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Sustained Expression of Exendin-4 Does Not Perturb Glucose Homeostasis, β-Cell Mass, or Food Intake in Metallothionein-Preproexendin Transgenic Mice*

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Activation of glucagon-like peptide (GLP)-1 receptor signaling promotes glucose lowering via multiple mechanisms, including regulation of food intake, glucose-dependent insulin secretion, and stimulation of β-cell mass. As GLP-1 exhibits a short $t_{1/2}$ *in vivo*, the biological consequences of prolonged GLP-1 receptor signaling remains unclear. To address this question, we have now generated metallothionein promoter-preproexendin (MT-Ex) transgenic mice. MT-Ex mice process preproexendin correctly, as is made evident by detection of circulating plasma exendin-4 immunoreactivity using high pressure liquid chromatography and an exendin-4-specific radioimmunoassay. Despite elevated levels of exendin-4, fasting plasma glucose and glucose clearance following oral and intraperitoneal glucose tolerance tests are normal in MT-Ex mice. Induction of transgene expression significantly reduced glycemic excursion during both oral and intraperitoneal glucose tolerance tests ($p < 0.05$) and increased levels of glucose-stimulated insulin following oral glucose administration ($p < 0.05$). Despite evidence that exendin-4 may induce β-cell proliferation, β-cell mass and islet histology were normal in MT-Ex mice. MT-Ex mice exhibited no differences in basal food intake or body weight; however, induction of exendin-4 expression was associated with reduced short term food ingestion ($p < 0.05$). In contrast, short term water intake was significantly reduced in the absence of zinc in fluid-restricted MT-Ex mice ($p < 0.05$). These findings illustrate that sustained elevation of circulating exendin-4 is not invariably associated with changes in glucose homeostasis, increased β-cell mass, or reduction in food intake in mice *in vivo*.

Glucagon-like peptide-1 (GLP-1),[1] a product of the proglucagon gene, is released from gut endocrine cells and potentiates glucose-dependent insulin secretion (1). GLP-1 also regulates gastric emptying, food intake, glucagon secretion, and islet proliferation and hence is currently under investigation as a therapeutic agent for the treatment of diabetes (1). However, a significant limitation to potential GLP-1 therapy in diabetic subjects is the short biological half-life of this peptide (2–4), limiting its ability to control blood glucose for an extended period of time. These considerations have prompted investigation of strategies designed to prolong the duration of GLP-1 action *in vivo* (5, 6).

Exendin-4, a peptide structurally related to but distinct from GLP-1 (7) was originally purified from the venom of a *Heloderma suspectum* lizard (8, 9). Subsequent characterization of exendin-4 activity demonstrated that the lizard peptide was a potent agonist for the mammalian glucagon-like peptide-1 receptor (GLP-1R) (8–11). Exendin-4 exhibits a much longer *in vivo* half-life and prolonged duration of action (11), rendering it more potent for continuous stimulation of GLP-1 receptor signaling and sustained improvement in glucose homeostasis *in vivo*. Despite the structural homology of lizard exendin-4 and mammalian GLP-1, a mammalian exendin-4 gene has not yet been identified (7, 12).

The finding that exendin-4 represents a potent GLP-1 analogue has prompted studies of exendin-4 activity in normal and diabetic rodents. Exendin-4 potentiates glucose-stimulated insulin secretion and lowers blood glucose in both rats and mice (11, 13–16). Exendin-4 also inhibits food and water intake, raising the possibility that chronic exendin-4 treatment may decrease satiety and promote weight loss *in vivo* (17, 18). Furthermore, recent studies demonstrate that exendin-4 administration leads to induction of pancreatic endocrine cell differentiation, islet proliferation, and expansion of β-cell mass (11, 13–16).

Although the biological activities of exendin-4 and GLP-1 have been examined in numerous short term studies, limited information is available regarding the physiological actions of these peptides in experimental paradigms characterized by prolonged exposure to increased levels of GLP-1R agonists. To assess the feasibility and physiological effects of chronic expression of lizard exendin-4 *in vivo*, we have generated transgenic mice in which lizard exendin-4 expression is under the control of the mouse metallothionein I promoter. We now report the characterization and metabolic consequences of sustained exendin-4 expression in mice *in vivo*.

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[1] The abbreviations used are: GLP, glucagon-like peptide; GLP-1R, GLP-1 receptor; MT-Ex, metallothionein promoter-preproexendin; Ex-4-IR, exendin-4-like immunoreactivity; HPLC, high pressure liquid chromatography.

