

Protein Engineering Strategies for Sustained Glucagon-Like Peptide-1 Receptor–Dependent Control of Glucose Homeostasis

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OBJECTIVE—We have developed a novel platform for display and delivery of bioactive peptides that links the biological properties of the peptide to the pharmacokinetic properties of an antibody. Peptides engineered in the MIMETIBODY platform have improved biochemical and biophysical properties that are quite distinct from those of Fc-fusion proteins. CNTO736 is a glucagon-like peptide 1 (GLP-1) receptor agonist engineered in our MIMETIBODY platform. It retains many activities of native GLP-1 yet has a significantly enhanced pharmacokinetic profile. Our goal was to develop a long-acting GLP-1 receptor agonist with sustained efficacy.

RESEARCH DESIGN AND METHODS—In vitro and in vivo activity of CNTO736 was evaluated using a variety of rodent cell lines and diabetic animal models.

RESULTS—Acute pharmacodynamic studies in diabetic rodents demonstrate that CNTO736 reduces fasting and postprandial glucose, decreases gastric emptying, and inhibits food intake in a GLP-1 receptor–specific manner. Reduction of food intake following CNTO736 dosing is coincident with detection of the molecule in the circumventricular organs of the brain and activation of *c-fos* in regions protected by the blood-brain barrier. Diabetic rodents dosed chronically with CNTO736 have lower fasting and postprandial glucose and reduced body weight.

CONCLUSIONS—Taken together, our data demonstrate that CNTO736 produces a spectrum of GLP-1 receptor–dependent actions while exhibiting significantly improved pharmacokinetics relative to the native GLP-1 peptide. *Diabetes* 57:1926–1934, 2008

Drug development strategies for therapeutic peptides continue to be challenging despite advances in technologies such as pegylation and lipidation (1–4). Although important biological processes are regulated by peptides, successful development of peptide drugs has been limited and transformation of a metabolically labile peptide into a drug remains challenging. In contrast, considerable advances have been

made in the development of antibody therapeutics (5,6). A technology that could link the activity of a target peptide with the pharmacokinetic characteristics of an antibody would be a valuable addition to tools available for drug discovery. To address this need, we developed the MIMETIBODY platform as a novel technology for the display and delivery of bioactive peptides. Using protein design tools, we linked an antibody Fc domain to a bioactive glucagon-like peptide 1 (GLP-1) analog and engineered the construct for optimal biochemical and biophysical properties.

GLP-1 is a 30–amino acid peptide secreted from L-cells of the intestine following nutrient ingestion (7–10). GLP-1 is rapidly degraded in vivo with a half-life of <2 min and cleared via the kidney (11,12). When circulating glucose concentrations are elevated, GLP-1 increases insulin and decreases glucagon secretion from the pancreas and slows gastric emptying, thereby reducing glucose appearance in the circulation and enhancing glucose clearance from the circulation (13–15). In rodent models, GLP-1 expands β -cell mass via induction of β -cell proliferation and neogenesis and reduction of β -cell apoptosis (16–20). The cytoprotective actions of GLP-1 also promote survival of human islets (21,22). Furthermore, GLP-1 reduces food intake, and therapy with GLP-1 receptor agonists has been associated with weight loss in clinical studies (23,24). Thus, GLP-1 receptor agonists are attractive therapeutic candidates for the treatment of type 2 diabetes.

CNTO736 is a GLP-1 receptor agonist engineered in our MIMETIBODY platform that incorporates a GLP-1 peptide analogue genetically fused to a domain that includes the Fc portion of an antibody (25,26). In addition to an amino acid substitution in the peptide rendering it resistant to dipeptidyl peptidase IV (27,28), the increased molecular weight and pharmacokinetic properties of an Fc were expected to enable sustained delivery of a GLP-1 receptor agonist. We demonstrate that CNTO736 dose-dependently increases cAMP and insulin secretion from islets in a glucose-dependent manner. In rodent models of type 2 diabetes, acute dosing with CNTO736 lowers fasting and postprandial blood glucose with a significantly longer duration of action than native GLP-1, and chronic dosing with CNTO736 decreases body weight. Although CNTO736 is a large molecule that is not likely to efficiently cross the blood-brain barrier, it can be detected in the circumventricular organs of the brain following peripheral dosing, and *c-fos* expression is detected in regions that are protected by the blood-brain barrier. Food intake is reduced in mice and rats following peripheral dosing with CNTO736, correlating with the appearance of the molecule

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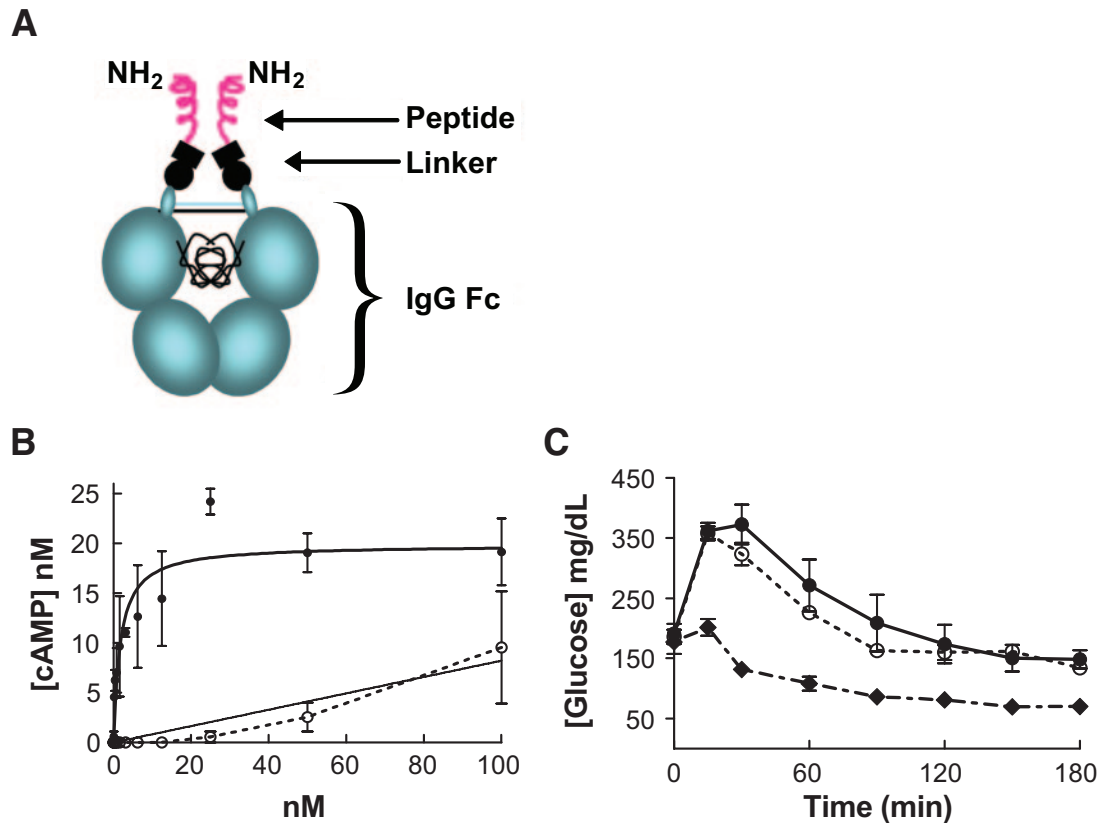


