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Novel GLP-1 analog supaglutide improves glucose homeostasis in diabetic monkeys

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Abstract

Glucagon-like peptide 1 (GLP-1) is an insulinotropic hormone and plays an important role in regulating glucose homeostasis. GLP-1 has a short half-life ($t_{1/2} < 2$ min) due to degrading enzyme dipeptidyl peptidase-IV and rapid kidney clearance, which limits its clinical application as a therapeutic reagent. We demonstrated recently that supaglutide, a novel GLP-1 mimetic generated by recombinant fusion protein techniques, exerted hypoglycemic and β -cell trophic effects in type 2 diabetes db/db mice. In the present study, we examined supaglutide's therapeutic efficacy and pharmacokinetics in diabetic rhesus monkeys. We found that a single subcutaneous injection of supaglutide of tested doses transiently and significantly reduced blood glucose levels in a dose-dependent fashion in the diabetic monkeys. During a 4-week intervention period, treatment of supaglutide of weekly dosing dose-dependently decreased fasting and random blood glucose levels. This was associated with significantly declined plasma fructosamine levels. The repeated administration of supaglutide remarkably also decreased body weight in a dose-dependent fashion accompanied by decreased food intake. Intravenous glucose tolerance test results showed that supaglutide improved glucose tolerance. The intervention also showed enhanced glucose-stimulated insulin secretion and improved lipid profile in diabetic rhesus monkeys. These results reveal that supaglutide exerts beneficial effects in regulating blood glucose and lipid homeostasis in diabetic rhesus monkeys.

Key Words

- ▶ GLP-1
- ▶ supaglutide
- ▶ diabetes
- ▶ glucose homeostasis
- ▶ obesity

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Introduction

Glucagon-like peptide 1 (GLP-1) is an incretin hormone produced and secreted by intestinal L cells in response to nutrients ingestion. GLP-1 regulates glucose metabolism mainly by stimulating postprandial insulin secretion and suppressing glucagon release (Drucker *et al.* 1987,

Ritzel *et al.* 1995, Drucker 2018, Muller *et al.* 2019). GLP-1 also decreases gastric emptying, inhibits food intake, and modulates rodent β -cell proliferation (Li *et al.* 2005, Brubaker 2010, Rajeev & Wilding 2016, Taylor 2018, Holst 2020). The endogenous GLP-1 has a short

in vivo half-life ($t_{1/2} < 2$ min) due to proteolytic cleavage primarily by dipeptidyl peptidase-IV (DPP-IV), and rapid renal clearance, which limits its therapeutic potential of native GLP-1 (Orskov *et al.* 1993, Deacon *et al.* 1995, Drucker 2018, 2020). To circumvent this limitation, great efforts have been made in the past decade to prolong the *in vivo* GLP-1 action and two therapeutic strategies that are developed and clinically accessible: one is the use of DPP-IV inhibitors, and the other is the development of DPP-IV-resistant long-acting GLP-1 mimetics (Drucker & Nauck 2006, Drucker 2018). There are clinically available GLP-1 receptor agonists (GLP-1RAs). According to their half-life and amino acid sequence homology with human counterparts, GLP-1RAs can be divided into short-term and long-term, animal-derived and human-derived (Uccellatore *et al.* 2015), which show distinguished pharmacokinetic and pharmacodynamic characteristics as well as clinical compliance (Campbell & Drucker 2013, Gentilella *et al.* 2019, Overgaard *et al.* 2019). It is noted, long-last and human-derived rather than animal-derived either short- or long-lasting (Holman *et al.* 2017, Bethel *et al.* 2020) GLP-1RAs showed significant cardiac and renal beneficial effects (Marso *et al.* 2016a,b, Mann *et al.* 2017, Gerstein *et al.* 2019).