MATERIALS AND METHODS

MT-Exendin Transgene Construction and Generation of Transgenic Mice—To generate the MT-exendin transgene, a 492-base pair cDNA encoding lizard preproexendin-4 (7) was cloned into the *Bgl*II site of the pEV142 expression vector (19), under the control of an inducible mouse metallothionein-I promoter. A 1.9-kilobase *Eco*RI fragment containing the MT-exendin transgene was electroeluted from a 1% (w/v) agarose gel and further purified on an Elutip-d column (Schleicher & Schuell). Transgenic mice were generated by Chrysalis (DNX Transgenic Sciences, Princeton, NJ) on a C57BL/6 × SJL genetic background. Two lines of MT-exendin mice were generated that exhibited comparable phenotypes. All mice used in these studies were 16–20 weeks old. Control animals were age- and sex-matched transgene-negative mice from the same litter or family. For induction of metallothionein-I promoter activity, drinking water was supplemented with 25 mM $ZnSO_4$ for a minimum of 72 h. All procedures were conducted according to protocols and guidelines approved by the Toronto Hospital Animal Care Committee.

Plasma Extraction—Blood samples were obtained by cardiac puncture and mixed with 10% (v/v) TED (500,000 IU/ml Trasylol, 1.2 mg/ml EDTA, and 0.1 mM Diprotin A). Plasma was collected by centrifugation at 4 °C and mixed with 2 volumes of 1% (v/v) trifluoroacetic acid, pH 2.5. Peptides and small proteins were adsorbed from plasma extracts by passage through a C18 silica cartridge (Waters Associates, Milford, MA). Adsorbed peptides were eluted with 4 ml of 80% (v/v) isopropanol containing 0.1% (v/v) trifluoroacetic acid.

High Pressure Liquid Chromatography (HPLC) and Radioimmunoassay—HPLC was performed on a Waters system using a C18 μBondapak column. Radioimmunoassay for exendin-4-like immunoreactivity was carried out using a rabbit anti-exendin-4 antiserum (Cocalico Biologicals Inc., Reamstown, PA), synthetic exendin-4 (California Peptide Research Inc., Napa, CA) as standard, and ^{125}I-exendin-4, prepared by the chloramine T method (20, 21).

Glucose Tolerance Tests and Measurement of Plasma Insulin Levels—Oral and intraperitoneal glucose tolerance tests were carried out following an overnight fast (16–18 h). Glucose (1.5 mg/g of body weight) was administered orally through a gavage tube or via injection into the peritoneal cavity. Blood was drawn from a tail vein at 0, 10, 20, 30, 60, 90, and 120 min after glucose administration, and blood glucose levels were measured by the glucose oxidase method using a One Touch Basic Glucometer (Lifescan Ltd., Burnaby, British Columbia, Canada). To measure plasma insulin, a blood sample was removed from the tail vein during the 10–20 min time period following oral or intraperitoneal glucose administration. Plasma was assayed for insulin content using a rat insulin enzyme-linked immunosorbent assay kit (Crystal Chem Inc., Chicago, IL) with mouse insulin as a standard.

Measurement of Food and Water Intake—For feeding studies, mice were fasted for 18 h and then placed into individual cages containing preweighed rodent chow, with free access to water. At the indicated time points, the chow was reweighed, and total food intake (g/g of body weight) was calculated. Food intake was monitored for a total of 24 h. For drinking studies, mice were water deprived for 13 h and then placed into individual cages containing preweighed water bottles, with free access to food. At 0.5, 1, 2, and 24 h, the water bottles were reweighed, and water intake (ml) was determined.

Histology and Immunohistochemistry—The pancreas was removed, fixed overnight in either 10% buffered formalin or 4% paraformaldehyde, and embedded in paraffin. Sections were obtained and stained with hematoxylin and eosin using standard protocols. Immunostaining for insulin and glucagon was carried out as described previously (22–24).

Estimation of β-Cell Mass—The entire pancreas was removed, weighed, fixed in acidic formalin, and paraffin-embedded. Paraffin blocks were sectioned and a set of 6–9 sections from each pancreas was sampled by systematic uniform random sampling. The sampled sections were immunostained for insulin using guinea-pig anti-insulin (Dako Diagnostics Canada, Mississauga, Ontario, Canada) as primary antibody (1:100 dilution) and rabbit anti-guinea-pig immunoglobulin (Dako Diagnostics Canada) as secondary antibody (1:50 dilution). Antibody binding was visualized by 3,3-diaminobenzidine, and sections were counterstained by Meyers hematoxylin. The volume fraction of β-cells within tissue blocks was estimated according to the principle of Delesse (25). The sections were examined using an Olympus BH-2 microscope equipped with a video camera and connected to a computer with C.A.S.T.-grid software (Olympus, Melville, NJ). Sampling within sections was also performed by systematic uniform random sampling. A coherent double-lattice grid was used for point counting. Sampling and grid density was calibrated such that approximately 100–200 points hitting β-cells and approximately the same number of points hitting pancreas were counted per pancreas (26). Estimates of β-cell mass were determined in a blinded manner.