FIG. 1. CNTO736 has improved activity relative to constructs lacking a linker. **A:** The schematic outlines the structure of CNTO736 including the CH2 and CH3 domains of the Fc, the hinge including the disulfide bonds, a linker containing a partial VH region, and two GLP-1 peptides. The amino acid sequence of CNTO736 beginning at the NH₂-terminus is as follows: HSEGTFTSDVSSYLEGQAAKEFIAWLVKGRGGGSGGGSGTLVT VSSESKYGPPCPPAPEAA... Fc. **B:** The concentration of cAMP was measured in INS-1E cells after addition of increasing concentrations of CNTO736 or a molecule lacking the hinge. The fit to the CNTO736 data provided an EC₅₀ of 1.8 nmol/l. **C:** Fasted mice (DIO, *n* = 5) were dosed intravenously with vehicle (■), CNTO736 (0.5 mg/kg) (◆), or a construct lacking a hinge (○) 10 min before an ipGTT. The results are presented as the means ± SE (*n* = 5).

in the hypothalamus. Hence, the generation of stable bioactive peptide therapeutics with optimized pharmacokinetic properties may provide a new option for the treatment of metabolic disorders.

RESEARCH DESIGN AND METHODS

Animal studies. All animal studies were performed according to the National Research Council's *Guide for the Care and Use of Laboratory Animals* and were approved by the internal institutional animal care and use committee. *db/db* mice were purchased from The Jackson Laboratories. For diet-induced obesity (DIO) mice experiments, C57BL/6J mice were maintained on a diet containing 60.9% kcal from fat from 4 weeks of age, and all animals achieved 3 consecutive weeks of diabetic fasting blood glucose (FBG) values (>120 mg/dl).

Expression and purification of CNTO736. CNTO736 was constructed by fusing a GLP-1 peptide analogue to a flexible Gly/Ser linker and a fragment of a V region heavy chain (VH) domain linked directly to the CH2 and CH3 domains of an Fc. The amino acid sequence is shown in Fig. 1A. Genes encoding CNTO736 or CNTO1996, a negative control molecule that lacks a GLP-1 segment, were cloned into a vector for mammalian expression under control of the cytomegalovirus promoter. For transient expression, HEK 293E cells were expanded (Dulbecco's modified Eagle's medium [Invitrogen] + 10% fetal bovine serum) and used to seed a 10-tier cell factory (5 × 10⁷ cells in growth medium [1,200 ml]). Twenty-four hours later, the cells were transfected. The transfection mix was replaced with 293-SFMII medium (Invitrogen) with 5 mmol/l sodium butyrate (1,200 ml) after 24 h. Four days later, the conditioned medium was harvested, filtered, and stored at 4°C. CNTO736 was purified using a Protein A MabSelect column (GE Healthcare) and Immunopure Gentle Ag/Ab binding and elution buffers (Pierce). The purified product was dialyzed into 20 mmol/l Tris, pH 7.4. The final column was a Superdex 200 column (GE Healthcare) in PBS. Selected fractions were pooled and concentrated.

Cells and islets. INS-1E cells were kindly provided by C.B. Wollheim. Human and Sprague-Dawley rat islets were isolated using an enzymatic digestion and density gradient purification as described (29).

cAMP. INS-1E cells were plated in 96-well plates (1 × 10⁵ cells/well) in RPMI-1640 (Invitrogen) containing fetal bovine serum (10%), L-glutamine (1%), sodium pyruvate (1%), nonessential amino acids (1%), and β-mercaptoethanol (50 μmol/l). The cells recovered for 4 days at 37°C with 5% CO₂. Media was aspirated from the wells, and 24 μl of Alexa Fluor 647 anti-cAMP antibody (LANCER cAMP Kit; Perkin Elmer) was added followed by 24 μl of CNTO736 or a construct lacking a linker (in PBS/0.5% BSA/0.5 mmol/l isobutylmethyl-xanthine). The cells were stimulated at room temperature for 7 min and lysed per the manufacturer's protocol (Perkin Elmer). The plates were incubated at room temperature for 1 h, and the fluorescence intensity was measured at 665 nm. cAMP concentrations were determined using a standard curve.

Insulin secretion from rat or human islets. Islets were suspended at a density of 20 islets/ml in functionality/viability media (MediaTech) containing BSA (1%), L-glutamine (1%), penicillin/streptomycin (1%), and glucose (0.5 mmol/l). Approximately 20 islets were added to each well of a 24-well plate and incubated at 37°C in 5% CO₂. After 2 h, functionality/viability media containing BSA (1%), L-glutamine (1%), penicillin/streptomycin (1%), CNTO736 (50 nmol/l), and glucose (3.5 or 15 mmol/l) was added. Supernatant was collected at baseline and after 4 h and was stored at -20°C. Rat insulin was quantitated using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (Crystal Chem), and human insulin was quantitated by ELISA (Linco Research).

