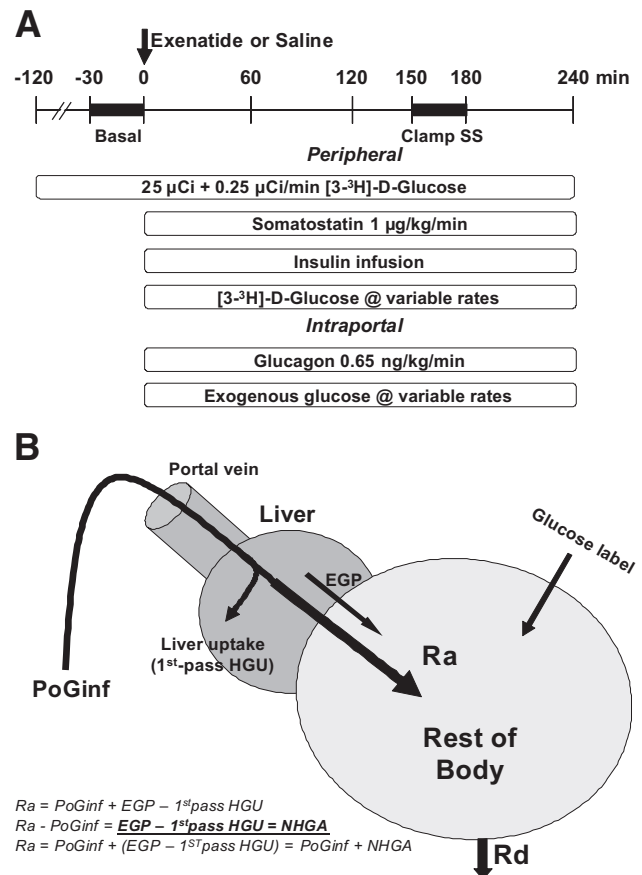


FIG. 1. A: Intraportal glucose infusion clamp. Basal and clamp SS indicate the steady state during the basal and clamp period, respectively. Porcine insulin was infused at either 0.75 (studies 1 and 2) or 0.15 mU · kg^{–1} · min^{–1} (study 3) to achieve postprandial hyperinsulinemia or maintain basal insulin levels. Exogenous glucose was given into portal vein to achieve systemic hyperglycemia (studies 1 and 3) or maintain systemic euglycemia (study 2). B: Calculation of NHGA. Glucose label indicates the constant and the variable tracer infusions.

glucose clamp was used to measure glucose turnover as discussed in study 1, except that plasma insulin was maintained at basal levels by a low infusion rate of 0.15 mU · kg^{–1} · min^{–1} while systemic glucose was raised to 150 mg/dl (Fig. 1A).

Blood sampling. Samples for the determination of glucose, free fatty acids (FFAs), insulin, C-peptide, glucagon, and tracer were collected as previously described (16,23). All samples were immediately centrifuged, and plasma was separated and stored at –80°C until analysis. To prevent triglyceride breakdown, FFAs samples were kept on ice and either immediately assayed or kept at –80°C for a limited time before assay, as previously described (16).

Assays. Glucose was measured with a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin was measured using a human insulin ELISA kit (Linco/Millipore, Billerica, MA) adapted for dog plasma (24). C-peptide and glucagon were measured using radioimmunoassay kits (Linco/Millipore). FFAs were determined using an enzymatic colorimetric assay (NEFA C; Wako Pure Chemical Industries, Richmond, VA). Samples for [3 -H]-D-glucose were deproteinized, dried, resuspended in scintillation fluid (ReadySafe; Beckman, Fullerton, CA), and then read in a β -scintillation counter.

Calculations. Two metabolic parameters can be calculated from the intraportal glucose infusion clamp: whole-body glucose disposal and a parameter we named “net hepatic glucose addition” (NHGA). The time course of whole-body glucose disposal [rate of glucose disappearance (R_d)] was calculated using Steele’s equation with a labeled glucose infusion (25) after data smoothing using OOPSEG (26). Because exogenous glucose was infused directly into portal vein, a fraction of intraportally given glucose would be taken up by the liver without entering the systemic circulation; referred to as 1st-pass hepatic glucose uptake (1st-pass HGU). Therefore, the R_a equals portal glucose infusion (PoGinf) plus endogenous glucose production (EGP) minus 1st-pass HGU (Eq. 1). The difference between EGP and 1st-pass HGU, which is defined as NHGA, is thus derived from the difference between R_a