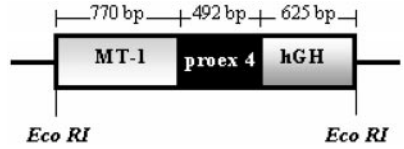

FIG. 1. **Structure of the MT-exendin transgene.** The *H. suspectum* proexendin-4 cDNA (7) was cloned into the pEV142 expression vector (58), downstream of an inducible mouse metallothionein-I promoter (*MT-1*) and upstream of 3′ flanking sequences from the human growth hormone (*hGH*) gene. The 1.9-kilobase Eco*RI* fragment containing the MT-exendin transgene was purified and used to generate transgenic mice.

Statistics—Results are expressed as mean ± S.E. Statistical significance was calculated by analysis of variance and Student's *t* test using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA). A p value <0.05 was considered to be statistically significant.

RESULTS

To study the generation of MT-exendin transgenic mice, we used a 1.9-kilobase fragment (Fig. 1) containing the following: (i) 770 base pairs of the mouse metallothionein I promoter (including 5′ flanking and exon 1 sequences) (27), (ii) the 492-base pair lizard proexendin-4 cDNA (7), and (iii) 625 base pairs of the human growth hormone gene (containing the polyadenylation signal and 3′-flanking sequences) (28). Transgenic mice were identified by Southern blot analysis (data not shown). Male and female MT-exendin transgenic mice were viable and fertile and appeared to develop normally.

Northern blot analysis detected transgene expression in several tissues, including heart, duodenum, jejunum, colon, and adipose tissue (data not shown). Tissue and plasma extracts from MT-exendin mice were analyzed by radioimmunoassay for exendin-4-like immunoreactivity (Ex-4-IR) using exendin-4 antiserum generated in our laboratory.[2] The exendin-4 antiserum used for these studies does not cross-react with glucagon, glicentin, oxyntomodulin, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, GLP-1, or GLP-2, nor does it require a free N terminus for binding.[3] In wild-type nontransgenic mice, basal levels of Ex-4-IR were less than 27 pg/ml. In contrast, basal plasma levels of Ex-4-IR were 434 ± 39 and 330 ± 84 pg/ml in male and female transgenic mice, respectively (Fig. 2*A*), and induction of transgene expression with zinc treatment resulted in an ~2.5-fold increase in the circulating levels of Ex-4-IR in both male and female mice ($p < 0.01$, Fig. 2*A*).

To determine whether preproexendin was both processed appropriately and secreted into the circulation, HPLC and radioimmunoassay analyses were used to characterize the molecular forms of circulating Ex-4-IR. The major exendin-immunoreactive peptide detected in plasma extracts from MT-exendin-4 transgenic mice eluted at the same position as synthetic exendin-4 (Fig. 2*B*). Significant amounts of exendin-4-immunoreactivity eluting in the same position as synthetic exendin-4 were also detected in several tissues.[3]

As GLP-1 receptor signaling is essential for control of blood glucose and glucose-stimulated insulin secretion (1), we examined these parameters in control and MT-exendin transgenic mice. Fasting blood glucose levels were normal in MT-exendin mice under conditions of either basal or induced transgene expression (Fig. 3). Despite clearly detectable levels of circulating exendin-4 immunoreactivity, blood glucose excursion and glucose-stimulated insulin was comparable in +/+ and MT-Ex

[2] D. J. Drucker and P. L. Brubaker, unpublished observations.
[3] P. L. Brubaker, manuscript in preparation.