Pharmacokinetics of CNTO736 in mice. C57BL/6 mice were dosed intravenously with CNTO736 (1 mg/kg) or GLP-1 peptide (0.062 mg/kg; Sigma). At various times, three animals were killed and blood was collected via cardiac puncture in 3.8% sodium citrate containing protease inhibitors (Roche Complete EDTA free; Roche Applied Science). Plasma was isolated and stored at -80°C. The concentration of intact CNTO736 was measured using a modified form of a purchased ELISA designed to detect intact GLP-1 (Linco). Briefly, CNTO736 was captured as described in the protocol, but a goat anti-human H + L alk-phos conjugate (Jackson Immunoresearch) was used to detect intact

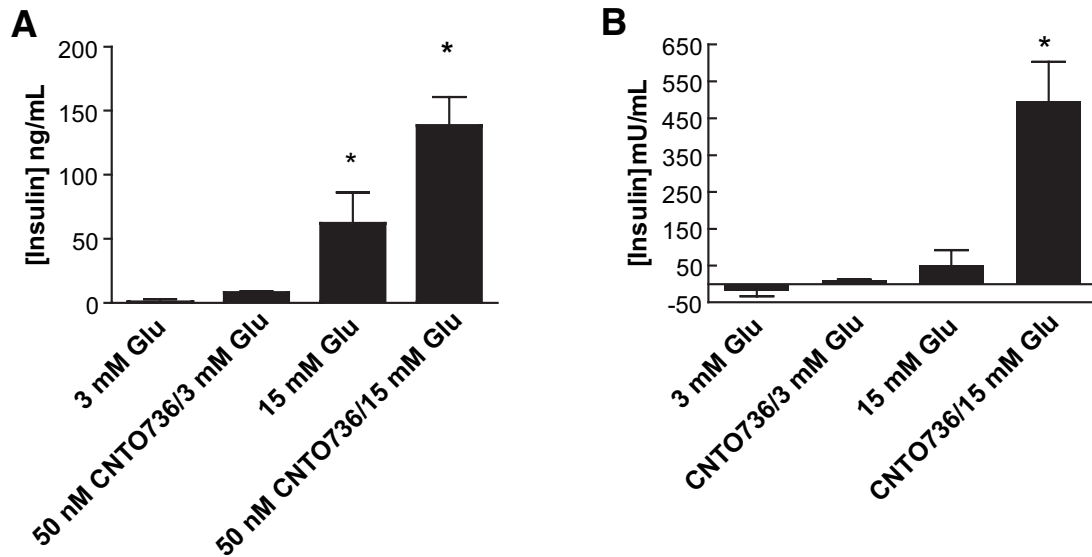


FIG. 2. CNTO736 induces glucose-dependent insulin secretion. Rat islets (A) or human islets (B) were incubated with CNTO736 (50 nmol/l) in the presence of low or high glucose, and the concentration of secreted insulin was measured after 4 h.

CNTO736. Fluorescence was read and the data analyzed with Softmax-Pro (Molecular Devices).

Intraperitoneal insulin glucose test in DIO mice. Male DIO mice were randomized ($n = 5$) based on FBG. Mice were dosed intravenously with CNTO736, the negative control lacking the GLP-1 peptide, or PBS 10 min before an intraperitoneal glucose tolerance test (ipGTT).

ipGTT and food intake in GLP-1 receptor knockout mice. Fasted male GLP-1 receptor knockout mice ($n = 5$) (30) and C57BL/6 age-matched

controls ($n = 6$) were dosed intravenously with CNTO736 (1 mg/kg) or a negative control lacking the GLP-1 peptide. An ipGTT was done 30 min after dosing. In a separate study, fasted male GLP-1 receptor knockout mice ($n = 5$) and C57BL/6 age-matched controls ($n = 6$) were dosed subcutaneously with CNTO736 (1 mg/kg) or a negative control lacking the GLP-1 peptide, and food intake was measured over the next 24 h.

Gastric emptying in wild-type mice. Mice ($n = 5$) were fasted overnight (16–18 h), and fed preweighed food in the morning. After 1 h of refeeding, the

