Glucose-Lowering and Insulin-Sensitizing Actions of Exendin-4

Studies in Obese Diabetic (*ob/ob*, *db/db*) Mice, Diabetic Fatty Zucker Rats, and Diabetic Rhesus Monkeys (*Macaca mulatta*)

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Exendin-4 is a 39 amino acid peptide isolated from the salivary secretions of the Gila monster (*Heloderma suspectum*). It shows 53% sequence similarity to glucagon-like peptide (GLP)-1. Unlike GLP-1, exendin-4 has a prolonged glucose-lowering action in vivo. We compared the potency and duration of glucose-lowering effects of exendin-4 and GLP-1 in hyperglycemic *db/db* and *ob/ob* mice. Whereas reductions in plasma glucose of up to 35% vanished within 1 h with most doses of GLP-1, the same doses of exendin-4 resulted in a similar glucose-lowering effect that persisted for >4 h. Exendin-4 was 5,530-fold more potent than GLP-1 in *db/db* mice (effective doses, 50% [ED_{50}s] of 0.059 µg/kg ± 0.15 log and 329 µg/kg ± 0.22 log, respectively) and was 5,480-fold more potent in *ob/ob* mice (ED_{50}s of 0.136 µg/kg ± 0.10 log and 744 µg/kg ± 0.21 log, respectively) when the percentage fall in plasma glucose at 1 h was used as the indicator response. Exendin-4 dose-dependently accelerated glucose lowering in diabetic rhesus monkeys by up to 37% with an ED_{50} of 0.25 µg/kg ± 0.09 log. In two experiments in which diabetic fatty Zucker rats were injected subcutaneously twice daily for 5–6 weeks with doses of exendin-4 up to 100 µg · rat^{-1} · day^{-1} (~250 µg/kg), HbA_{1c} was reduced relative to saline-injected control rats. Exendin-4 treatment was also associated in each of these experiments with weight loss and improved insulin sensitivity, as demonstrated by increases of up to 32 and 49%, respectively, in the glucose infusion rate (GIR) in the hyperinsulinemic euglycemic clamp. ED_{50}s for weight loss and the increase in clamp GIR were 1.0 µg/kg ± 0.15 log and 2.4 µg/kg ± 0.41 log, respectively. In conclusion, acute and chronic administration of exendin-4 has demonstrated an antidiabetic effect in several animal models of type 2 diabetes. *Diabetes* 48:1026–1034, 1999

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ANOVA, analysis of variance; CV, coefficient of variation; ED_{50}, effective dose, 50%; GIR, glucose infusion rate; GLP, glucagon-like peptide.

Exendin-4 is a 39 amino acid peptide isolated from the salivary secretions of *Heloderma suspectum* (1), a venomous lizard commonly known as the Gila monster. Exendin-4 shows 53% amino acid identity to mammalian glucagon-like peptide (GLP)-1. It is reported to show agonist activity at GLP-1 receptors in vitro (2–4), and the truncated molecule exendin(9-39) has been used as a specific antagonist of the GLP-1 receptor (3,4). Exendin-4, however, is encoded by a separate gene in the Gila monster (5), is not the lizard homolog of GLP-1 (for which separate genes exist), and has significantly different characteristics that make it attractive as a potential antidiabetic agent.

Because of a favorable spectrum of antidiabetic actions, including a glucose-dependent insulinotropic action (6), restoration of islet glucosensitivity (7), a glucagonostatic action (8), an effect to slow gastric emptying (9,10), and a possible role in appetite control (11), GLP-1 has been widely explored as a potential therapy in metabolic disease (12). But its short duration of action appears to preclude the maintenance of therapeutic levels by subcutaneous dosing (13), prompting the search for analogs with longer durations of action (14,15).

Exendin-4 has been shown to have a glucose-lowering action that endured for several hours in *db/db* mice (16). In the present study, we explored the potential utility of exendin-4 in type 2 diabetes by examining the dynamics and potency of glucose-lowering actions in diabetic mice and monkeys (comparing these properties to those of GLP-1 in mice), and by examining the effects of chronic administration of exendin-4 on glycemic control and insulin sensitivity in a rat model of type 2 diabetes, the diabetic fatty Zucker rat. We find that subcutaneously injected exendin-4 exhibits potent acute and chronic antidiabetic actions in these animal models of diabetes. Some of the data presented here have been previously reported in abstract form (17–20).

RESEARCH DESIGN AND METHODS

Materials. The exendin-4 used in these studies was obtained from Bachem (Torrance, CA; Cat H8730, Lot 506189), American Peptides (Sunnyvale, CA; Cat 301577, Lot K1005ITI), and from in-house solid-phase synthesis (Lot AR1374-11; peptide content 93.3%). GLP-1 was obtained from Bachem (Cat PGAS242B, Lot WM680).

Acute time-course experiments in *db/db* mice

Animals. Time-course experiments used C57BL/6J-m+/+ Lepr^{db} (*db/db*) mice, 8–12 weeks of age, mean weight 53.7 g, obtained from Jackson Laboratories (Bar Harbor, ME). Animals were housed in groups of 10 for ~3–4 weeks to habituate to vivarium conditions (22–24°C, 45–55% relative humidity, 12:12-h light:dark cycle with lights on at 6:00 A.M.)

Procedures. After 2 h of food deprivation, mice were lightly anesthetized with metaphane, 50 µl of blood was collected from the retro-orbital sinus, and plasma was analyzed for glucose (YSI 2300-Stat; YSI, Yellow Springs, OH). Mice were then injected intraperitoneally with exendin-4, saline, or GLP-1. Between 30 min and 4 h after the initial glucose sampling and drug injections, animals were again lightly anesthetized with metaphane and 50 µl retro-orbital blood was collected and assayed for glucose. In exendin-4–treated mice, blood sampled at 1 and 4 h was from the same animals (0.001 µg, n = 12; 0.01 µg, n = 16; 0.1 µg, n = 27; 1 µg, n = 13; 10 µg/100 µl, n = 13). For saline- and GLP-1–treated mice, data were pooled from animals that were sampled at all or some of the 30 min, 1 h, or 4 h time points after injection. For saline-treated mice, n = 26, 66, and 48, respectively, for these time points. For GLP-1, administered at doses of 0.1, 1, 10, 100, or 1,000 µg/100 µl per animal, n was 14–18, 12–21, and 4–8, respectively.