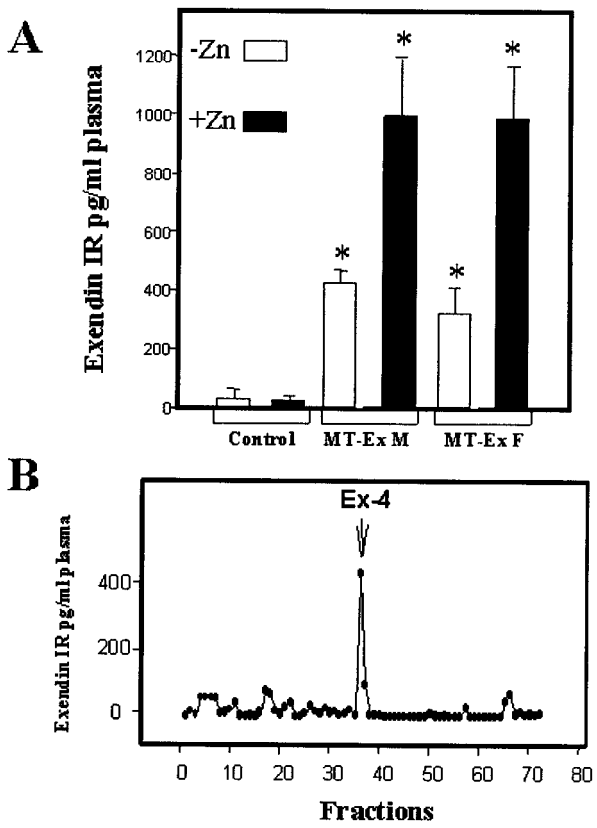

FIG. 2. **Detection of exendin-4-like immunoreactivity (*exendin IR*) in the plasma of transgenic mice.** *A,* radioimmunoassay for detection of exendin-like immunoreactivity in plasma from control littermates (nontransgenic) and transgenic male (*MT-Ex M*) and female (*MT-Ex F*) mice. Mice were given either standard drinking water (*–Zn*) or water supplemented with 25 mM $ZnSO_4$ (*+Zn*) to up-regulate transgene expression. Zinc supplementation was for a period of 72 h. Values are expressed as means ± S.E. **, $p < .01$, transgenic *versus* control (nontransgenic). *B,* HPLC elution profile of exendin-like immunoreactivity extracted from the plasma of a 4-month-old zinc-treated MT-exendin male mouse. The elution position of synthetic exendin-4 is indicated by the *arrow*.

transgenic mice following either oral (Fig. 3*A*) or intraperitoneal (Fig. 3*C*) glucose challenge. In contrast, induction of transgene expression with zinc treatment resulted in a significant reduction in glycemic excursion following oral (Fig. 3*B*) and intraperitoneal (Fig. 3*D*) glucose loading. The reduced glycemic excursion was associated with a significant increase in plasma levels of glucose-stimulated insulin after oral but not intraperitoneal glucose challenge (0.38 ± 0.04 *versus* 0.21 ± 0.02 ng/ml, for insulin in Mt-Ex *versus* control mice, respectively; Fig. 3*B*).

The physiological importance of GLP-1 receptor signaling for central nervous system control of food intake and body weight remains unclear (29). Administration of intracerebroventricular GLP-1 or exendin-4 inhibits short term feeding, whereas repeated administration of the GLP-1 receptor antagonist exendin (9–39) increases food intake and promotes weight gain in rats (30, 31). In contrast, mice with complete disruption of GLP-1R signaling do not exhibit defects in feeding control or body weight homeostasis (32, 33). Basal levels of exendin expression had no effect on short term (2 h) or long term (24 h) food intake (Fig. 4, *A* and *B*). However, up-regulation of transgene expression following zinc treatment led to a small but significant reduction in short term (2 h) food intake (0.026 ± 0.003 g/g of body weight in transgenic *versus* 0.034 ± 0.001 g/g of body weight in control mice; $p < 0.05$; Fig. 4, *C* and *D*). Basal levels of transgene expression were also associated with a significant reduction in short term (up to 2 h) water intake (Fig. 5, *A* and *B*). In contrast to recent studies demonstrating weight loss in exendin-treated rats (18), no significant differences in body weight were observed in MT-Ex transgenic mice compared with nontransgenic littermates at 4, 8, 16, or 20 weeks of age (data not shown).

Increasing evidence suggests that both GLP-1 and exendin-4 stimulate β-cell replication and neogenesis, enhance islet size, and promote differentiation of pancreatic precursor cells into islet cells (14–16, 34). To examine the effects of transgene expression on islet growth, we examined pancreata from MT-exendin transgenic mice. Islet histology and islet cell numbers appeared normal and comparable in transgenic (Fig. 6*B*) and +/+ control mice (Fig. 6*A*), with no evidence for islet neogenesis or abnormal distribution of endocrine cell types within the islets. Furthermore, quantitative analysis demonstrated no differences in β-cell mass in MT-Ex transgenic compared with +/+ control mice (Fig. 6*C*).

DISCUSSION

The observation that GLP-1 exhibits a very short plasma half-life due to its rapid degradation by dipeptidyl peptidase IV (2, 3) has prompted a search for DP IV-resistant GLP-1 analogues that exhibit longer durations of action and enhanced potency *in vivo*. Several GLP-1 analogues have now been reported that exhibit improved potency in both normal and diabetic rodents (5, 35). Furthermore, fatty acid derivatives of GLP-1 may also result in enhanced albumin binding and more prolonged bioactivity *in vivo* (36). The naturally occurring lizard exendin-4 peptide is not a substrate for DP IV and consequently exhibits a much longer half-life and greater potency *in vivo* (9, 11, 13).

GLP-1 and exendin-4 have been administered daily to humans and diabetic rodents for periods of up to several weeks (11, 13, 16, 18, 37, 38); however, the long term consequences of prolonged exendin-4 administration have not been examined. Although cell-based delivery systems for GLP-1 and exendin-4 have been proposed (39), there is little information available on the viability or efficacy of this strategy in rodents *in vivo*. The generation of mice expressing lizard preproexendin-4 provides an opportunity to assess the safety and feasibility of continuous exendin-4 delivery in mice *in vivo*. Although studies of the molecular determinants of preproexendin-4 processing have not yet been reported, the finding of detectable levels of circulating exendin-4 in MT-exendin transgenic mice is consistent with the correct processing and secretion of the lizard preproexendin precursor in murine tissues *in vivo*. Furthermore, the levels of circulating bioactive exendin-4 detected in MT-exendin-4 transgenic mice are clearly much higher than plasma levels of less potent GLP-1 (1) and are certainly within the range of or higher than the plasma levels of exendin-4 noted to decrease blood glucose in diabetic db/db mice (11, 40). Hence, the findings observed in our studies cannot simply be attributable to a failure to achieve sufficient levels of bioactive exendin-4 *in vivo*.