TABLE 1

Intraportal glucose infusion clamp in the presence of postprandial hyperinsulinemia and hyperglycemia: basal and clamp steady-state parameters in the saline- and exenatide-treated groups

	Basal		Clamp steady state	
	Saline	Exenatide	Saline	Exenatide
Glucose (mg/dl)	92.6 ± 1.6	89.4 ± 2.1*	150.2 ± 2.8†	157.4 ± 4.3†
Insulin (pmol/l)	41.3 ± 4.5	45.8 ± 4.0	231.5 ± 10.5†	217.9 ± 11.5*†
C-peptide (ng/ml)	0.24 ± 0.06	0.25 ± 0.06	0.06 ± 0.06†	0.05 ± 0.04†
Glucagon (ng/l)	42.5 ± 4.2	50.8 ± 6.4	33.3 ± 3.2	33.5 ± 3.9†
FFAs (mmol/l)	0.58 ± 0.06	0.55 ± 0.11	0.02 ± 0.01†	0.03 ± 0.03†

Data are means ± SE. * $P < 0.05$ significantly different from the saline group. † $P < 0.05$ significantly different from basal in each group.

and PoGinf (Eq. 2; Fig. 1B). R_a itself was calculated using Steele's equation, similar to R_d .

$$R_a = \text{PoGinf} + \text{EGP} - \text{1st-pass HGU} \quad (1)$$

$$R_a - \text{PoGinf} = \text{EGP} - \text{1st-pass HGU} = \text{NHGA} \quad (2)$$

$$R_a = \text{PoGinf} + (\text{EGP} - \text{1st-pass HGU}) = \text{PoGinf} + \text{NHGA} \quad (3)$$

In an intraportal glucose infusion–glucose clamp, NHGA, as the name implies, reflects the net addition of glucose by the liver to portally infused exogenous glucose (Eq. 3). During the basal period, PoGinf and 1st-pass HGU both equal 0; thus, $R_a = \text{EGP} = \text{NHGA}$. During the experimental period, under the influence of elevated insulin and/or glucose levels, EGP decreases while 1st-pass HGU increases. A positive NHGA indicates $\text{EGP} > \text{1st-pass HGU}$, i.e., there is a net addition of glucose by the liver to portally infused glucose and thus systemic $R_a > \text{PoGinf}$. A negative NHGA indicates $\text{EGP} < \text{1st-pass HGU}$, i.e., there is a net uptake of glucose by the liver from portally infused glucose and thus systemic $R_a < \text{PoGinf}$. Physiologically, the parameter NHGA ($\text{EGP} - \text{1st-pass HGU}$) represents, in postprandial situations, the net effect of the liver on R_a in the systemic circulation by changing EGP and 1st-pass HGU ($R_a = \text{PoGinf} + \text{NHGA}$). It is noteworthy that NHGA is different from net hepatic glucose balance (NHGB). The latter is calculated as the arteriovenous difference of hepatic glucose input and output; $\text{NHGB} = \text{EGP} - \text{total HGU}$ (not just 1st-pass HGU).

Statistics. All experimental data are expressed as means ± SE. Two-way ANOVA was used to compare time course data with or without exenatide. Paired Student's t test was used to compare the basal or clamp steady-state parameters between saline and exenatide treatments. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Study 1: The effect of exenatide on glucose turnover in the presence of postprandial hyperinsulinemia and hyperglycemia. We first studied the effect of exenatide on glucose turnover in the presence of postprandial insulin and glucose levels. The time courses of plasma insulin and glucose were matched between the saline and exenatide groups, with insulin raised to ~225 pmol/l and glucose raised to ~150 mg/dl at the clamp steady state (Table 1).

The total glucose turnover, as indicated by portal glucose infusion rate, was elevated with exenatide, increasing ~30% at steady state (15.9 ± 1.6 saline vs. 20.4 ± 2.1 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.001$; Fig. 2A). Increased glucose turnover was a result of both increased whole-body glucose disposal (R_d) and increased net hepatic uptake of portal exogenous glucose. R_d was increased by ~20% (15.5 ± 1.5 saline vs. 18.3 ± 2.1 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$; Fig. 2B). NHGA was quickly switched from net addition to net uptake (1st-pass), which was increased by ~80% with exenatide (-1.2 ± 0.2 saline vs. -2.2 ± 0.3 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$; Fig. 2C).