Acute dose-response experiments in *db/db* and *ob/ob* mice. Data obtained from 231 *db/db* mice at the 1-h time point in the experiment just described above were subjected to dose-response analysis. Mice were injected intraperitoneally with either 0.15 mol/l saline (100 µl; n = 66); exendin-4 at 0.001 (n = 12), 0.01 (n = 16), 0.1 (n = 27), 1 (n = 13), or 10 µg/100 µl (n = 13) per animal; or GLP-1 at 0.1 (n = 12), 1 (n = 17), 10 (n = 20), 100 (n = 21), or 1,000 µg/100 µl (n = 14) per animal. Blood samples from a subset of the exendin-4–treated *db/db* mice (n = 22, 8, 18, 27, 19, or 8 for saline, 0.01, 0.1, 1, 10, and 100 µg doses) were tested for insulin concentration using a rat insulin radioimmunoassay (kit RI-13K; Linco, St. Charles, MO).

In a separate set of experiments, 149 C57Bl/6J-lep^{ob} (*ob/ob*) mice, mean weight 65.6 g, obtained and housed as in the single-dose time-course experiments, were initially sampled for plasma glucose concentration, injected with saline (n = 62), exendin-4, or GLP-1 as described above, and had blood taken again 1 h later for plasma glucose concentration. Exendin-4 doses were 0.001 (n = 10), 0.01 (n = 6), 0.1 (n = 6), 1 (n = 9), or 10 µg (n = 14). GLP-1 doses were 0.1 (n = 8), 1 (n = 11), 10 (n = 11), 100 (n = 8), or 1,000 µg (n = 4).

Acute dose-response experiments in diabetic rhesus monkeys

Animals. Four rhesus monkeys (*Macaca mulatta*), three males and one female, aged 17.1–29.1 years, weighing 5.7–17.3 kg, and previously characterized as diabetic (fasting glucose >140 mg/dl; $K_{glucose}$ <1.5%/min) were used in these studies. Animals had ad libitum access to monkey food (Monkey Diet #5038; PMI Feeds, St. Louis, MO) and water. Monkeys were individually housed, and care and husbandry were provided according to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Two of the monkeys were treated with insulin (~24 U/day Humulin-R and ~33 U/day NPH Humulin, respectively).

Procedures. Animals were fasted for ~16 h before each of five experiments that were spaced ~2 weeks apart. Monkeys were anesthetized with ketamine hydrochloride (10 mg/kg body wt, supplemented as needed) before insertion of a venous catheter in an antecubital vein for blood withdrawal for measurement of glucose concentration at the intervals shown in Fig. 4*A*. At t = 0, exendin-4 dissolved in 0.15 mol/l saline was injected subcutaneously at a dose of 0 (vehicle only), 0.1, 1, 10, or 100 µg/kg, doses rising with subsequent experiments. At a given dose, the initial (predose) overnight-fasted plasma glucose concentration was highly variable between monkeys (coefficients of variation [CVs] ranged from 34 to 51% for different doses). Within each monkey, between experiments, the CV for fasted plasma glucose was 9–21%. Glucose responses were quantified for analysis as percent of predose glucose concentration.

Chronic single-dose experiments in ZDF rats

Animals. Male ZDF/Gmi-(*fa/fa*) rats (n = 12) and lean control rats (n = 12) obtained from Genetic Models (Indianapolis, IN) were housed at 22.7 ± 0.8°C in a 12:12-h light:dark cycle and were fed and watered ad libitum (diet: 19% protein, 5% fat, LM-485; Teklad, Madison, WI). Experiments began at 75–77 days of age when rats had reached body weights of 354 ± 22 and 283 ± 9 g, respectively (ZDF versus lean).

Procedures. Blood was sampled from the tip of the topically anesthetized tail (Hurricaine brand of 20% topical benzocaine solution; Beutlich, Waukegan, IL) of conscious overnight-fasted rats before treatment and at weekly intervals for 5 weeks during treatment for analysis of HbA_{1c} (DCA2000 latex immunoagglutination inhibition; Bayer Diagnostics, Tarrytown, NY). Body weight was measured daily.

Hyperinsulinemic-euglycemic clamps. After 5 weeks of treatment, ~22 h after the last exendin-4 (or saline) dose, and after an overnight fast, hyperinsulinemic-euglycemic clamps (21) were performed on halothane-anesthetized rats. Rats were thermoregulated, tracheotomized, and catheterized via the saphenous vein for infusion of 20% D-glucose and insulin and via the femoral artery for blood sampling and blood pressure monitoring (P23XL transducer; Spectramed, Oxnard, CA) (universal amplifier; Gould, Valley View, OH) (A/D conversion; DataTranslation, Wilmington, DE). Insulin (Humulin-R; Eli Lilly, Indianapolis, IN) was infused at 16.6 mU · kg^{-1} · min^{-1}, beginning at t = –30 min and continuing until t = +180 min. Glucose was infused at a variable rate to maintain euglycemia, as determined by glucose sampling and analysis at 5-min intervals (immobilized glucose oxidase method, YSI 2300-Stat Analyzer; Yellow Springs, OH). Mean plasma glucose was maintained at 105.6 mg/dl over the 60- to 180-min period (mean CV 4.6%). Glucose infusion rate (GIR) data for analysis were taken from t = 60–180 min when responses had approached a steady state.

Treatments. The 12 ZDF rats and 12 lean control rats were each divided into two groups of n = 6 for chronic treatment with either exendin-4 (100 µg/0.1 ml 0.15 mol/l saline) or saline alone. Injections were given intraperitoneally at ~8:00 A.M. and 4:00 P.M. from Monday through Friday and at ~10:00 A.M. on Saturday and Sunday.