Supaglutide is a novel long-acting human-derived GLP-1RAs, produced by recombinant fusion protein techniques. This human-derived GLP-1 fusion protein contains two GLP-1 molecules with an immunoglobulin IgG2 constant region and partial hinge chains to form a spatial structure of ligand-receptor binding (Kumar *et al.* 2007, Soltani *et al.* 2007, Wang *et al.* 2010). Our previous studies under *in vitro* and *in vivo* conditions showed that supaglutide was relatively resistant to DPP-IV enzymatic degradation and delayed renal clearance rate due to enlarged molecular mass (>60 kDa), retaining native GLP-1 biological activities (Wang *et al.* 2010).

We demonstrated recently that supaglutide stimulates insulin secretion in mouse and human islet β -cells, and enlarges β -cell mass, and improves glucose homeostasis in diabetic db/db mice (Ren *et al.* 2019). We also showed recently that supaglutide exerts a beneficial effect on established obesity in mice induced by high-fat diet feeding through reducing energy intake while enhancing brown remodeling of white adipose tissue via uncoupling protein 1 (Ucp1) dependent signaling pathway (Wan *et al.* 2017). In the present study, we investigated the therapeutic efficacy of supaglutide and pharmacokinetics in diabetic rhesus monkeys.

Materials and methods

Animals

Male rhesus monkeys with spontaneous type 2 diabetes were provided and housed in Sichuan Primed Shines Biotech Co., Ltd. (SPSB). Before the experiments, the animals were quarantined and received a physical examination, two tuberculosis (TB) tests, and tests for parasite, *Salmonella*, *Klebsiella* and B virus. Thirty-five qualified animals were screened according to guidelines for the development and management of a colony of monkeys with naturally occurring type 2 diabetes (Hansen 2012).

Exclusion criteria

Monkeys met the following exclusion criteria were excluded: type 1 diabetes, pancreatitis, diabetic ketoacidosis, chronic gastrointestinal diseases, obvious hematological diseases, severe trauma and infection.

Inclusion criteria

Monkeys were examined for intravenous glucose tolerance test (IVGTT) and insulin levels during IVGTT. Monkeys did not receive any drug treatment within 1 year after the onset of either impaired glucose tolerance (IGT), or impaired fasting glucose (IFG), or obvious hyperglycemia were selected, excluding those with advanced type 2 diabetes (Hansen 2012). During the adaptation period, male rhesus monkeys were selected with the age of 8–21 years, body weight ranging from 9.25–15.70 kg, fasting plasma glucose (FPG): 5.50–8.58 mmol/L, glycosylated hemoglobin (HbA1c): 4.5–5.0%, with normal liver and kidney functions. The animals were randomly divided into placebo and intervention groups.

Feeding conditions

Monkeys were housed under controlled temperature, 12 h light:12 h darkness cycle, humidity of 40–70%, ventilation of fresh air at least eight times per hour, with free access to food and water. In addition to the chow diet (Primed Monkey Chow 4#, containing ~18% proteins, ~12% fat, ~62% carbohydrate), monkeys were provided with 100 g of green feed (fruits and vegetables) every day. As a reward after domestication, snacks such as peanuts, melon seeds and other nuts are also provided. Two weeks prior to experiments, animals were domesticated for quiet and volunteer to reach out for blood collection (one to three times/week). Animal care procedures were

approved by the Institutional Animal Care and Utilization Committee (IACUC) of SPSB accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

Supaglutide treatment

A pilot efficacy study was conducted in 6 diabetic rhesus monkeys divided into three dosing groups. Animals have received one subcutaneous (SC) injection of supaglutide (25, 50 and 100 µg/kg, $n = 2$), self-baseline as a control, and monitoring FPG, random plasma glucose (RPG), body weight, and food intake during a follow-up period of 14 days. In the pharmacodynamic study of multiple administration, 15 rhesus monkeys with type 2 diabetes were randomly divided into three groups (0, 25, 50 µg/kg, $n=5$). Supaglutide (placebo as the control) was injected subcutaneously once a week, four times in total, followed by a washing period of 15 days. FPG, RPG, fructosamine (FRA), HbA1c, fasting plasma insulin (FPI), random plasma insulin (RPI), body weight, and food intake were monitored. IVGTT was conducted on the third day after the last injection. Figure 1 shows the experimental design, showing the injection time and the blood sampling time points used for laboratory examinations.