Exogenous GLP-1/exendin-4 treatment has been shown to reduce both fasting and postprandial blood glucose levels and enhance glucose-stimulated insulin secretion in both human and rodent studies (1, 41–46). In complementary experiments, mice with a targeted disruption of the GLP-1 receptor gene exhibit mild fasting hyperglycemia (32), and immunoneutralization or blockade of GLP-1 action increased fasting blood glucose in baboon, rodent, and human studies (47–49). These findings implicate an important role for basal GLP-1 signaling, even in the fasting state, for control of glucose homeostasis. Although basal levels of circulating exendin-4 were clearly detectable in MT-exendin mice, fasting blood glucose was nor-

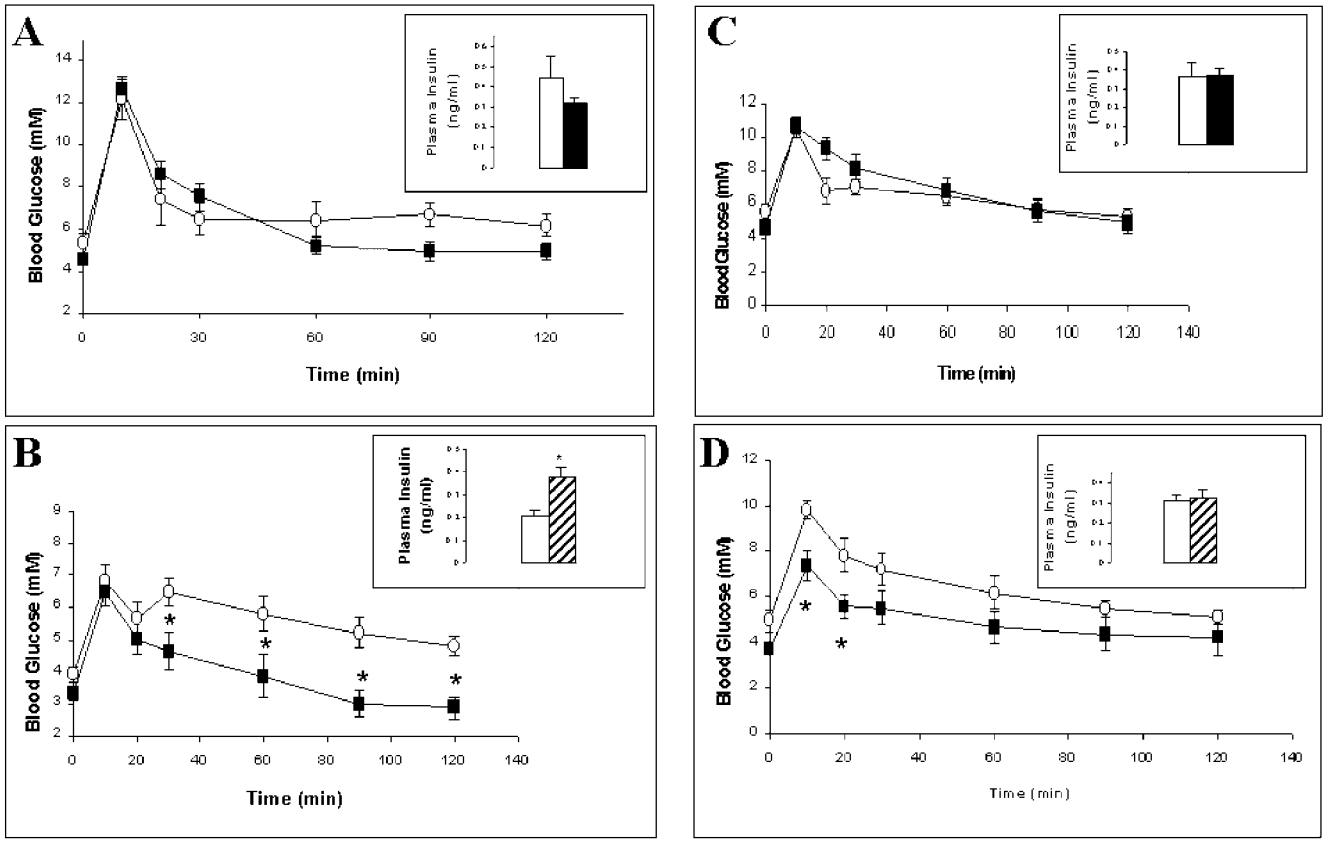

FIG. 3. **Oral and intraperitoneal glucose tolerance and levels of plasma insulin in control and MT-exendin transgenic female mice.** Values, averaged over three independent experiments, are expressed as means ± S.E.; $n = 8–12$ mice/group. *, $p < 0.05$, transgenic *versus* control mice. *A,* oral glucose tolerance in control (*open circles*) and MT-exendin (*solid squares*) mice. Plasma insulin concentrations (*inset*) following oral glucose in control (*open bar*) and MT-exendin (*solid bar*) mice were measured in plasma obtained at the 10–20 min time point. *B,* oral glucose tolerance in control (*open circles*) and MT-exendin (*solid squares*) mice following treatment with 25 mM $ZnSO_4$ to up-regulate transgene expression. Plasma insulin concentrations (*inset*) in control (*open bar*) and MT-exendin (*hatched bar*) mice were obtained at the 10–20 min time point following oral glucose. *C,* intraperitoneal glucose tolerance in control (*open circles*) and MT-exendin (*solid squares*) mice. Plasma insulin concentration (*inset*) were obtained at the 10–20 min time point following intraperitoneal glucose in control (*open bar*) and MT-exendin (*solid bar*) mice. *D,* intraperitoneal glucose tolerance in control (*open circles*) and MT-exendin (*solid squares*) mice following treatment with 25 mM $ZnSO_4$ to up-regulate transgene expression. Plasma insulin concentrations (*inset*) were measured in samples obtained at the 10–20 min time point following intraperitoneal glucose in control (*open bar*) and MT-exendin (*hatched bar*) mice.

mal. Furthermore, hypoglycemia was not observed in MT-exendin mice despite further induction of transgene expression with zinc. As exendin-4 has been estimated to be up to 5000 times more potent than GLP-1 with respect to glucose lowering *in vivo* (11), our findings of normoglycemia in MT-Ex mice further emphasize the glucose dependence of GLP-1R signaling for glucoregulation *in vivo* (1, 46).