Chronic dose-response experiments in ZDF rats

Animals. A total of 39 male ZDF/Gmi-(*fa/fa*) rats (age 116 ± 20 days; weight 441 ± 39 g) were assigned to five treatment groups: saline injections only (n = 9) or exendin-4 injections of 0.1, 1, 10, or 100 µg (n = 9, 10, 6, and 5, respectively). Of these, 35 rats were used in hyperinsulinemic-euglycemic clamp studies (n = 9, 7, 9, 5, and 5). Experimental conditions and procedures were the same as those in the single-dose ZDF study, except that *1*) rats were treated for 6 weeks instead of 5 weeks; *2*) the interval between the last exendin-4 dose and the clamp procedure was ~16 h; *3*) the insulin infusion rate in the clamp was 50 mU · kg^{-1} · min^{-1}; and *4*) plasma lactate data, obtained from an immobilized lactate oxidase sensor incorporated in the glucose analyzer, were also collected. Mean plasma glucose during clamps was 103.9 mg/dl (mean CV was 5.8%).

Numerical methods. Pairwise statistical analyses were performed using Student's t test routines (Instat v3.0; GraphPad Software, San Diego, CA) using $P < 0.05$ as the level of significance. Dose-response analyses used four-parameter logistic regression, and general effects were tested using one-way analysis of variance (ANOVA) (Prism v3.0, GraphPad Software).

RESULTS

Acute time-course experiments in *db/db* mice. Both exendin-4 and GLP-1 lowered plasma glucose concentration to below preinjection values at some dose and time (Fig. 1). The maximal fall in plasma glucose with each peptide approached 35% of initial values. Intraperitoneal GLP-1 injection resulted in a statistically significant lowering of plasma glucose 1 h after injection only with doses of 100 µg or higher, while exendin-4 injections significantly lowered plasma glucose 1 h later with doses as low as 0.01 µg. Plasma glucose was significantly lower 4 h after injection with exendin-4 at doses of 0.1 µg or higher, but not at this time with any dose of GLP-1. Doses of GLP-1 as low as 1 µg significantly lowered plasma glucose 30 min after injection (Fig. 1), indicating that the absence of effect at 1 h with doses <100 µg was attributable to waning of an early response.

The relative decline in plasma glucose after an intraperitoneal injection of 1 µg exendin-4 is plotted in Fig. 2 as a function of pretreatment plasma glucose concentration. In both animal models, the absolute fall in plasma glucose was greater in the mice that were most hyperglycemic. That is, the glucose-lowering effect of exendin-4 in *db/db* and *ob/ob* mice was glucose-dependent (r = 0.802, $P < 0.0001$; r = 0.951, $P <$ 0.0001, respectively). In *db/db* and *ob/ob* mice, the plasma glucose concentrations below which no responses to exendin-4 were predicted were close to normal plasma glucose concentrations. Because absolute pretreatment plasma glucose concentrations varied widely, but changes appeared proportionate to the pretreatment value, percent change was used as the response in dose-response analyses.

Acute dose-response experiments in *db/db* and *ob/ob* mice. Using the percent fall from preinjection glucose concentration after 1 h as the glucose-lowering response, dose responses for exendin-4 and GLP-1 in diabetic *db/db* mice were constructed from the data in Fig. 1 and are shown in Fig. 3*A*. In *db/db* mice, exendin-4 lowered plasma glucose with an ED_{50} of 0.0032 µg (0.059 µg/kg) ± 0.19 log, ~5,530 times more potent than GLP-1 (ED_{50} of 17.7 µg [329 µg/kg] ±