Intravenous glucose tolerance test (IVGTT)

After 12 h of overnight fasting, monkeys were anesthetized with ketamine hydrochloride (10 mg/kg) by intramuscular injection. In total, 50% glucose solution (0.25 g/kg) was injected into the lower extremity vein. Blood samples were collected through the forearm vein of the monkey at 3, 5, 7, 10, 20, 30 and 60 min after glucose injection. Blood samples were taken twice, 5 and 3 min before glucose injection as baseline control.

Glucose-stimulated insulin secretion (GSIS) was evaluated by measuring plasma insulin levels during IVGTT at the time points as indicated.

We calculated area under glycemic curve ($AUC_{Glu0-60min}$), glucose disposal rate (GDR) (Jen & Hansen 1988) during the IVGTT. Acute insulin response (AIR) was estimated by calculating the average concentrations of the plasma insulin from 3 to 10 min after the glucose bolus. Homeostatic model assessment for β -cell function ($HOMA-\beta$) and for insulin resistance ($HOMA-IR$) was estimated by equations: $HOMA-\beta = 20 \times FINS / (FPG - 3.5)$ and $HOMA-IR = FPG \times FINS / 22.5$, respectively, and FINS denoted fasting insulin levels.

Serology studies

Blood samples were collected by EDTA-2K anticoagulant vacuum tube (13 × 75 mm, 3.0 mL BD Vacutainer® plastic whole blood tube containing 3.4 mg of spray-coated K2EDTA) and centrifuged at 1600 *g* for 10 min at 4°C. Plasma glucose was detected by Roche Cobas 6000 analyzer series C501 (Roche Diagnostics GmbH), and plasma insulin by Roche Cobas 6000 analyzer series E601 (Roche Diagnostics GmbH). HbA1c was detected by Siemens DCA vantage glycosylated hemoglobin analyzer. Roche Cobas 6000 analyzer series C501/E601 was used to detect FRA and other blood biochemical tests including triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), blood urea nitrogen (BUN), creatinine (Cr) and creatine kinase (CK).

Metabolic measurements

Food intake was measured and calculated (food intake = feed – discard – surplus) during the entire study period. Four feedings of total 300–600 g feeds were made every day (09:00, 10:30, 15:00 and 16:30). Body weight measuring times: once in the adaptation period (before grouping), four times in the administration observation period (D7, D14, D21 and D28), and twice in the washout

Figure 1

Supaglutide treatment design. The experiment is consisted of three periods: adaptation (43 days), observation (28 days) and washout (15 days). The solid arrows indicate the time points of drug injection. The dotted arrows indicate time points of blood sampling for PK (black dots) and other laboratory examinations as indicated. The red dots indicate the time points for the intravenous glucose tolerance test (IVGTT).

period (D35 and D42), a total of seven times, using METTLER TOLEDO electronic platform scale.

Detection of plasma drug concentration

Supaglutide of monkey blood samples was determined by ELISA, using primary monoclonal mouse anti-GLP-1 (Bronner *et al.* 2015) (Abcam, lot# GR305368-1), and secondary antibody horseradish peroxidase-labeled goat anti-human IgG (Fc) (Thermo, lot# QK2117736), and detected by TMB Colorimetric Kit (Thermo, lot# RJ2281281). Optical density measurements are taken at 630 nm using SpectraMax 340PC384 microplate reader (Molecular Device, Sunnyvale, CA, USA) and the reading compared to a calibration curve, according to manufacturer instructions. The standard drug samples of all concentration points were prepared from 100% rhesus monkey plasma. The quantitative range of the analytical method was 3.91–500.00 ng/mL. The concentration of the samples tested as well as drug concentration were automatically calculated by Watson LIMS v.7.3.0.01 (Thermo Scientific Inc.) according to the standard curve.