Endogenous insulin secretion was suppressed with somatostatin, as reflected by plasma C-peptide levels, which were significantly reduced at steady state in both groups. Plasma glucagon was replaced near basal levels and matched between groups. Plasma FFAs were quickly and completely suppressed in both groups (Table 1).

Study 2: The effect of exenatide on glucose turnover in the presence of hyperinsulinemia but systemic euglycemia. To dissect the role of hyperglycemia in exenatide-mediated glucose turnover, the effect of exenatide on glucose turnover was examined in the presence of hyperinsulinemia but systemic euglycemia (reduced glycemia intraportally). The time courses of plasma insulin and glucose were superimposable between the saline and exenatide groups, with insulin raised to ~245 pmol/l and glucose clamped at basal (~94 mg/dl) at the clamp steady state (Table 2). At systemic euglycemia, total glucose turnover was still increased with exenatide but to a lesser degree, increasing ~20% at steady state (13.2 ± 1.9 saline vs. 15.6 ± 2.1 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$; Fig. 3A). Accordingly, R_d was only increased by ~12%, but the

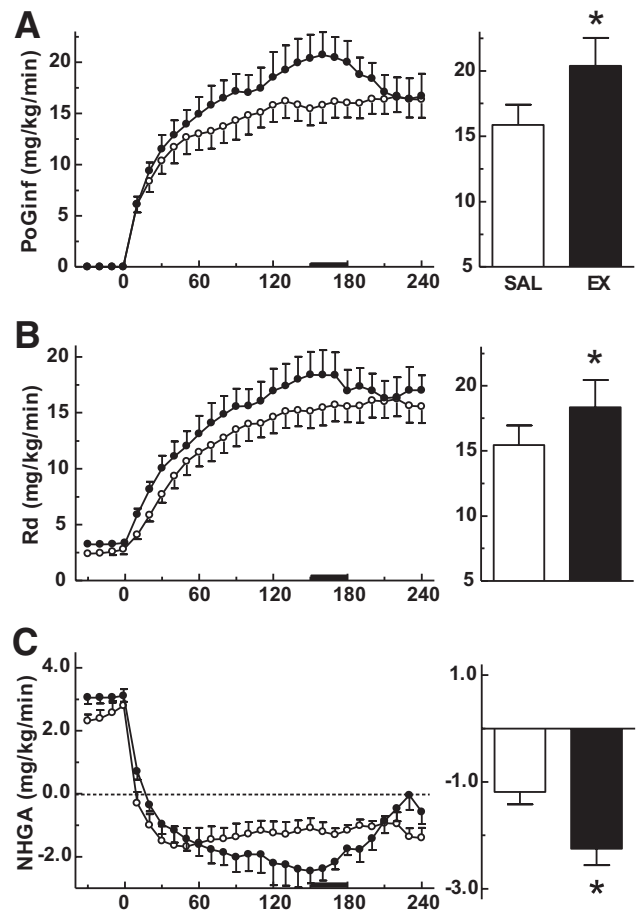


FIG. 2. Intraportal glucose infusion clamp in the presence of postprandial hyperinsulinemia and hyperglycemia. Time course (left) and clamp steady-state (right) data of intraportal glucose infusion rate (PoGinf) (A), R_d (B), and NHGA (C) in the saline (SAL, ○) and exenatide (EX, ●) groups. * $P < 0.05$, significantly different from saline.

TABLE 2

Intraportal glucose infusion clamp in the presence of hyperinsulinemia but systemic euglycemia: basal and clamp steady-state parameters in the saline- and exenatide-treated groups

	Basal		Clamp steady state	
	Saline	Exenatide	Saline	Exenatide
Glucose (mg/dl)	93.1 ± 1.9	93.0 ± 1.5	91.2 ± 2.9	96.2 ± 2.5
Insulin (pmol/l)	39.5 ± 5.5	47.7 ± 4.0	246.3 ± 11.7*	242.8 ± 10.9*
C-peptide (ng/ml)	0.21 ± 0.05	0.23 ± 0.05	0.04 ± 0.03*	0.06 ± 0.03*
Glucagon (ng/l)	47.3 ± 4.6	47.8 ± 5.8	31.1 ± 4.0*	30.1 ± 5.2*
FFAs (mmol/l)	0.64 ± 0.06	0.57 ± 0.07	0.04 ± 0.01*	0.03 ± 0.01*

Data are means ± SE. * $P < 0.05$ significantly different from basal in each group.

increment was still significant (13.0 ± 2.4 saline vs. 14.5 ± 2.4 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$; Fig. 3B). The increment in net hepatic uptake of portal exogenous glucose was 45%, which was significant over the entire time course (-1.1 ± 0.1 saline vs. -1.6 ± 0.5 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.005$ time course, whereas $P = 0.33$ steady state; Fig. 3C). Again, no difference was found in plasma C-peptide, glucagon, and FFAs between groups (Table 2).