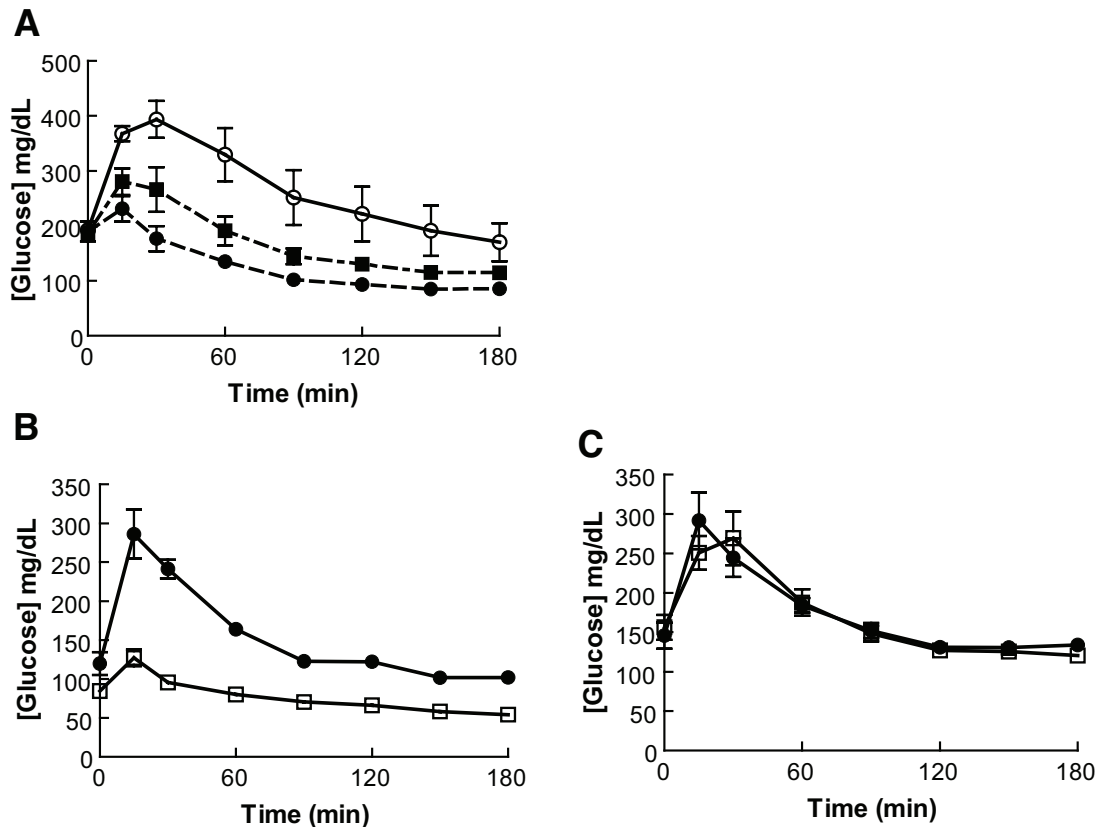


FIG. 3. CNTO736 improves glucose tolerance in normal and DIO mice but not GLP-1 receptor knockout mice. A: Fasted mice (DIO, $n = 5$) were dosed intravenously with CNTO736 [0.02 (■) and 0.1 (●) mg/kg] or the negative control lacking the GLP-1 peptide (○) 10 min before an ipGTT. B: Fasted mice (C57BL/6, $n = 6$) were dosed intravenously with CNTO736 (1 mg/kg) (□) or the negative control (1 mg/kg) (●) 30 min before an ipGTT. C: Fasted mice (GLP-1 receptor knockout, $n = 5$) were dosed intravenously with CNTO736 (1 mg/kg) (□) or control (1 mg/kg) (●) 30 min before an ipGTT. The results are presented as the means \pm SE ($n = 5$).

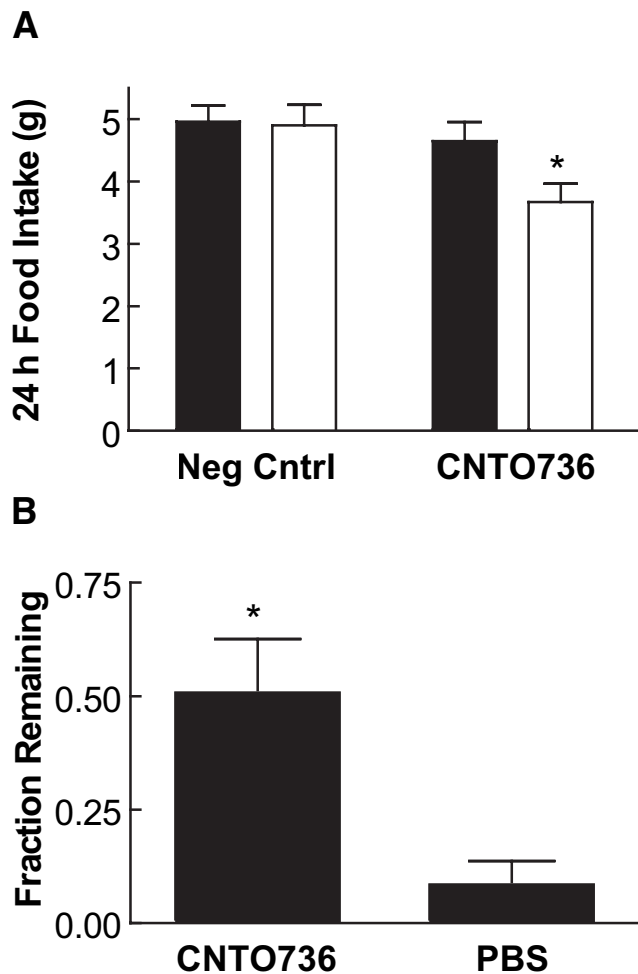


FIG. 4. CNT0736 decreases food intake and delays gastric emptying. **A:** Fasted GLP-1 receptor knockout ($n = 5$) (■) or C57BL/6 mice ($n = 6$) (□) were dosed subcutaneously with CNT0736 (1 mg/kg) or a negative control (1 mg/kg). Food intake was monitored over 24 h. The results are presented as the means \pm SE ($n = 5$). * P value < 0.05 . **B:** Fasted mice ($n = 5$) were given a preweighed meal and the amount of food consumed in 1 h was measured. The mice were dosed intravenously with CNT0736 (2.4 mg/kg) or vehicle and were killed after 4 h. The wet weight of the stomach contents was measured to determine what percent of the food remained in the stomach as an estimate of gastric emptying. The results are presented as means \pm SE ($n = 10$). * P value < 0.05 .

remaining food was weighed for measurement of food intake, and mice were dosed intravenously with CNT0736 (2.4 mg/kg) or PBS (31). The animals were deprived of food for the remainder of the study. Four hours postdosing, the stomach was exposed by laparotomy, quickly ligated at both the pylorus and cardia, and removed. The wet weight of the stomach content was determined, and the amount of food retained in the stomach was calculated.

Immunohistochemistry of anti-human IgG in brain sections of rats. Sprague-Dawley rats ($n = 3$) were fasted for 24 h and dosed intravenously with PBS or CNT0736 (1 mg/kg) just before the dark cycle. After 30 min, 2 h, and 6 h, food and water intake measurements were recorded and rats were anesthetized with pentobarbital. Transcardial perfusion included PBS followed by paraformaldehyde (PFA) (4%) with sucrose. Following decapitation, rat skulls were stored overnight in PFA (4%) at 4°C. Brains were dissected, stored in PBS, and shipped to NeuroScience Associates (Knoxville, TN) for immunohistochemistry analysis. A multibrain block was prepared using MultiBrain Technology (NeuroScience Associates), frozen by immersion in chilled isopentane, and mounted on a freezing stage of an AO 860 sliding microtome. The MultiBrain block was sectioned coronally at 35 μ m with sequential collection into a 4 \times 5 array of containers filled with antigen preserve solution (50% PBS, pH 7.0; 50% ethylene glycol, 1% polyvinyl pyrrolidone). Sections were stained with anti-human Fc (Jackson ImmunoResearch) and anti-*c-fos* (Calbiochem) reagents for CNT0736 and *c-fos*, respectively. Antibody binding was visualized using appropriate secondary

antibodies, avidin-horseradish peroxidase and diaminobenzidine tetrahydrochloride with hydrogen peroxide.