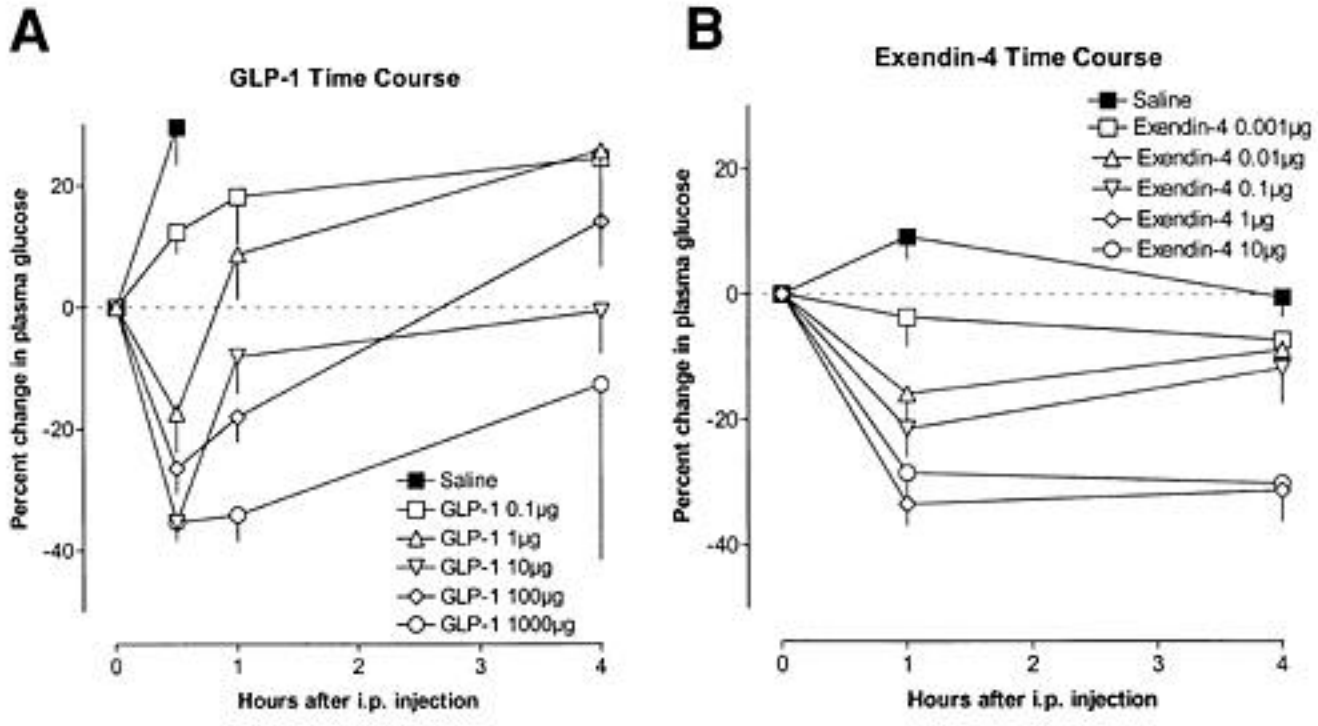

FIG. 1. Time course of glucose-lowering effects of GLP-1 (*A*) or exendin-4 (*B*) injected intraperitoneally in diabetic *db/db* mice. Initial plasma glucose concentrations were 558 ± 14 and 567 ± 18 mg/dl for mice treated with GLP-1 and exendin-4, respectively.

0.22 log; $P < 0.0002$). Dose-response data for GLP-1 30 min after injection, shown as a broken line, are left-shifted 34-fold from those for 1 h, indicating that the comparative lack of potency for GLP-1 is due to the decay of a more potent earlier response.

Dose-response data for exendin-4 and GLP-1 in *ob/ob* mice, which are also diabetic but less hyperglycemic than *db/db* mice, are shown in Fig. 3*B*. In *ob/ob* mice, exendin-4 lowered plasma glucose with an ED_{50} of 0.0089 µg (0.136 µg/kg) ± 0.10 log, ~5,480 times more

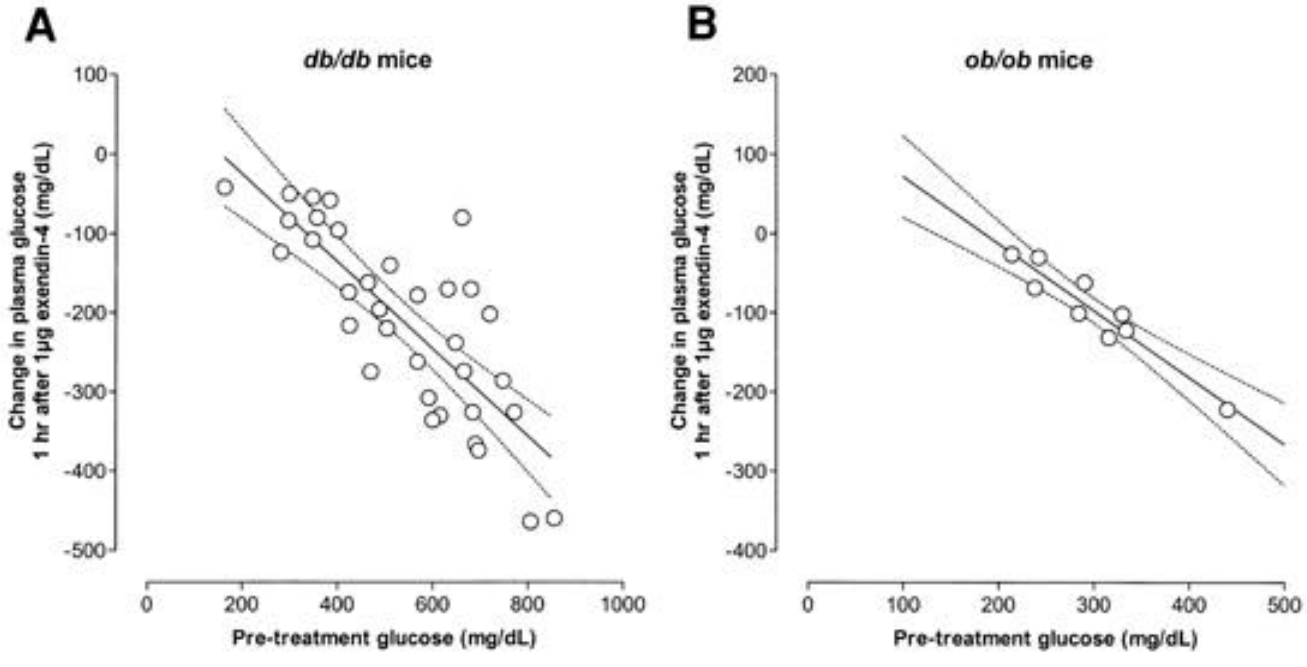

FIG. 2. Dependence of relative fall in plasma glucose after subcutaneous injection of exendin-4 upon pretreatment plasma glucose concentration in *db/db* mice (*A*) and *ob/ob* mice (*B*).