Statistical analysis

Statistical analysis was performed using Microsoft Office Excel, IBM SPSS Statistics V22.0, and GraphPad Prism 6 (GraphPad Software). Student's *t*-test or one-way ANOVA

or two-way ANOVA with Dunnett *post hoc* test were used as appropriate. Pharmacokinetic parameters calculation and statistical analysis were made by the non-chamber model of WinNonLin v.6.4 (Certara Corp.); the serum drug concentration values of all individuals less than the lowest concentration of the standard curve (LLOQ, 3.91 ng/mL) were recorded as below the quantification limit of the assay (BQL) when pharmacokinetic parameters were calculated. Data are mean $\pm$ S.E.M. *P* values < 0.05 are considered significant.

Results

Long-term treatment of supaglutide reduces blood glucose, circulating insulin and body weight

Pilot studies exploring effective dosing showed that a single SC injection of supaglutide decreased FPG (Fig. 2A and Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article) and RPG (Fig. 2B and Supplementary Table 1) levels. Within the testing dose range (25, 50, 100 μ g/kg), the effect appears to be dose-related. The single SC injection also reduced body weight (Fig. 2C and Supplementary Table 1) and food intake (Fig. 2D and Supplementary Table 1). The clinical indexes of those diabetic monkeys were shown in Supplementary Table 2. It is worth noting that from the first day to the second day after the injection, subjects receiving high

Figure 2

A single subcutaneous injection of supaglutide reduces blood glucose, food intake and body weight. (A) Changes of fasting plasma glucose (FPG) and (B) random plasma glucose (RPG) in diabetic rhesus monkeys during the treatment of supaglutide. Baseline ranges of FPG and RPG were 5.04–9.86 mmol/L, and 5.21–23.35 mmol/L, respectively. (C) Changes of body weight and (D) food intake and after a single subcutaneous injection of supaglutide. The areas under curves (AUC) are shown in the right panels. Data are shown as mean $\pm$ S.E.M. **P* < 0.05 , ***P* < 0.01 , ****P* < 0.001 (*n* = 2).

Figure 3

Long-term supaglutide treatment dose-dependently reduces blood glucose, insulin levels, body weight and food intake. (A) Change of fasting plasma glucose (FPG) and (B) random plasma glucose (RPG) in diabetic rhesus monkeys during the treatment of supaglutide or placebo. Baseline ranges of FPG and RPG were 5.38–7.25 mmol/L, and 4.5–6.72 mmol/L, respectively. (C) Changes of random plasma insulin (RPI). (D) Changes of fructosamine (FRA) from baselines. Changes of body weight (E) and food intake (F). The areas under curves (AUC) are shown in the right panels. Data are shown as mean $\pm$ s.e.m. SC, subcutaneous of supaglutide. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ ($n = 5$).

doses (100 µg/kg) did not eat chow diet, although they did not refuse fruits and vegetables provided daily, indicating that 100 µg/kg of supaglutide strongly inhibited the appetite of obese monkeys. The monkeys regained their appetite on the third day after the injection.

We then examined the hypoglycemic effect of two doses of supaglutide (25, 50 µg/kg) by repeatedly SC injections dosing weekly, for 4 weeks and a subsequent follow-up of 2 weeks. The results showed that long-term treatment of supaglutide markedly reduced FPG and RPG in a dose-related pattern (Fig. 3A, B and Supplementary Table 3). This was associated with significantly decreased RPI levels (Fig. 3C and Supplementary Table 3) and FPI levels (Supplementary Fig. 1A and Supplementary Table 3). During the intervention, although there was no significant change in HbA1c (Supplementary Fig. 1B), the plasma FRA was significantly reduced in a dose-dependent manner (Fig. 3D and Supplementary Table 3).

There was a continuous decline in body weight during the intervention period (Fig. 3E and Supplementary Table 4). It is noted that within 24 h of injection, a significant reduction in food intake was observed, but recovery began the next day (Fig. 3F and Supplementary Table 4).