Although incretins such as gastric inhibitory polypeptide and GLP-1 have been proposed as possible treatments for patients with diabetes, short term infusion of gastric inhibitory polypeptide has been associated with diminished effectiveness in diabetic patients (50) and desensitization of the gastric inhibitory polypeptide receptor in diabetic rats *in vivo* (51). Both homologous and heterologous desensitization of GLP-1 receptor signaling has also been observed in islet cell lines *in vitro* (52–54). However, daily administration of exendin-4 to diabetic mice for 13 weeks reduced levels of blood glucose and decreased glycosylated hemoglobin and increased plasma insulin (13), demonstrating that a single daily exendin-4 injection does not produce significant desensitization *in vivo*. The results of our studies in MT-exendin transgenic mice extend these observations by demonstrating that despite continuous exposure to transgene-derived exendin-4 for several months, acute induction of transgene expression in older mice led to reduced glycemic excursion and significantly increased levels of glucose-stimulated insulin following oral glucose challenge. These findings suggest that ongoing continuous exposure to exendin-4 in the mouse is not associated with significant impairment of GLP-1 receptor-dependent actions, such as loss of the glucose-lowering effects of exendin-4 *in vivo*. Nevertheless, whether β-cell desensitization to GLP-1 receptor agonists will prove to be an issue in long term human studies cannot be inferred from our transgenic mouse studies.

The physiological importance of GLP-1 receptor signaling for control of food and water intake remains unclear (29); however, several studies have demonstrated that exogenous administration of GLP-1 or exendin-4 clearly reduces food intake. Intracerebroventricular administration of GLP-1 reduced short but not long term food and water intake (17, 30, 55, 56), whereas peripheral GLP-1 administration inhibited water intake but had no effect on feeding in rodents (17). In both normal and type 2 diabetic humans, intravenous administration of GLP-1 was found to promote satiety and reduce energy intake (56, 57).

Although chronic intracerebroventricular administration of exendin (9–39) increased feeding and weight gain in rats (31), we found no evidence for sustained dysregulation of food intake or change in body weight in MT-exendin transgenic mice. The effects of exendin-4 on food intake may be related to the mode and timing of exendin-4 delivery and the variation in the levels of systemic exendin-4. Rats treated with a single daily dose of exendin-4 exhibited no significant changes in food intake or body weight after the first few days of exendin-4 administra-