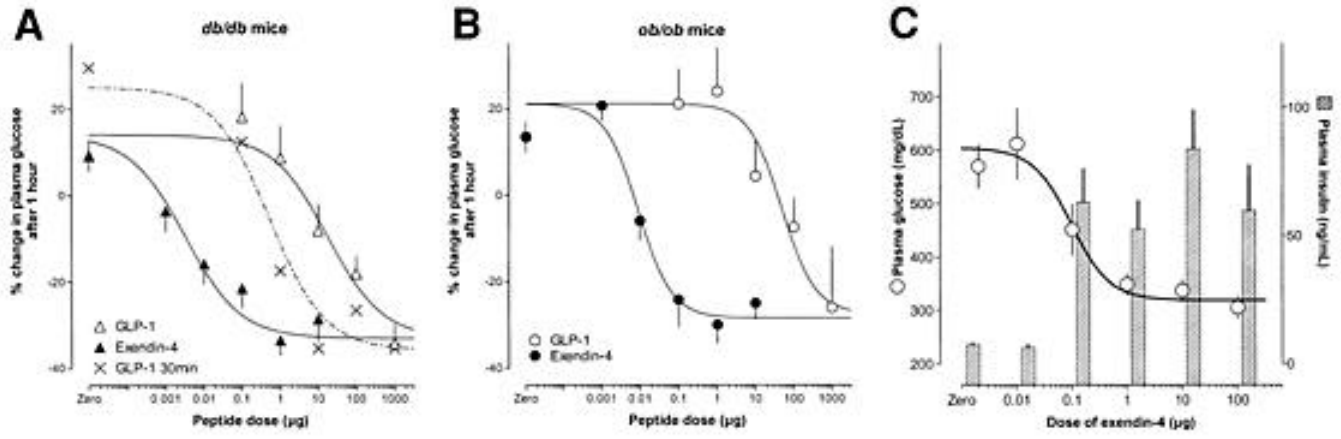

FIG. 3. Dose response for glucose-lowering effects of GLP-1 (open triangle, open circle) or exendin-4 (closed triangle, closed circle) in hyperglycemic *db/db* mice (*A*) or *ob/ob* mice (*B*). Percent lowering of plasma glucose was measured 1 h after intraperitoneal injection. In a subset of *db/db* mice, insulin was also measured after 1 h and is plotted with absolute plasma glucose (*C*).

potent than GLP-1 (ED_{50} of 48.8 µg [744 µg/kg] ± 0.21 log; $P < 0.002$).

Plasma insulin concentration 1 h after exendin-4 injection, measured in a subset of exendin-treated *db/db* mice, is plotted on the same *x*-axis as absolute plasma glucose at 1 h in Fig. 3*C*. A decrease in plasma glucose was observed at the same 0.1 µg exendin-4 dose at which an 8.5-fold increase in plasma insulin concentration was observed, the coincidence of which raises the possibility that the decrease in glucose was a consequence of stimulation of insulin secretion.

Acute dose-response experiments in diabetic rhesus monkeys. Under overnight-fasted control conditions, after saline injection in control experiments, there was a 12.6 ± 3.4% decline in plasma glucose over the 60–120 min after injection (Fig. 4*A*). After subcutaneous exendin-4 injection, the rate of fall of plasma glucose was dose-dependently accelerated beyond this, so that the 60- to 120-min mean plasma glucose concentration was lowered from preinjection values by up to 36.7 ± 3.3%. The exendin-4 dose response for fall in plasma glucose (Fig. 4*B*) indicated an ED_{50} of 0.25 µg/kg ± 0.09 log. Plasma glucose tended to rise again toward the control level ~2–3 h after injection of exendin-4.

Chronic single-dose experiments in ZDF rats. HbA_{1c} measured from weekly tail bleeds is shown in Fig. 5. Compared with saline-treated control rats, chronic injections of exendin-4 resulted in a reduction in HbA_{1c} in both lean ($P < 0.002$) and obese ($P < 0.01$) rats after 35 days of treatment. The greatest fall, constituting a 41.3% reduction in initial HbA_{1c} over 35 days, was observed in obese rats treated with exendin-4. The relative reduction in HbA_{1c} was 2.4-fold

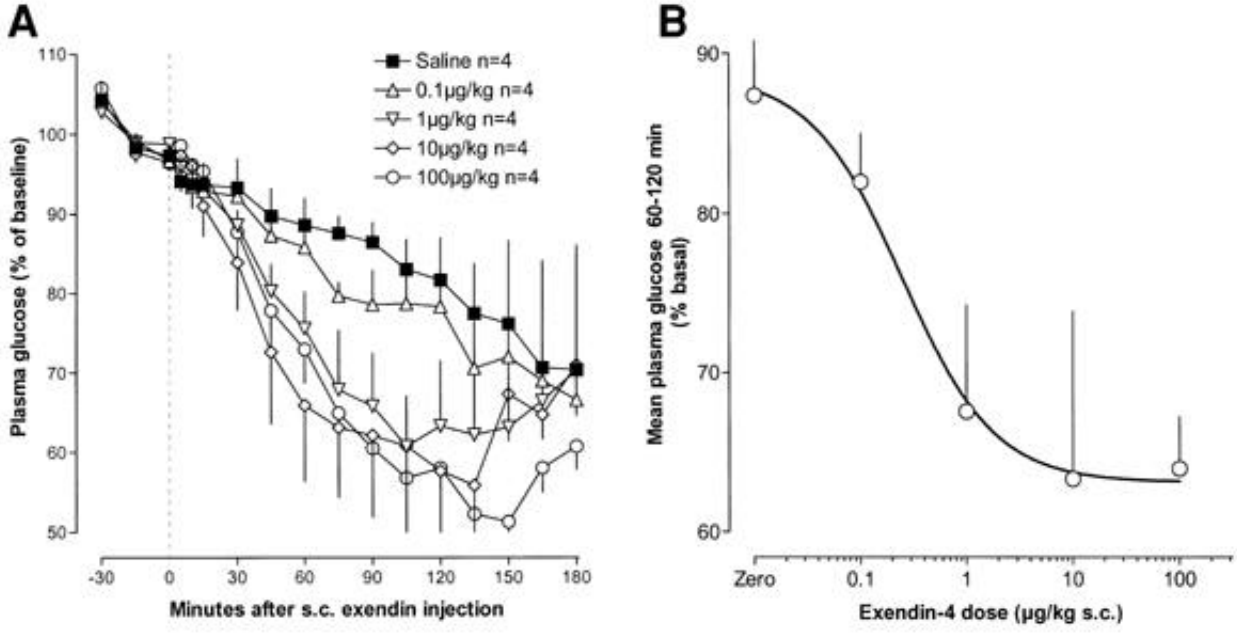

FIG. 4. Acute glucose-lowering effect of exendin-4 in rhesus monkeys with type 2 diabetes (*A*). Change in plasma glucose, averaged from 60 to 120 min after dosing, was used to construct the dose-response relationship (*B*).