Long-term treatment of supaglutide improves glucose tolerance, enhances glucose-stimulated insulin secretion, and β -cell function

IVGTT was performed 3 days after the last injection (D24), and the results showed that long-term treatment of supaglutide significantly improved glucose excursion rates (Fig. 4A). GSIS evaluated by measuring insulin levels during IVGTT showed that glucose-stimulated insulin secretion, particularly the first phase was significantly increased (Fig. 4B).

Metabolic analysis, using homeostatic model assessment showed enhanced β -cell function (HOMA- β , Fig. 4C), increased acute insulin response (AIR, Fig. 4D), improved insulin resistance (HOMA-IR, Fig. 4E), and increased glucose disposal rate (GDR, Fig. 4F) in the diabetic monkeys received long-term supaglutide treatment.

Supaglutide alters lipid profile in the diabetic rhesus monkeys

The results of laboratory biochemical tests of diabetic rhesus monkey are summarized in Supplementary Table 5. After 4 weeks of repeated SC injection by weekly

Figure 4

Long-term treatment of supaglutide improves glucose tolerance, enhances glucose-stimulated insulin secretion, and β -cell function. (A) Blood glucose levels measured during IVGTT conducted on the third day after the last injection (D24). (B) The plasma insulin levels measured during the IVGTT (D24). (C) The homeostasis model assessment- β (HOMA- β). (D) The acute insulin secretion ($\text{AIR}_{3-10\text{min}}$). (E) The Homeostasis model assessment-IR (HOMA-IR). (F) The glucose disposal rate ($\text{GDR}_{5-20\text{min}}$). The areas under curves (AUC) are shown in the right panels. Data are shown as mean $\pm$ S.E.M. * $P < 0.05$ ($n = 5$).

dosing, supaglutide decreased total cholesterol by 27.06 and 20.85% in the monkeys received 25 and 50 µg/kg doses, respectively (Fig. 5A, $P = 0.06$, $P = 0.1$). Supaglutide also lowered LDL levels by 35% (25 µg/kg) and 37% (50 µg/kg, $P < 0.05$) in the diabetes monkeys (Fig. 5B). HDL levels were not significantly changed in both dose groups (Fig. 5C).

Other biochemical indexes such as ALT, AST, BUN and Cr were not significantly changed in both dosing groups. Compared with baseline, supaglutide decreased CK values in both dosing group, but statistically significance only found in the 25 µg/kg group (130 ± 13 vs 87 ± 3 , $P < 0.05$), but not the 50 µg/kg group (93 ± 8 vs 76 ± 5 , $P > 0.05$). Supaglutide also decreased TBIL measurements in both

dosing groups (25 µg/kg, 2.5 ± 0.5 vs 1.9 ± 0.4 , $P > 0.05$; 50 µg/kg, 3.2 ± 0.3 vs 2.2 ± 0.2 , $P < 0.05$) when compared with the baseline (Supplementary Table 5), suggesting that the investigational doses of supaglutide had no adverse effect on the liver and kidney function of diabetic rhesus monkeys.

Pharmacokinetic outcomes of supaglutide repeatedly injections in diabetic rhesus monkeys

The major pharmacokinetic parameters of supaglutide in diabetic rhesus monkeys are summarized in Table 1. The maximal concentration C_{max} is 84 and 181 ng/mL for 25 and 50 µg/kg, respectively. The time of observed

Figure 5

Supaglutide alters lipid profile in diabetic rhesus monkeys. (A) The levels of the total cholesterol (TC). (B) Low-density lipoprotein (LDL) levels. (C) High-density lipoprotein (HDL) levels. (D) The levels of the alanine aminotransferase (ALT). (E) Aspartate aminotransferase (AST) levels. Data are shown as mean $\pm$ S.E.M. * $P < 0.05$ ($n = 5$). (F) Supaglutide blood concentrations measured during the intervention period in the blood sampled at the time points as indicated (54, 72, 168, 336, 504, 576 and 672 h). Data are shown as mean $\pm$ S.E.M. SC, subcutaneous of supaglutide. * $P < 0.05$ ($n = 3$).