Study 3: The effect of exenatide on glucose turnover in the presence of hyperglycemia but basal insulin levels. To investigate whether hyperinsulinemia is required for the effect of exenatide on glucose disposal, the

effect of the compound was examined in the presence of hyperglycemia but basal insulin levels. The time courses of plasma insulin and glucose were matched between the saline and exenatide groups, with insulin maintained at basal levels (~ 45 pmol/l) and systemic glucose elevated to ~ 150 mg/dl (Table 3). In sharp contrast to studies 1 and 2, in the absence of hyperinsulinemia, exenatide did not cause an increase in total glucose turnover despite elevated glycemia (2.9 ± 0.6 saline vs. 2.3 ± 0.3 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ at steady state, $P = 0.29$; Fig. 4A). Hence, without hyperinsulinemia, whole-body glucose disposal did not differ between groups; a slight increment from basal levels resulting from elevated glycemia occurred similarly in both groups (4.1 ± 0.5 saline vs. 4.0 ± 0.3 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.77$; Fig. 4B). Without hyperinsulinemia, exenatide did not induce net hepatic uptake of portally infused glucose either; net addition was simply suppressed to $\sim 50\%$ of basal levels (1.3 ± 0.1 saline vs. 1.4 ± 0.2 exenatide $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = 0.77$; Fig. 4C). Again, no difference was found in plasma C-peptide, glucagon, and FFAs profiles between groups (Table 3).

In Fig. 5, we summarized clamp steady-state values of R_d and NHGA versus their respective plasma insulin or glucose levels from the three studies. In the absence of elevated insulin levels (study 3), no difference was induced by exenatide either in R_d or in NHGA despite the presence of hyperglycemia (R_d , Fig. 5A; and NHGA, Fig. 5C). In contrast, raising insulin to postprandial levels, a significant enhancement in whole-body glucose disposal with exenatide occurred at similar systemic hyperglycemia (study 3 vs. study 1; Fig. 5A). At hyperinsulinemia, a greater net hepatic uptake of portal exogenous glucose was also induced with exenatide facing similar hepatic glucose load (estimated by plasma glucose \times hepatic flow + portal glucose infusion; study 3 vs. study 2; Fig. 5C). Hyperinsulinemia is a prerequisite for exenatide-mediated glucose disposal, pointing to an insulin-sensitizing effect of exenatide. In addition, at similar hyperinsulinemia, raising systemic glycemia from basal levels to ~ 150 mg/dl (study 2 vs. study 1), the increments in R_d (Fig. 5B) and in net hepatic uptake of exogenous glucose (Fig. 5D) were further increased. Hyperglycemia further enhanced exenatide-mediated glucose disposal only in the presence of elevated insulinemia, again indicating increased insulin sensitivity with exenatide.