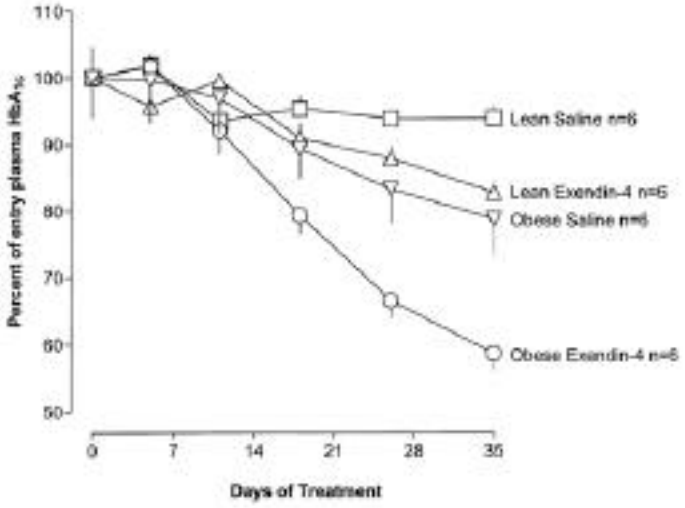

FIG. 5. Time-related change in plasma HbA_{1c} in obese ZDF rats and in lean littermate control rats administered either 100 µg i.p. exendin-4 or saline twice daily. Initial HbA_{1c} in obese ZDF rats was 6.44 ± 0.25% and in lean rats was 3.73 ± 0.37% ($P < 0.0001$).

greater in obese than in lean exendin-treated rats ($P < 0.0001$), consistent with exendin-4 having a greater effect in hyperglycemic than in normoglycemic conditions.

After 35 days of treatment, euglycemic clamps performed 22–24 h after the last exendin-4/saline injection indicated *1*) that ZDF rats were extremely insulin-resistant, in that the GIR required to maintain euglycemia in saline-treated obese rats was only 31% of that observed in saline-treated lean rats (6.37 ± 0.36 vs. 20.71 ± 0.71 mg · kg^{-1} · min^{-1}; $P < 0.0001$) and *2*) that exendin-4 administration increased GIR in the clamp by 32% in obese rats ($P < 0.02$) and by a nonsignificant 15% in lean rats ($P = 0.06$).

Chronic dose-response experiments in ZDF rats. In a separate group of ZDF rats treated with different doses of exendin-4 for 6 weeks, there was a dose-dependent reduction in food intake (ED_{50} of 0.14 µg ± 0.15 log) (Fig. 6*A*) and in body weight (ED_{50} of 0.42 µg ± 0.15 log) (Fig. 6*B*) of up to 27 ± 2 g, representing a 5.6 ± 0.5% decrease in body weight relative to saline-injected control rats.

In this group of rats, which was nearly 90 g heavier than the ZDF rats used in the 35-day single-dose study described above, the diabetic course appeared more progressive, since HbA_{1c} initially rose in all groups. Injection of exendin-4 nonetheless appeared to dose-dependently arrest and reverse the rise in HbA_{1c} (Fig. 6*C*). The exendin-4 dose response for effect on HbA_{1c} measured during the last 2 weeks of treatment was generally significant ($P = 0.05$ by ANOVA), specifically at 1 and 100 µg doses ($P < 0.005$, $P < 0.02$, respectively). A similar pattern was observed in relation to fasting plasma triglycerides in the last 2 weeks of treatment, where plasma concentrations were significantly reduced at all doses by between 51 and 65% ($P < 0.002$ by ANOVA).

A total of 35 rats progressed to a hyperinsulinemic-euglycemic clamp ~16 h after their last treatment. Initial fasting plasma glucose concentrations, higher in saline-treated (489 ± 28 mg/dl) than in exendin-treated rats, fell with insulin infusion and were subsequently clamped at similar plasma glucose concentrations (105.6 mg/dl at 60–180 min; mean CV 4.6%) (Fig. 7*A*). GIR required to maintain euglycemia was dose-dependently increased by prior treatment with exendin-4 (ED_{50} of 1.0 µg ± 0.41 log) (Fig. 7*B*). Exendin-4 treatment increased GIR by up to 48% relative to saline-treated control rats.

Plasma lactate concentration before and during the clamp procedure was dose-dependently reduced by prior treatment with exendin-4 (ED_{50} of 4 µg ± 0.25 log) (Fig. 7*C*). This effect, representing up to a 42% reduction in mean lactate between 60 and 180 min of the clamp, appeared primarily due to a reduction in preclamp (basal) lactate concentration; increments in plasma lactate during hyperinsulinemia were similar in all treatment groups. There were no treatment-related differences in mean arterial pressure measured before or during clamp procedures.

DISCUSSION

In the present study, we have shown in three animal models of type 2 diabetes (*db*/*db* mice, *ob*/*ob* mice, and rhesus monkeys) that a single dose of exendin-4, a peptide with some sequence similarity to GLP-1, can dose-dependently reduce plasma glucose concentration. Analysis of data from mice revealed that the reduction in plasma glucose concentration was greatest in animals that were initially most hyperglycemic and was least effective in animals with near-normal plasma glucose concentrations. That is, the glucose-lowering effect was glucose-dependent. The potency of the acute glucose-lowering effects of exendin-4 was comparable in *db*/*db* mice, *ob*/*ob* mice, and rhesus monkeys (0.07, 0.13, and 0.25 µg/kg, respectively). The magnitude of the fall in plasma glucose after acute exendin-4 injection was comparable in each diabetic model, approaching 35–40%.

Two separate studies (one single-dose, one multiple-dose) investigated the metabolic effects of chronic (5- to 6-week) exendin-4 administration in ZDF rats. In both studies, exendin-4 treatment was associated with a reduction in glycated hemoglobin compared with saline treatment. A comparable reduction in HbA_{1c} has recently been reported after 13 weeks of treatment with exendin-4 in diabetic *db*/*db* mice (22). In the present study, exendin-4 treatment was also associated with a decrease in fasting triglycerides, a reduction in food intake, and a reduction in body weight. In hyperinsulinemic-euglycemic clamp studies performed 16–22 h after the last injection, rats treated with exendin-4 showed an ~50% improvement in insulin sensitivity, accompanied by reductions in plasma lactate concentration. There was no dose-dependent change in endogenous glucose production (data not shown).