Table 1 Pharmacodynamics associated with pharmacodynamics of supaglutide repeatedly injections for four times in rhesus monkeys.

Dosing	C _{max} (ng/mL)	T _{max} (h)	AUC _{0-inf} (ng/h/mL)	T _{1/2} (h)	CL/F (mL/h/kg)	V _{ss} /F (mL/kg)
25 µg/kg	84.01 ± 31.39	576	11,323.69 ± 4934.76	59.36 ± 3.77	2.39 ^a	178.67 ^a
50 µg/kg	181.28 ± 13.63	576	247,979.53 ± 1407.04	58.46 ± 2.44	2.53 ± 0.16	214.22 ± 20.78

Data are shown as mean ± S.E.M.

^aMedian.

AUC, area under the curve; C_{max}, maximum plasma concentration; CL/F, clearance/fraction absorbed (bioavailability); T_{max}, time to maximum plasma concentration; T_{1/2}, terminal half-life; V_{ss}/F, volume of distribution at steady-state/fraction absorbed (bioavailability).

maximal drug concentration T_{max} is 576 h. The half-life is approximately 59 h. These data revealed that the peak concentration of supaglutide and the level of exposure increased with the increase of dose.

During the intervention, monitoring animal behavior showed no abnormal change related to administration including behavioral activities, mental state, body surface hair color, and animal feces.

Discussion

The present study demonstrates that supaglutide administrated once a week for 4 weeks significantly improved glucose homeostasis in rhesus monkeys with naturally occurred type 2 diabetes.

Single SC injection of supaglutide showed the tested doses (25, 50 and 100 µg/kg) that were effective in lowering both FPG and RPG associated with body weight sparing effects in a dose-dependent fashion. The efficacy peak appeared at 24 h after the injection and it continued for about a week and gradually returned to the basal levels. Weight loss is one recognized efficacy of GLP-1 therapy (Drucker 2018). A significant reduction in food intake in dose-dependent manner was noted. Within the first 48 h after the high dose injection (100 µg/kg), animals were reluctant to chow diet, indicating strongly inhibited appetite, although they did not refuse fruits and vegetables, and regained their appetite on the third day after the injection. Our observations are consistent with previous studies demonstrating that therapies with GLP-1RAs, that is, exendin-4 (Scott & Moran 2007), and/or dulaglutide (Vahle *et al.* 2015) significantly and dose-dependently reduced food intake and body weight in non-diabetic rhesus monkeys.

Repeated administration with weekly dosing (25, 50 µg/kg), supaglutide reached a steady state of blood concentration after the second injections. The observed decrease in FPG and RPG levels was accompanied by a decrease in FPI and RPI levels. Type 2 diabetes is usually

associated with obesity and insulin resistance which is characterized by basal hyperinsulinemia (Shanik *et al.* 2008). Obese diabetic monkeys treated with supaglutide displayed improved blood glucose and increased glucose-stimulated insulin secretion (during IVGTT). Therefore, it is likely, the decreased fasting insulin (and RPI) may be attributed to improved glycemic control that leading to decreased demand for insulin (Hasnain *et al.* 2014). In addition, the improvement of obesity may also contribute to the decrease of basal insulin (Shanik *et al.* 2008). Since we did not perform ITT or hyperinsulinemic normoglycemic clamp, and we are unable to directly address whether supaglutide therapy improved insulin sensitivity in these diabetic monkeys. Nevertheless, our observations are consistent with other's study demonstrating that treatment with GLP-1RA liraglutide produced beneficial effects on hyperglycemia and insulin sensitivity parameters (i.e. HOMA-IR and HOMA-β) in patients with type 2 diabetes (Díaz-Soto *et al.* 2014). It is interesting to note, although the causal relationships between insulin levels and insulin resistance are not fully elucidated, a number of studies showed that improved insulin sensitivity is associated with reduced fasting glucose and decreased insulin levels in mice (Templeman *et al.* 2017), non-human primates (Henderson *et al.* 2016) and humans (Wang *et al.* 2018, Contreras *et al.* 2019). On the other hand, elevated insulin contributes to insulin resistance (Templeman *et al.* 2017).