Chronic dosing in DIO mice. Male DIO mice ($n = 7$) were subcutaneously dosed daily with vehicle or CNT0736 (0.1 and 1 mg/kg) for 6 weeks. FBG was measured from tail vein blood using a hand-held glucometer (Lifescan) twice per week. Body weights were measured daily during the 6 weeks of dosing. Dual-energy X-ray absorptiometry analysis was done at the end of the 6-week study on the CNT0736 (1 mg/kg) and vehicle-treated groups.

Statistical analysis. All values are presented as the means \pm SE. Comparisons among groups were made using ANOVA followed by unpaired, nonparametric two-tailed Student's *t* test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

MIMETIBODY engineering. Various constructs were engineered to balance optimization of GLP-1 activity with maximal in vivo stability, concentrating the design on the linker because it is such a critical element. The linker must be flexible enough to enable effective engagement of the GLP-1 receptor yet stable enough to maintain in vivo attachment to the Fc portion of the molecule since it is responsible for imparting extended pharmacokinetic properties (Fig. 1A). To illustrate the importance of the linker, constructs with different linkers were evaluated. One variant in which the GLP-1 segment was linked directly to the Fc hinge had improved pharmacokinetic properties but was linked to significant decreases in receptor activity (Fig. 1B) and in vivo efficacy (Fig. 1C). The optimal variant, CNT0736, maintained the bioactivity of GLP-1, while extending the half life $\sim 1,000$ -fold compared with GLP-1 in mice (30 h vs. 1–2 min) (12).

In vitro characterization. CNT0736 increased cAMP accumulation in INS-1 cells in a dose-dependent manner with an EC_{50} of 4.9 ± 2.5 nmol/l ($n = 6$) compared with 0.2 ± 0.1 nmol/l ($n = 5$) for native GLP-1 (Fig. 1B shows one representative dataset). To directly compare the affinity of CNT0736 and GLP-1 binding to the GLP-1 receptor, competitive binding experiments using 125 I-labeled GLP-1 demonstrated that binding of CNT0736 to GLP-1 receptor on INS-1 cells was approximately eightfold weaker than native GLP-1 (data not shown). Both the cAMP and binding studies indicate that addition of the hinge and Fc onto the GLP-1 peptide results in a decreased affinity for the receptor. Nevertheless, CNT0736 stimulated insulin secretion from INS-1 cells with an EC_{50} of 0.03 nmol/l (data not shown) and enhanced insulin secretion from rat and human islets in a glucose-dependent manner (Fig. 2A and B).

ipGTT in DIO mice. The in vivo activity of CNT0736 was evaluated in an acute study to ascertain whether the molecule was able to reduce blood glucose levels. CNT0736 or a negative control lacking the GLP-1 peptide was dosed intravenously to DIO mice, and an ipGTT was performed 10 min later. CNT0736 reduced the glucose excursion in a dose-dependent manner (Fig. 3A). At 20 μ g/kg CNT0736, the glucose area under the curve was reduced $> 35\%$ relative to control animals, while the glucose area under the curve for mice treated with 100 μ g/kg was reduced by 53%.

ipGTT in wild-type and GLP-1 receptor knockout mice. To determine whether the glucoregulatory actions of CNT0736 were GLP-1 receptor dependent, we evaluated the activity of CNT0736 in GLP-1 receptor knockout (Glp1r $^{-/-}$) mice (30). As previously demonstrated in DIO mice, wild-type mice dosed with CNT0736 (1 mg/kg) had significantly reduced circulating glucose following an ipGTT (Fig. 3B), while no change in blood glucose was