The mechanisms responsible for the improvement in glycemic control and insulin sensitivity after chronic exendin-4 are presently unclear. Potential mechanisms include those secondary to a reduction in food intake (17,23), a slowing of gastric emptying (24), and stimulation of insulin secretion (25). Preliminary pair-feeding studies suggest that reduced food intake contributes to, but cannot fully explain, the improvement in metabolic indices after chronic treatment with exendin-4 (S.B., B.R.G., C.J., unpublished observations).

The ~50% increase in insulin sensitivity observed after chronic administration of exendin-4 was surprising in view of observations that exendin-4 has no acute effect in insulin-sensitive tissues in vitro (i.e., no effect on basal or insulin-stimulated incorporation of radiolabeled glucose into glycogen in isolated soleus muscle or into lipid in isolated adipocytes; R. Pittner and J.-N. Ma, unpublished observations). We cannot exclude the possibility that the increase in insulin sensitivity may have been a consequence of improved glycemic control

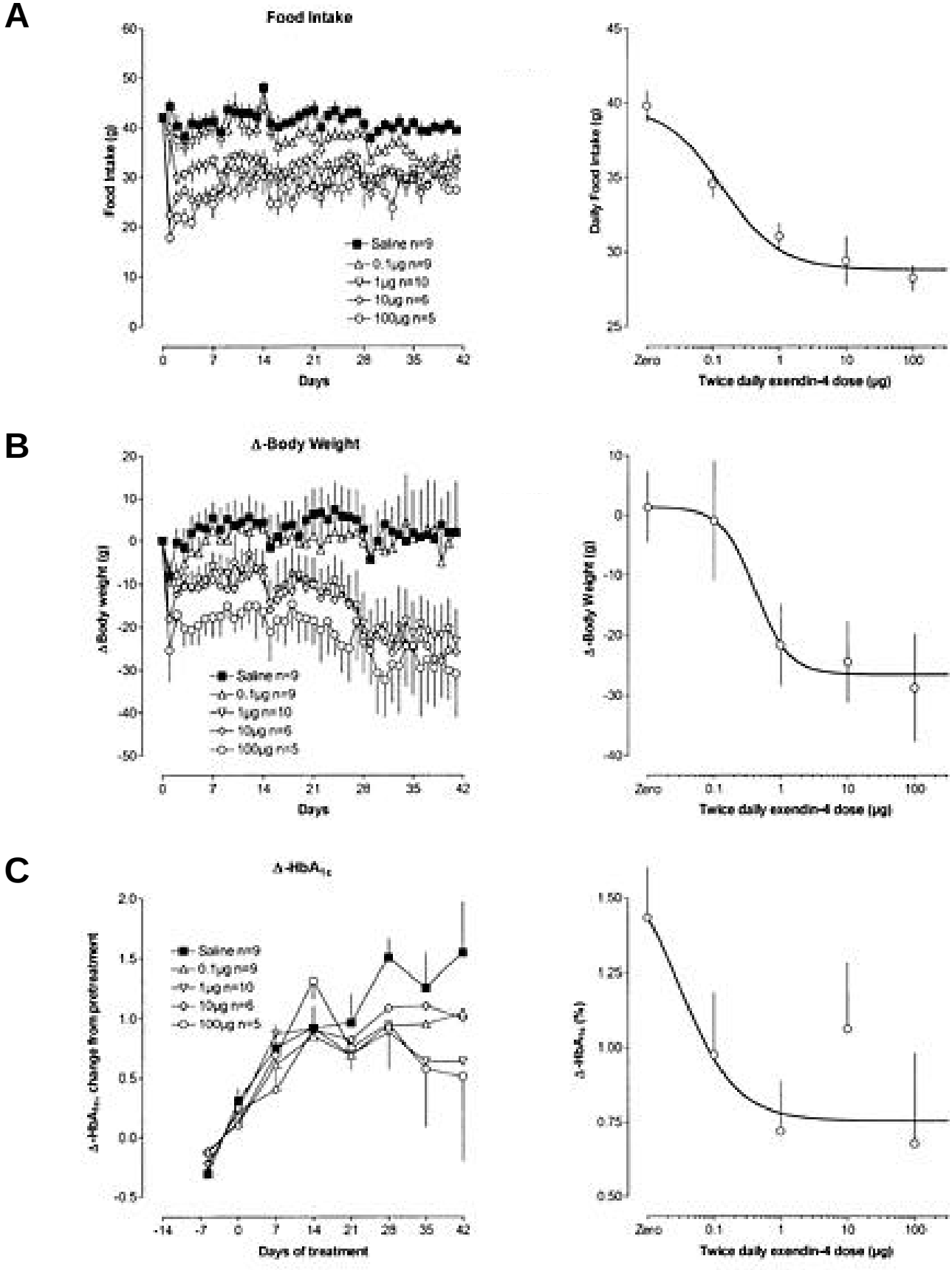

FIG. 6. Effect of exendin-4 (administered intraperitoneally twice daily) on food intake (*A*), change in body weight (*B*) (initial body weight 441 ± 39 g), or change in HbA_{1c} (*C*) (7.68 ± 0.20% at 0 weeks). Dose responses (right panels) are for the means over the last 2 of 6 weeks of treatment.