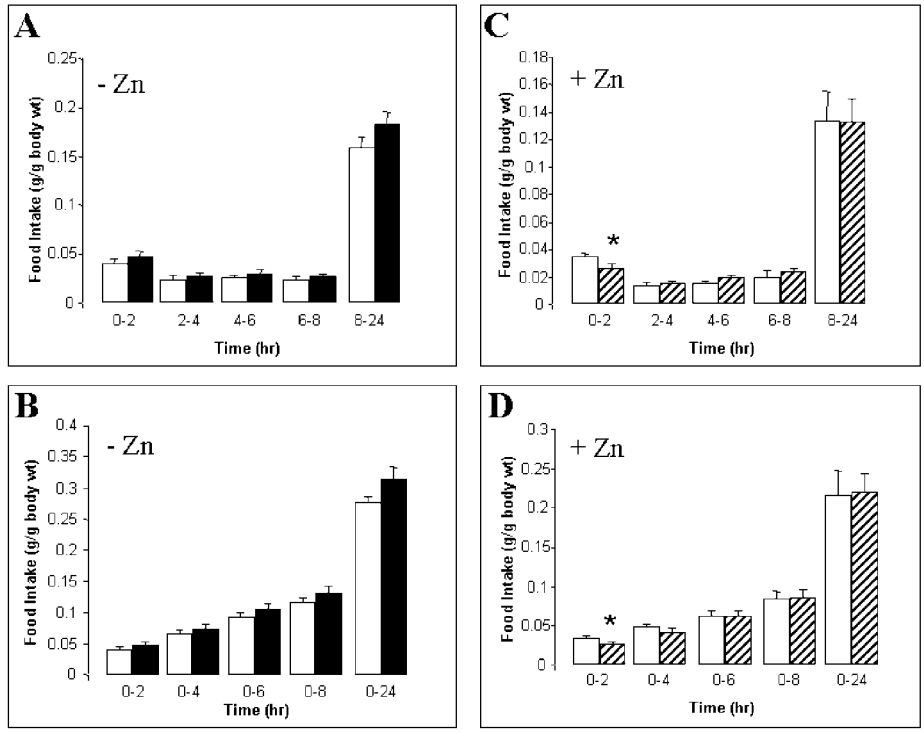

FIG. 4. **Food intake in control and MT-exendin mice.** Following an overnight fast, food intake was monitored during specific time intervals (*A* and *C*) as well as cumulatively (*B* and *D*) for a total period of 24 h in control (*open bars*) and MT-exendin transgenic (*solid* or *hatched bars*) mice. *+Zn* denotes mice treated with zinc supplementation as described under "Materials and Methods." Values are expressed as means ± S.E.; $n = 6$ mice/group. *, $p < 0.05$, transgenic *versus* control mice.

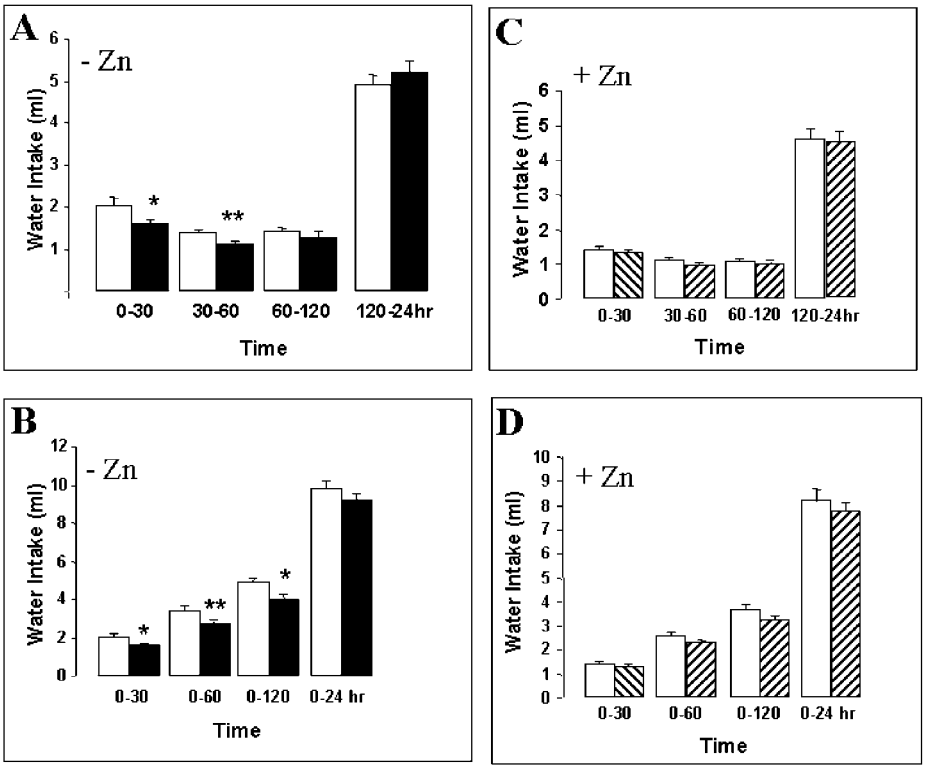

FIG. 5. **Water intake in control and MT-exendin mice.** Following a 13-h period of water deprivation, water intake was monitored during specific time intervals (*A* and *C*), as well as cumulatively (*B* and *D*) for a total period of 24 h in control (*open bars*) and MT-exendin transgenic (*solid* or *hatched bars*) mice. *+Zn* denotes mice treated with zinc supplementation as described under "Materials and Methods." Values are expressed as means ± S.E.; $n = 5$–7 mice/group. *, $p < 0.05$, transgenic *versus* control mice. Zinc supplementation alone decreased water intake in both control and transgenic mice (data not shown).

tion, whereas twice daily exendin-4 dosing led to a sustained reduction in food intake and body weight (18). In contrast, basal transgenic expression of exendin-4 in MT-Ex mice was associated with a significant reduction in short term water intake; however, only induced (but not basal) exendin-4 expression was associated with a significant reduction in short term food intake. These findings have implications for future studies designed to deliver therapeutic levels of exendin-4 that promote sustained reductions in food intake and body weight over a long term treatment period.