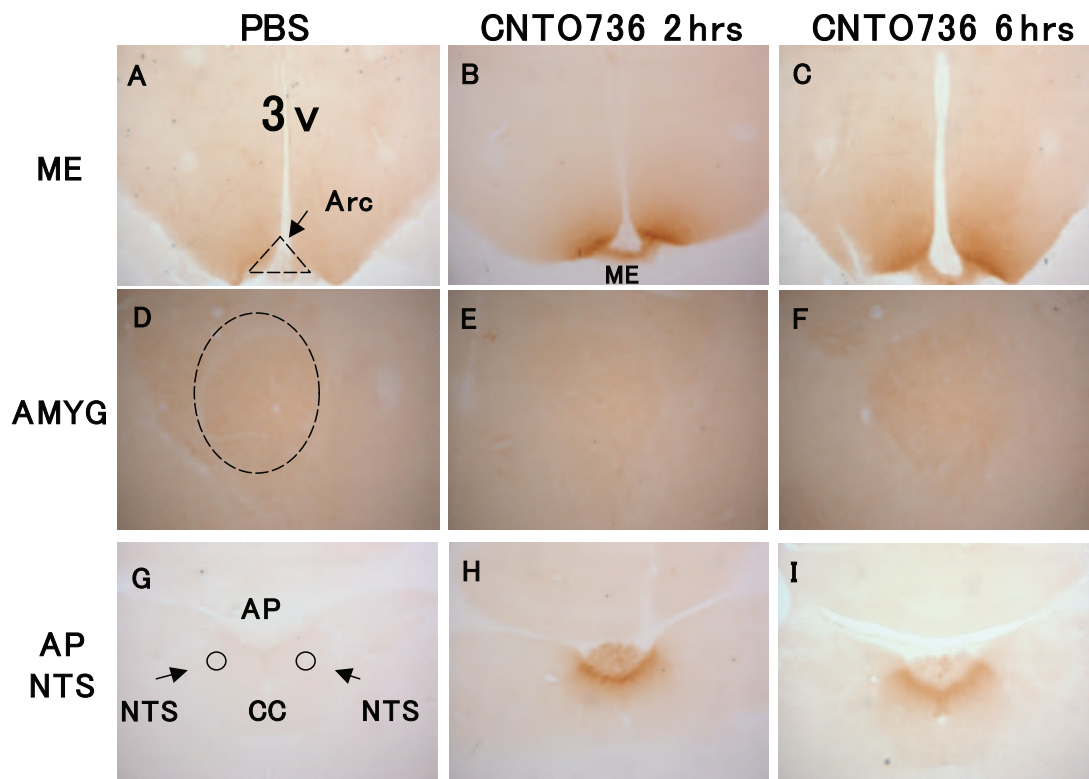


FIG. 5. CNTO736 is present in the area of the hypothalamus and circumventricular organs but not in the amygdale following peripheral dosing. Brain sections from rats ($n = 3$) dosed intravenously with PBS (A, D, and G), CNTO736 for 2 h (B, E, and H) and CNTO736 for 6 h (C, F, and I). Brain sections were taken at the level of the median eminence (ME) (A–C), central nucleus of the amygdale (AMYG) (D–F), and area postrema (AP), including nucleus of the solitary tract (NTS) (G–I). Coronal brain sections were stained with an anti-human IgG antibody to detect the presence of CNTO736. The images are representative of sections taken from three rats per group. Picture magnification was 4 \times for A–C and G–I and 10 \times for D–F. Staining in PBS treated animals at 2 and 6 h postinjection were identical. Additional abbreviations include central canal (CC), third ventricle (3v), and arcuate nucleus (Arc).

observed following CNTO736 administration in $Glp1r^{-/-}$ mice (Fig. 3C). In addition, FBG was reduced significantly 30 min after administration of CNTO736 in wild-type mice (85 ± 13 vs. 120 ± 25 mg/dl) but not in $Glp1r^{-/-}$ mice (156 ± 38 vs. 145 ± 37 mg/dl).

Food intake in wild-type and GLP-1 receptor knock-out mice and gastric emptying in wild-type mice. Treatment with CNTO736 (1 mg/kg) reduced 24-h food intake by 25% in wild-type, but not in $Glp1r^{-/-}$, mice (Fig. 4A). In a separate study, gastric emptying was estimated by weighing stomach contents after mice were given a preweighed meal. There was a fivefold increase in retained stomach contents in animals dosed with CNTO736 relative to control animals (Fig. 4B).

Fc and *c-fos* immunohistochemistry in brain sections of wild-type rats. Some actions of small peptidic GLP-1 receptor agonists have been attributed to activation of central receptors that regulate food intake and satiety. We anticipated that the molecular weight of CNTO736 would inhibit its passage across the blood-brain barrier and limit its ability to regulate food intake. To assess whether peripherally administered CNTO736 could access central GLP-1 receptors and directly activate *c-fos* expression in the brain, rats were dosed intravenously with CNTO736 and brains were isolated 2 or 6 h after dosing. Brains were sectioned and stained for the presence of human Fc. CNTO736 was detected in the median eminence and area postrema of the nucleus of the solitary tract at both the 2- and 6-h time points but not in the central nucleus of the amygdale (Fig. 5A–I). However, *c-fos* expression could be detected via immunohistochemistry in the central nucleus

of the amygdale as well as in the hypothalamus and area postrema (Fig. 6A–I). The appearance of CNTO736 in the median eminence and area postrema and *c-fos* expression in the hypothalamus, area postrema, and amygdale correlated with reductions in food and water intake (data not shown).

Chronic dosing in DIO mice. DIO mice dosed chronically with CNTO736 demonstrated a dose-dependent reduction in FBG (Fig. 7A). In addition, treatment with CNTO736 over a 6-week period reduced body weight by 7.8 or 19.8% relative to control mice after 6 weeks for the 0.1 or 1 mg/kg doses, respectively (Fig. 7B). Dual-energy X-ray absorptiometry analysis indicated that the reduction in body weight was primarily due to a loss of fat mass, while muscle mass was unchanged (Fig. 7C).

DISCUSSION

Protein therapeutics represent the fastest-growing segment in the pharmaceutical industry. The increasing success rate of antibody therapeutics has fueled an interest in expanding the utility of protein therapeutics to address other unmet medical needs (32). Peptides represent a rich source of therapeutic targets, but unmodified peptides have not traditionally made good drugs because of their high rates of metabolism and clearance. A technology that could couple the desirable pharmacological properties of antibodies with the bioactivity of small peptides would be a valuable extension of antibody technology. The MIMETIBODY platform was developed to address the gap between large protein and small peptide molecules. A variety of