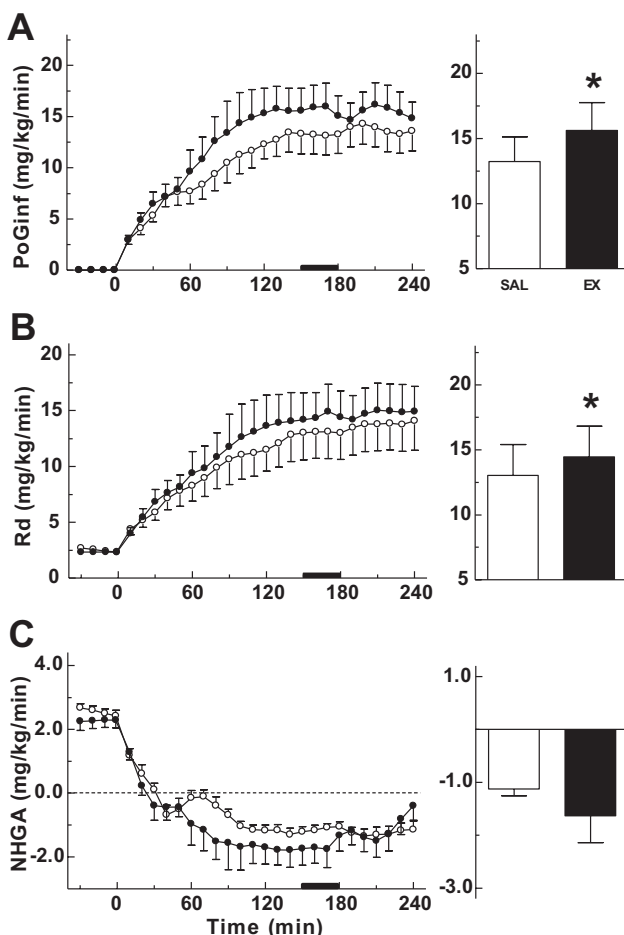


FIG. 3. Intraportal glucose infusion clamp in the presence of hyperinsulinemia but systemic euglycemia. Time course (left) and clamp steady-state (right) data of intraportal glucose infusion rate (PoGinf) (A), R_d (B), and NHGA (C) in the saline (SAL, \circ) and exenatide (EX, \bullet) groups. * $P < 0.05$, significantly different from saline.

DISCUSSION

It has been shown that exenatide improves glycemic control primarily by reducing postprandial hyperglycemia (27). Multiple mechanisms have been implicated in this

TABLE 3

Intraportal glucose infusion clamp in the presence of hyperglycemia but basal insulin levels: basal and clamp steady-state parameters in the saline- and exenatide-treated groups

	Basal		Clamp steady state	
	Saline	Exenatide	Saline	Exenatide
Glucose (mg/dl)	89.5 ± 0.8	90.0 ± 1.7	151.0 ± 2.8*	153.4 ± 4.3*
Insulin (pmol/l)	41.1 ± 4.5	54.3 ± 3.3†	44.4 ± 1.8	42.2 ± 2.7*
C-peptide (ng/ml)	0.20 ± 0.03	0.19 ± 0.05	0.01 ± 0.01*	0.00 ± 0.00*
Glucagon (ng/l)	45.3 ± 7.3	50.8 ± 8.9	38.1 ± 6.3*	39.2 ± 7.2*
FFAs (mmol/l)	0.44 ± 0.05	0.43 ± 0.07	0.23 ± 0.04*	0.22 ± 0.03*

Data are means ± SE. * $P < 0.05$ significantly different from basal in each group. † $P < 0.05$ significantly different from the saline group.

regard, including the effect of exenatides on the pancreas, the gastrointestinal tract, and the brain (3). The central finding of the present study is that exenatide also directly stimulates glucose turnover by enhancing insulin-mediated whole-body glucose disposal and increasing uptake of exogenous glucose by the liver, contributing to its overall action to lower postprandial glucose excursion.

Currently, available information regarding the effect of exenatide or GLP-1 on glucose turnover is not consistent (14,28–30). Although the discrepancies may be explained by different study designs or subjects used, one explanation could be a GLP-1/glucose sensor in the porto-hepatic region to mediate the glucose-lowering effect of exenatide

and GLP-1. The existence of such a GLP-1 sensing mechanism has been suggested by anatomical and functional studies, showing GLP-1 receptors expressed on nerve terminals in portal vein (21) and increased vagal discharge rate by intraportal GLP-1 infusion (20). Moreover, concurrent portal hyperglycemia appears to be required for the activation of GLP-1 sensor (16–19,22). We have shown that intraportal GLP-1 does not lower systemic glucose unless it is paired with intraportal (but not systemic) glucose infusion (16). Therefore, we used the intraportal glucose infusion clamp to measure exenatide-mediated glucose turnover, which mimics the postprandial nutritional entry into the systemic circulation, potentially stimulating the putative portal sensor with exenatide. Although administered subcutaneously, portal exenatide concentration should increase effectively because of its longer plasma half-life than GLP-1 (3). Exenatide was given at 20 µg, a recommended clinical daily dose. We have previously confirmed that this dose induces a significant improvement in glucose tolerance and is well tolerated in dogs (13), so we expected it to provide important information regarding the mechanism of action of exenatide. In the future, dose-response studies can be done to determine the relative importance of the different effects of this agent to enhance glucose tolerance (delayed gastric emptying, glucagon suppression, enhancement of insulin response, and now, improved insulin action).