Several experiments implicate a role for exogenous exendin-4 in the induction of β-cell neogenesis and proliferation. Treatment of pancreatic AR42J cells with exendin-4 induced differentiation into insulin-secreting islet cells (15), and exendin-4 stimulated β-cell replication and neogenesis, enhanced ductal pdx-1 expression, and improved glucose control in rats and mice (14, 16). In contrast, we observed no differences in islet morphology or β-cell mass in normoglycemic MT-exendin-4 transgenic mice. The findings of normal islet histology in MT-exendin-4 transgenic mice may reflect the need for ad-

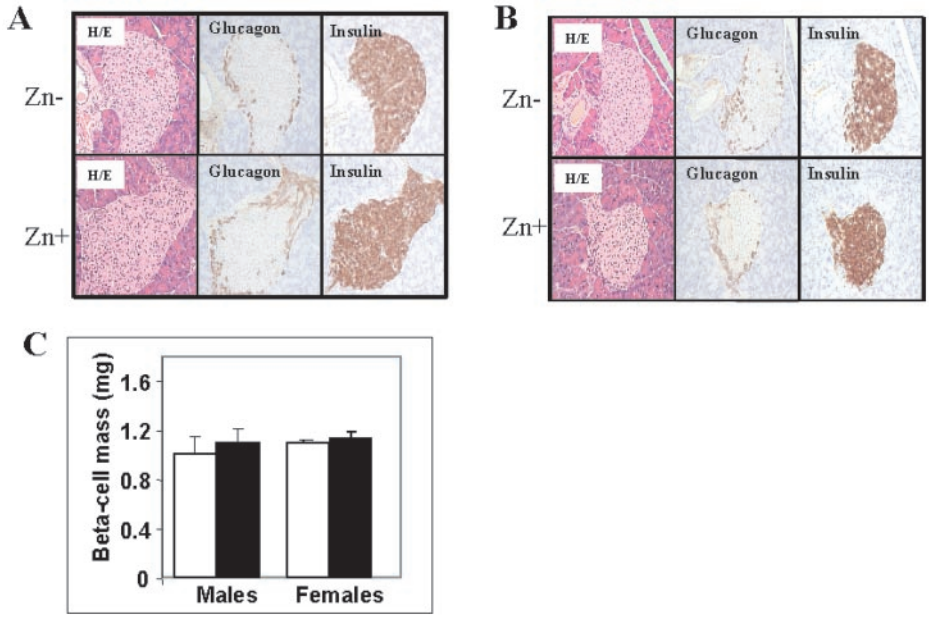

FIG. 6. **Normal islet morphology and β-cell mass in MT-exendin transgenic mice.** Hematoxylin & eosin (*H/E*) and immunohistochemical staining for glucagon and insulin in the pancreatic islets of control (*A*) and MT-exendin transgenic (*B*) mice. Pancreata were obtained from control and transgenic animals that were given either standard drinking water (*Zn–*) or water supplemented with 25 mM $ZnSO_4$ (*Zn+*) for 5–7 days to up-regulate transgene expression. *C,* β-cell mass in control (*open bars*) and MT-exendin transgenic (*solid bars*) mice. Values are expressed as means ± S.E.; $n = 3–8$ mice/group. All mice were maintained on water supplemented with 25 mM $ZnSO_4$ for 5–7 days to up-regulate transgene expression.

ditional metabolic conditions, such as hyperglycemia, to promote islet neogenesis following activation of GLP-1R signaling. Alternatively, ductal and islet cells chronically exposed to exendin-4 may compensate by down-regulating the GLP-1R-dependent signaling pathways leading to increased islet proliferation. Taken together, our data suggest that sustained exposure to circulating exendin-4 alone in normoglycemic transgenic mice is not sufficient for induction of islet proliferation or neogenesis.

As exendin-4 and long acting GLP-1 analogues have generated considerable interest as potential therapeutic agents for the treatment of diabetes, several questions about the safety and efficacy of these molecules remain unanswered. Our analyses of MT-Ex mice demonstrate that although bioactive exendin-4 is liberated into the circulation following transgene expression, sustained reductions in food intake or body weight, or induction of islet proliferation are not invariable consequences of prolonged exendin-4 expression in the mouse. Given the central importance of these biological actions for the potential treatment of diabetes, MT-exendin mice represent a useful new model for analysis of the physiological consequences of sustained activation of GLP-1 receptor signaling *in vivo*.

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