During the intervention period of 4 weeks, although HbA1c was not obviously changed, the plasma FRA, a validated-biomarker that reflects the average levels of glucose control over the past 2–3 weeks (Ren *et al.* 2019, Calderon *et al.* 2020), was significantly decreased, in a dose-dependent fashion. The metabolic cycle of erythrocytes in rhesus monkeys is about 90 days (Pasini *et al.* 2010). HbA1c of rhesus monkey is used to evaluate the blood glucose level over the past 2–3 months.

Compared with the placebo control, repeated injections with weekly dosing (25, 50 µg/kg) significantly reduced body weight in dose-dependent fashion,

which was accompanied by significantly decreased food intake in these obese diabetic monkeys. Reduction in body weight may be a consequence of lower food intake and/or enhanced energy expenditure. It is worth noting that during the intervention period, the body weight significantly and continuously decreased within 48 h after the first injection, but reached the plateau period after the second injection. This is in contrast to the significant reduction in food intake within 24 h after the first injection and recovery from the next day, suggesting that the weight loss may also be contributed by factors other than food intake, possibly due to changes in energy expenditure. Our recent study showed that in obese mice repeated injections of supaglutide significantly promoted body weight loss associated with increased energy expenditure (Wan *et al.* 2017). This is consistent with other's observations that in diet-induced obese mice and non-human primates, GLP-1 based therapy significantly reduced blood glucose, and reduced body weight mainly due to the increased lipid and carbohydrate oxidation and energy consumption (Elvert *et al.* 2018).

Supaglutide treatment improved glucose tolerance associated with improved β -cell function, exemplified by enhanced insulin secretion, particularly in the first phase during IVGTT in diabetic monkeys, consistent with our recent data showing that long-term treatment with supaglutide stimulates insulin secretion and enlarges β -cell mass in diabetic db/db mice (Ren *et al.* 2019).

Numerous evidence showing that GLP-1/GLP-1RAs stimulates insulin secretion and enhances β -cell mass in rodents (Lee & Jun 2014, Kawamori *et al.* 2017, Muller *et al.* 2019). However, whether incretin-based therapies sustain functional β -cell mass in human diabetic subjects remains unclear (Drucker 2013). Clinical studies demonstrated that GLP-1RAs enhance β -cell function and improve insulin resistance (Mari *et al.* 2007). Non-human primate islets are morphologically and structurally similar to human islets (Mohanasundaram *et al.* 2011), and there is experimental evidence of islet regeneration and restoration of β -cell mass in non-human primates under the condition of β -cell injury (Chen *et al.* 2014).

In summary, our results suggest that the human origin long-lasting GLP-1R agonist supaglutide by weekly dosing in diabetic rhesus monkeys exerts long-lasting hypoglycemic effects associated with enhanced β -cell function, and body weight sparing effects. The prolonged half-life and metabolically beneficial effect of supaglutide provides an alternative new tool in studying GLP-1 biology and potentially alternative therapy to treat type 2 diabetes and its associated metabolic disorders.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-20-0255>.

Declaration of interest

The authors declare that there is no duality of interest associated with this manuscript. Q W has GLP-1 related patents.

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Author contribution statement

W Z and Q W contributed to the conception and design of the study. Z Y, Y L, M W, Z Y and W Z contributed to performing the major body of the experiments. X H, W T, Q Z and A S contributed to supaglutide production. Q C, Y L, A M, Y J, Y Z, J L, Z W, and L Z analyzed the data. Y L, Y J, Q C and Q W contributed to writing manuscript. All authors reviewed and proved the manuscript.

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