Using such a modified glucose clamp, we first measured a significant increase in total glucose turnover with exenatide in the presence of postprandial insulin and glucose levels, as a result of increased whole-body glucose disposal and increased net hepatic uptake of portal exogenous glucose (study 1). This finding supports the concept that exenatide directly stimulates glucose turnover. This finding also raised the question of whether exenatide might increase glucose disposal via potentiating insulin action or enhancing glucose-mediated glucose disposal, or independent of elevated insulinemia and glycemia. When lowering systemic hyperglycemia to basal levels (study 2), the increased glucose disposal was maintained, although reduced, both in glucose utilization and in NHGA (net uptake) in the presence of elevated insulinemia. In sharp contrast, reducing insulinemia from postprandial to basal levels (study 3), the increased glucose disposal was completely abolished both for glucose utilization and NHGA despite elevated glycemia. Therefore, it appears that hyperinsulinemia is a prerequisite for exenatide-mediated glucose disposal, suggesting an insulin-sensitizing effect of exenatide. Hyperglycemia further enhances exenatide-mediated glucose disposal only in the presence of elevated insulinemia, again supporting increased insulin action

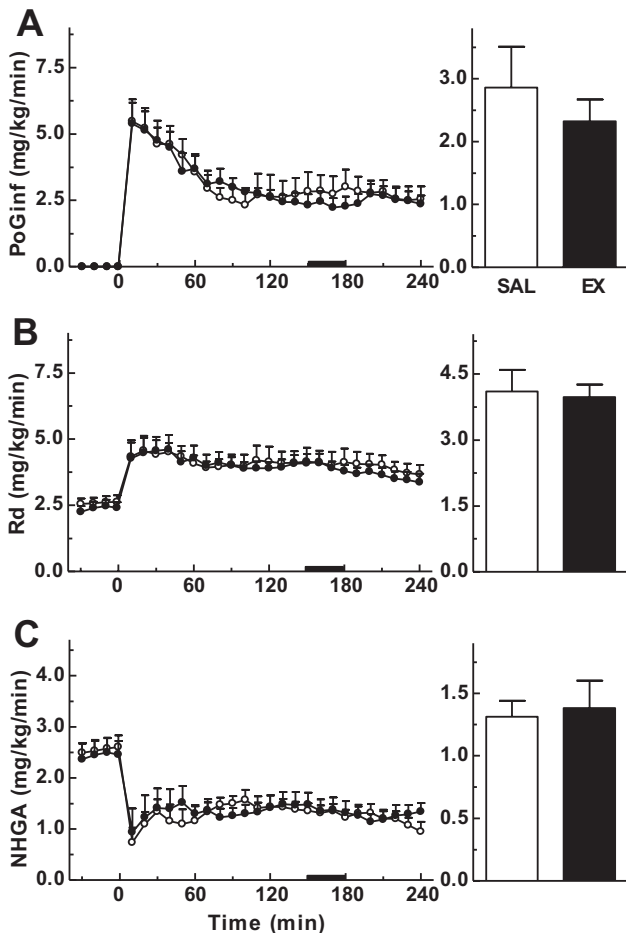


FIG. 4. Intraportal glucose infusion clamp in the presence of hyperglycemia but basal insulin levels. Time course (left) and clamp steady-state (right) data of intraportal glucose infusion rate (PoGinf) (A), R_d (B), and NHGA (C) in the saline (SAL, ○) and exenatide (EX, ●) groups.