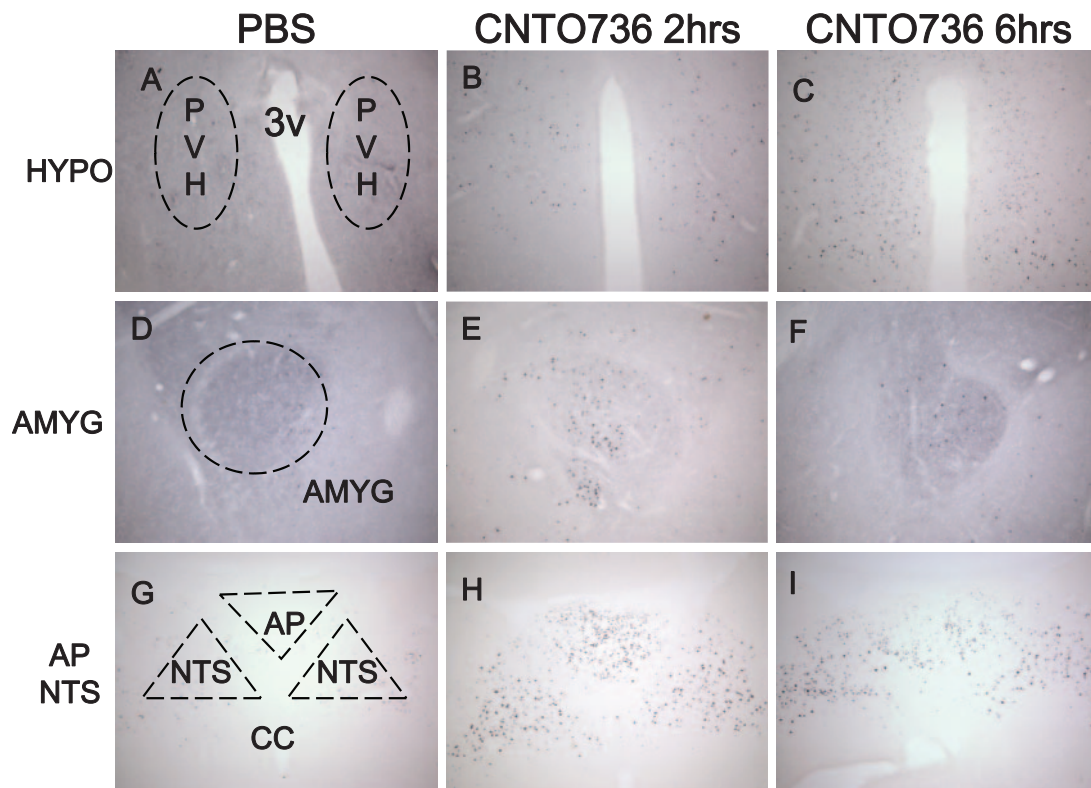


FIG. 6. Peripheral treatment of CNT0736 leads to neuronal activation of several brain areas as measured by *c-fos* immunohistochemical staining. Brain sections from rats ($n = 3$) dosed intravenously with PBS (A, D, and G), CNT0736 for 2 h (B, E, and H), and CNT0736 for 6 h (C, F, and I). Brain sections were taken at the level of the hypothalamus (HYPO) (A–C), central nucleus of the amygdala (AMYG) (D–F), area postrema (AP), and nucleus of the solitary tract (NTS) (G–I). The images are representative of sections taken from three rats per group. Coronal brain sections were stained with an anti-*c-fos* antibody. All pictures were taken at a $\times 10$ magnification. Staining of PBS-treated animals at 2 and 6 h postinjection were identical. PVH, paraventricular nucleus of the hypothalamus.

peptides were engineered into the platform to demonstrate the utility and versatility of the platform. This novel class of proteins provides a scaffold for display and delivery of bioactive peptides that is unique from other fusion protein technologies. The placement of the genetic fusion and the nature of the linker sequence are critical for maintenance of peptide activity. During engineering of CNT0736, we have tested a construct in which the GLP-1 moiety was fused directly to the hinge of the Fc; this construct exhibited very low activity (Fig. 1B). Similarly, others (31,33) have reported that fusion of a GLP-1 analogue to an IgG1 Fc or albumin results in nearly 100-fold lower activity. Thus, optimization of the linker sequence is an essential component of the MIMETIBODY technology.

We have used our novel MIMETIBODY platform to engineer a molecule with potential for application to type 2 diabetes. GLP-1 receptor agonists have gained significant attention because of several attractive properties of the peptide. GLP-1 stimulates insulin release from β -cells in a glucose-dependent manner (7,10,34), increases insulin biosynthesis in the β -cell (7), regulates gastric emptying (13–15), inhibits food intake (35–37), and increases β -cell mass in rodents (16,17,19–21). However, GLP-1 itself is not a desirable therapeutic because of its extremely short in vivo half-life ($T_{1/2} = \sim 1$ –2 min) (11,12). Our data demonstrate that CNT0736 maintains the bioactivity properties of the native hormone while significantly enhancing the half-life.

CNT0736 is a macromolecule containing two GLP-1 moieties, and our data demonstrate that its activity is similar to native GLP-1. CNT0736 binds to the GLP-1

receptor, induces cAMP production, and increases insulin secretion in a glucose-dependent manner. CNT0736 also reduces fasting and postprandial blood glucose and reduces A1C after chronic dosing.

One potential concern for any long-lived GLP-1 receptor agonist is that continual exposure to the peptide could result in receptor tachyphylaxis. However, continuous infusion of native GLP-1 in type 2 diabetic patients reduced blood glucose equally after 1 or 6 weeks of treatment, indicating that the receptor was present and accessible throughout treatment (23). A second study comparing a 16- vs. 24-h GLP-1 infusion in patients indicated that greater efficacy could be achieved with sustained 24-h coverage (38), with no evidence of tachyphylaxis or loss of activity. Finally, treatment with exendin-LAR has been reported to reduce A1C as much as 2% in the high-dose group (39). Since the half-life for exendin-LAR was reported to be 14–15 days, the data confirms that sustained exposure to a GLP-1 analogue has potential for profound efficacy. Diabetic mice dosed chronically with CNT0736 achieved a significant reduction in FBG, and an oral glucose tolerance test performed at the end of the study showed no evidence of tachyphylaxis. Consistent with a prolonged reduction in blood glucose levels, A1C was significantly lower in mice treated with CNT0736 compared with vehicle-treated *db/db* mice (7.2 ± 1.1 vs. $8.4 \pm 1.1\%$, $P < 0.05$) (data not shown).

A second issue relates to the potential for an immune response in animals treated with CNT0736 that contains a human Fc. We anticipated that an immune response could attenuate the activity of CNT0736 in mouse models. Thus,