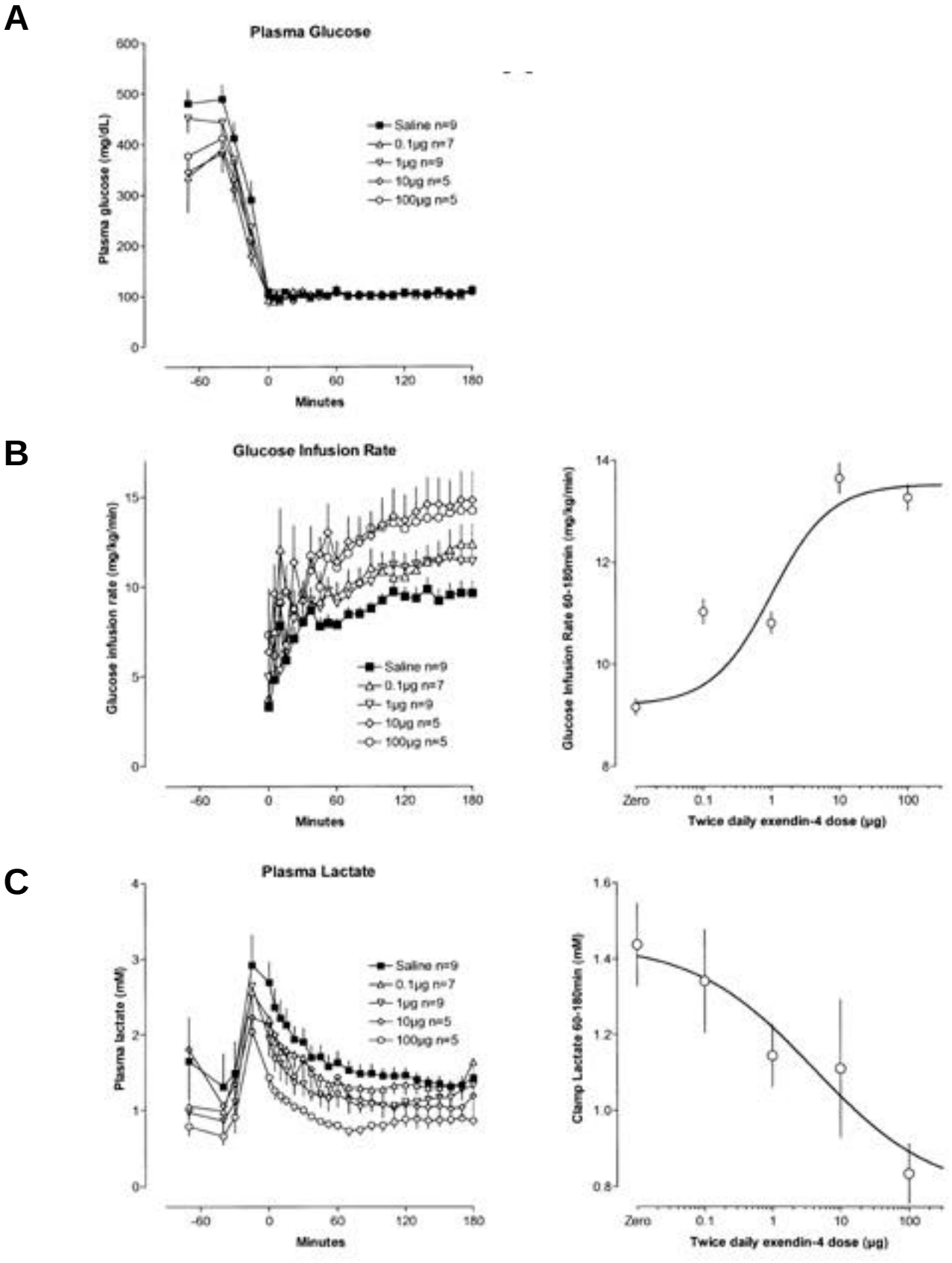

FIG. 7. Plasma glucose concentration (*A*), GIR required to maintain euglycemia (*B*), and plasma lactate concentration (*C*) in clamp procedures performed on ZDF rats previously treated for 6 weeks with the specified doses of exendin-4 (intraperitoneally twice daily). Dose responses for GIR and plasma lactate concentration are based upon mean values obtained between 60 and 180 min of the clamp procedure.

and reduced glucose toxicity. Many antidiabetic therapies, including those not classified as insulin sensitizing, have been reported to increase insulin sensitivity in diverse study designs in humans and rodents. Insulin therapy alone increased insulin sensitivity 10–39% in humans (26–28). Thiazolidinediones are reported to increase insulin sensitivity between 3 and 55% (median 32%) (29–35). Metformin is reported to increase it by 11 (27) to 32% (26), while sulfonylureas range from no effect (36,37) to a 42% increase (median 26%) (27,28,38–40). Acute treatment with GLP-1 appears not to immediately alter insulin sensitivity in humans (41–43), similar to our exendin-4 observations after acute dosing in rats (B.R.G., C.J., unpublished observations). But, consistent with the results reported with exendin-4 in the present study, chronic administration of GLP-1 in rats resulted in a large (88%) increase in insulin sensitivity (44). Thus, chronic (but not acute) administration of exendin-4 (and GLP-1) appears to be associated with increases in insulin sensitivity that are as great as, if not greater than, those observed with other therapies, including insulin-sensitizing drugs such as thiazolidinediones and metformin.

Interestingly, in the present study, plasma lactate concentrations before and during the clamp in ZDF rats were lowered toward values more typical for normal insulin-sensitive rats in a manner that was robustly associated with previous exendin-4 dose. The reason for the association between elevated basal plasma lactate concentrations and insulin resistance, apparent in humans (45) as well as rats, is unclear, but the inability of muscle to store transported glucose as glycogen, a major determinant of insulin resistance (46–48), may be contributory, since transported glucose that is not stored and passes through the glycolytic pathway emerges as lactate if it cannot be oxidized. Consistent with this idea are studies in LA/N corpulent versus lean rats using the lactate clamp (49,50) in which an approximately fourfold higher lactate release, rather than reduced lactate clearance, explained the hyperlactemia in the insulin-resistant animals. Whereas it appears to have no acute or direct effect upon muscle glycogen metabolism, the effect of chronic exendin-4 administration on carbohydrate storage via this pathway merits further study.

In summary, the present studies show an acute effect of the 39 amino acid peptide exendin-4 to acutely reduce plasma glucose in diabetic *ob/ob* mice, *db/db* mice, and diabetic rhesus monkeys. In addition, chronic administration of exendin-4 was associated with decreased food intake and body weight, decreased HbA_{1c}, decreased plasma lactate concentration, and increased insulin sensitivity in ZDF rats. We conclude that exendin-4 warrants further exploration as a potential therapy in metabolic disease.

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