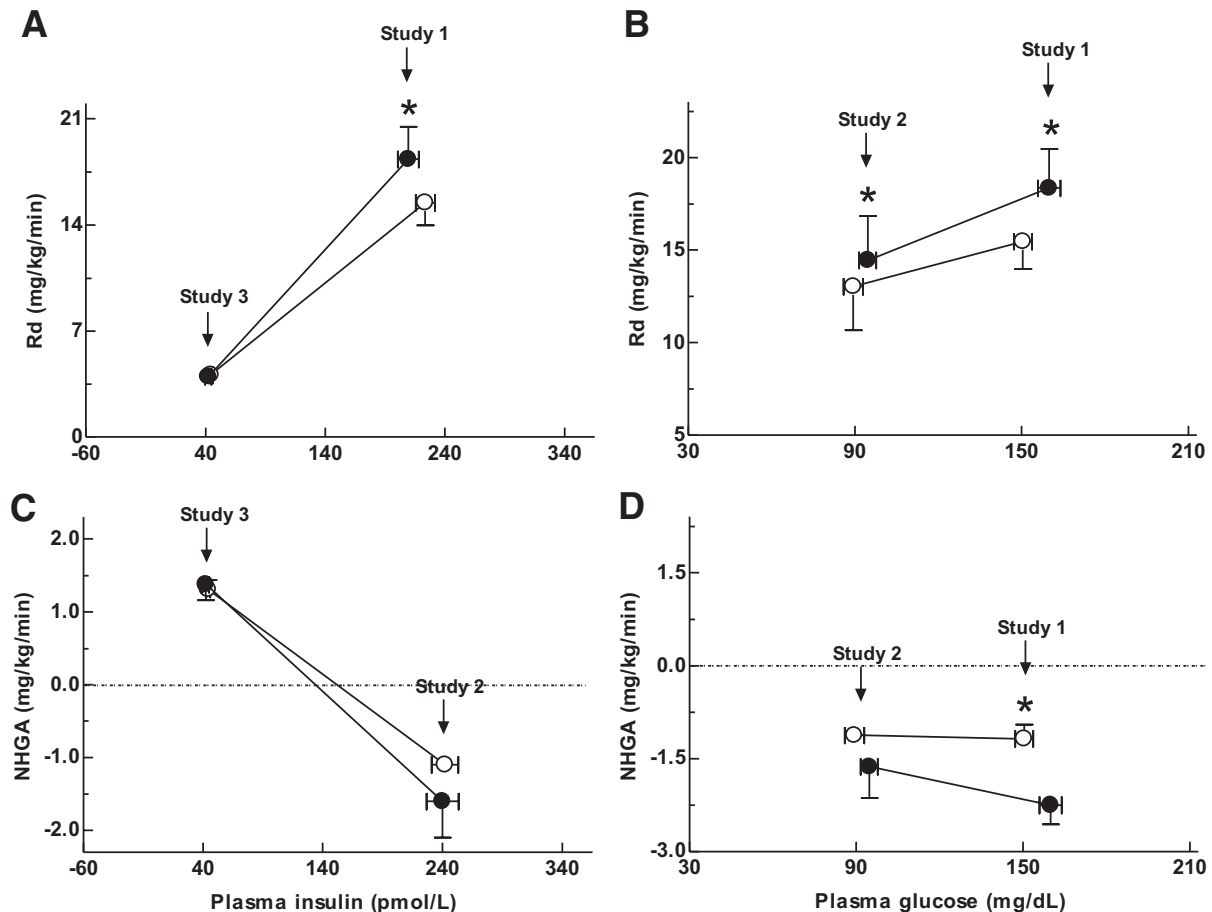


FIG. 5. The clamp steady-state data of R_d and NHGA expressed against their respective plasma insulin (A and C) or glucose (B and D) levels. \circ , saline; \bullet , exenatide. Study 1, postprandial hyperinsulinemia plus hyperglycemia; study 2, hyperinsulinemia plus systemic euglycemia; study 3, hyperglycemia plus basal insulin levels. * $P < 0.05$, significantly different from saline.

with exenatide that has been amplified by the elevation of glycemia.

An enhancement of insulin sensitivity with exenatide using the hyperinsulinemic-euglycemic clamp has been reported in chronic studies performed in diabetic or insulin-resistant animal models (12,28,31). But, concurrent changes in food intake, body weight, and metabolic parameters can affect insulin sensitivity and make it difficult to differentiate the direct versus indirect effects of exenatide on insulin action. In one study, food intake, body weight, plasma glucose, insulin, and lipid levels were matched in pair-fed animals (12). A $\sim 50\%$ increase in insulin sensitivity with exenatide was maintained, supporting the direct role of the peptide in insulin action. Enhancing insulin sensitivity by exenatide has also been suggested by other studies in which the effect of exenatide on postprandial glycemia was investigated (5,7,27). When exenatide is administered with a meal, significantly lower plasma glucose occurs without a corresponding increase in insulin secretion. Decreased gastric emptying and lower plasma glucagon level partially explain the lowered glucose excursion, although enhanced insulin sensitivity cannot be ruled out as a contributing factor. By simulating portal R_a in portal vein after meal with or without exenatide (thus bypassing gastrointestinal tract), we have shown that exenatide lowers systemic glucose levels in the absence of corresponding changes in plasma insulin and glucagon (13). Similar results have been found in studies using GLP-1, which leads to lowered postprandial plasma

glucose accompanied by lowered insulin levels (32,33). In one study, erythromycin was used to antagonize the effect of GLP-1 on gastric emptying (32). With matching gastric emptying between the GLP-1 plus erythromycin and the control groups, plasma insulin rose similarly in both groups after a meal. Yet, plasma glucose was still partially lower than the control. These results suggest a possible role of exenatide to enhance postprandial insulin sensitivity.