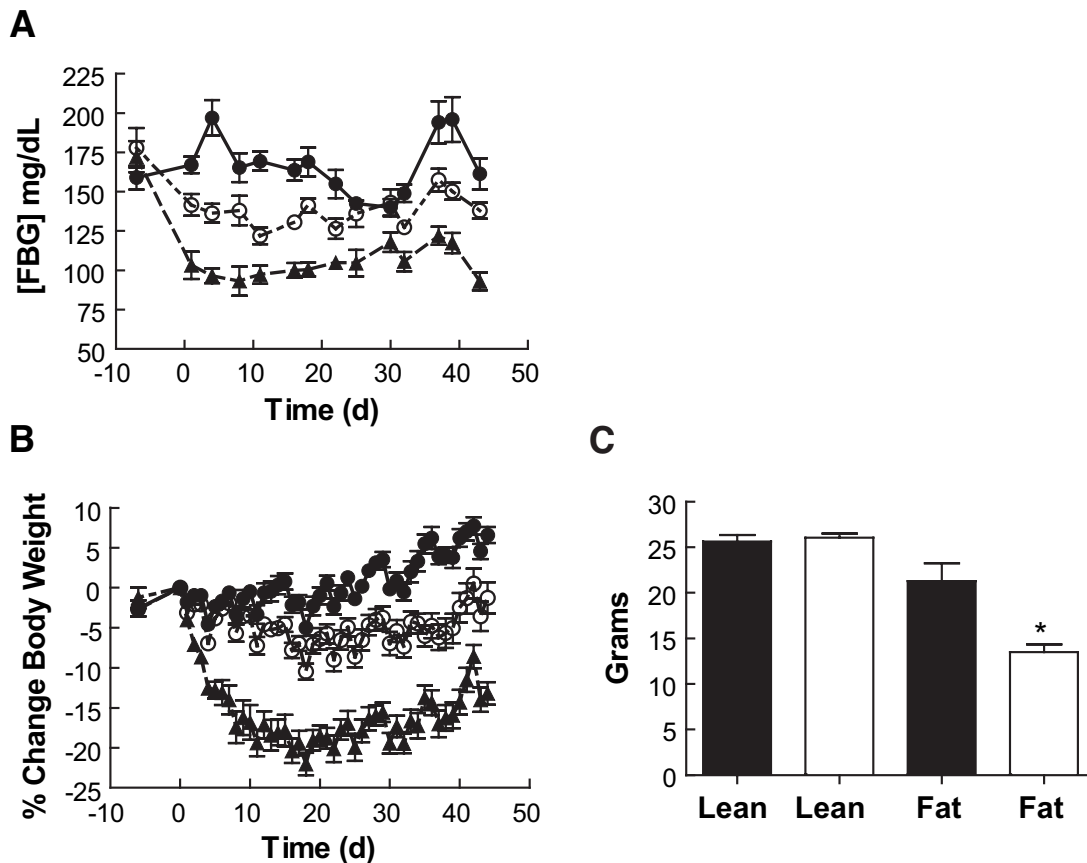


FIG. 7. Chronic CNTO736 dosing improves glucose control and decreases body weight in DIO mice. CNTO736 (0.1 mg/kg) (□) and (1 mg/kg) (▲) or vehicle (●) was dosed subcutaneously daily for 6 weeks ($n = 7$). A: Fasting blood glucose. B: Body weight. C: Dual-energy X-ray analysis for lean and fat mass in the vehicle (■) and CNTO736 groups (1 mg/kg) (□). The results are presented as the means \pm SE ($n = 5$). * P value < 0.05 .

although CNTO736 showed prolonged pharmacokinetics following a single dose in mice, animals were dosed daily to ensure continuous coverage in chronic experiments. Despite the likelihood of an anti-human Fc response, efficacy was maintained in mice dosed chronically with CNTO736.

A significant component of the observed efficacy in patients treated with byetta or liraglutide arises from a reduction in food intake and body weight (24,40–42). Our data demonstrate that peripheral dosing of CNTO736 decreases food intake and chronic dosing reduces body weight. It remains unclear whether peripherally or centrally located receptors mediate the GLP-1 effect on food intake, and we anticipated that the molecular weight of CNTO736 would preclude its passage across the blood-brain barrier. Immunohistochemistry was used to test the hypothesis directly, and CNTO736 was detected only in brain regions that are readily available to circulation (circumventricular organs). However, *c-fos* activation was observed in the amygdala. Therefore, GLP-1 receptors that influence food intake are likely located either in the periphery or in an area of the brain that is exposed to circulation. Our *c-fos* activation data are consistent with previous reports that neurons found in the area postrema are important for transmitting signals from the periphery to the central autonomic regulatory system (43).

A major adverse event associated with the incretin mimetics is nausea. Although the mechanism for nausea is not well understood, it has been speculated that a delay in gastric emptying may play a role. Alternatively, it is likely

that there is a central component to the nausea. Experiments in which GLP-1 was dosed directly into various regions of rat brains followed by a conditioned taste aversion test suggest that receptors located in the central nucleus of the amygdala mediate visceral illness (44). A significant difference between CNTO736 and many GLP-1 receptor agonists evaluated in the clinic thus far is its size. Byetta and liraglutide are low-molecular weight peptides that are likely to cross the blood-brain barrier. Since the central nucleus of the amygdala is an area of the brain that is protected by the blood-brain barrier, CNTO736 is unable to directly activate receptors there following peripheral dosing. However, *c-fos* is activated in the amygdala following peripheral dosing of CNTO736. Peripheral dosing of albugon, another large molecule GLP-1 receptor agonist, also activated *c-fos* in many areas of the brain that are not likely to be accessible to large molecules, including the central nucleus of the amygdala (31). Thus, it is likely that there is a peripheral mechanism that allows for activation of receptors in central nervous system centers. Since *c-fos* signaling from albugon was less robust than that of exenatide, it is possible that central and peripheral mechanisms act in concert to mediate the complete pharmacology of GLP-1. It may be possible to separate the negative (i.e., nausea) events associated with GLP-1 therapeutics by minimizing localization in the central nervous system. Regardless of the predominant mechanism, a correlation between C_{\max} and nausea has been observed for GLP-1 receptor peptide agonists. A molecule with an extended and flattened pharmacokinetic profile may be expected to

have fewer C_{\max} -related side effects. Taken together, we anticipate that the MIMETIBODY platform may enable the desired activities of GLP-1 with minimal adverse events.

In conclusion, the data presented suggest that our novel MIMETIBODY platform can be used to improve the properties of a bioactive peptide, rendering it more suitable for therapeutic use. CNTO736 is a potent GLP-1 analogue with potential to improve treatment of type 2 diabetes. The half-life of CNTO736 is substantially longer than native GLP-1, byetta, or liraglutide. It is tempting to speculate that sustaining superphysiological levels of a GLP-1 receptor agonist will translate into significant improvements in efficacy with less frequent dosing and the potential to inhibit disease progression. These data illustrate the potential for applying the MIMETIBODY platform to enhance the properties of agonist peptide therapeutics for metabolic diseases. More generally, identification of agonist antibodies or small molecule drugs for multitransmembrane receptors remains challenging, and the MIMETIBODY platform provides a technology to address this gap and enable peptide agonist drug development.

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