In contrast to positive results, some studies failed to reveal an acute effect of exenatide on insulin sensitivity using the hyperinsulinemic-euglycemic clamp (14,15). Several factors might explain the lack of an insulin-sensitizing effect in these negative studies. First, in these latter studies, intraportal glucose was not raised significantly, and thus, the putative portal GLP-1/glucose sensor may have been left inactive. As we mentioned, intraportal GLP-1 lowers systemic glucose only when paired with intraportal but not systemic glucose infusion (16,19). In addition, exendin 9–39 (GLP-1 receptor antagonist) infused portally at a low rate attenuates the glucose-lowering effect exenatide (17). It appears that the putative GLP-1/glucose sensor might at least partially mediate the glucose-lowering effect of GLP-1 and exenatide. In chronic studies, exenatide was given twice a day with meals for 6 weeks (12,28,31), continuously producing the putative portal signals. Significant changes in insulin signaling pathways and/or glucose transporting systems might occur (34), and thus, en-

hanced insulin sensitivity with exenatide could be more readily detected. Second, cortisol, known to induce insulin resistance, increased with the treatment of exendin-4 in these studies, which might offset possible exenadin-4-increased insulin action (14). Instead, in the present study, plasma cortisol levels were matched between the saline control and exenatide groups (data not shown).

An interesting aspect of the present study is the effect of exenatide on hepatic glucose turnover. In the presence of hyperinsulinemia and hyperglycemia, the liver quickly switched from net production to net uptake (1st-pass), as indicated by negative NHGA. The addition of exenatide further increased net hepatic uptake of portally infused glucose, reducing the amount of portal exogenous glucose entering the systemic circulation. A higher portal glucose infusion associated with exenatide treatment led to a slight increase in hepatic glucose load by ~12% compared with the saline control, but it cannot completely account for the 83 and 45% increase in NHGA (net uptake) in studies 1 and 2. Importantly, it is known that impaired suppression of endogenous glucose production and reduced splanchnic glucose uptake contribute to postprandial hyperglycemia in type 2 diabetic patients (35,36). The present work suggests that exenatide can increase uptake of exogenous glucose by the liver, limiting the appearance of exogenous glucose in the system similarly to its known action to reduce gastric emptying. The observation that exenatide significantly increased net hepatic glucose uptake (1st-pass) in the liver facing elevated insulin and glucose levels suggests that it might promote the conversion of glucose into glycogen at the cellular level. A few in vitro studies indicate that GLP-1 and exendin-4 exert a stimulatory effect on glycogen synthase a but an inhibitory effect on glycogen phosphorylase a in the liver and muscle (37,38). Glucokinase appears to be another good candidate because it is pivotal for hepatic glycogen synthesis and because its cytoplasmic activity has been shown to be regulated by insulin and glucose (39–42).

In summary, the present study was designed to understand the mechanisms of action of the glucose-lowering effect of exenatide in postprandial conditions. Our results reveal a novel mechanism: Exenatide potentiates insulin-mediated glucose disposal, acting as an insulin sensitizer. Specifically, exenatide enhances whole-body glucose disposal, increasing the R_d while it also increases the uptake of portally delivered exogenous glucose by the liver, decreasing the R_a . Although a direct enhancement of insulin sensitivity by exenatide is confined to healthy animals in the present study, it appears that such a mechanism might as well exist in insulin-resistant conditions. There is evidence that GLP-1 and exendin-4 enhance insulin action, acutely or chronically, in obese, insulin-resistant, or type 2 diabetic humans and animals (28,29,31,43–46). Nonetheless, whether exenatide is capable of reversing or improving insulin sensitivity in insulin-resistant conditions, e.g., a diet-induced obese dog model manifesting central obesity and deterioration of insulin action in the liver and periphery (36,47,48), should be further studied.

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