GLP-1 treatment reduces endogenous insulin resistance via activation of central GLP-1 receptors in mice fed a high-fat diet

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Parlevliet ET, de Leeuw van Weenen JE, Romijn JA, Pijl H.

GLP-1 treatment reduces endogenous insulin resistance via activation of central GLP-1 receptors in mice fed a high-fat diet. Am J Physiol Endocrinol Metab 299: E318–E324, 2010. First published June 8, 2010; doi:10.1152/ajpendo.00191.2010.—Glucagon-like peptide-1 (GLP-1) plays a major role in the control of postprandial metabolism, since it augments nutrient-induced insulin release (13, 21), inhibits glucagon secretion (36), and reduces endogenous glucose production (EGP) through (peripheral?) mechanistic routes that appear to be independent of islet hormones (32). Circulating GLP-1 also inhibits food intake probably via vagal afferents activating neurons in the nucleus of the solitary tract that subsequently project to hypothalamic neurons to blunt appetite (6, 40). Thus, circulating GLP-1 has multiple effects on postprandial behavior and metabolism to coordinate the systemic response to food intake.

GLP-1 is also produced in a discrete population of neurons in the hindbrain (11, 15), and GLP-1 fibers terminate in the arcuate and paraventricular nucleus of the hypothalamus (15), brain areas that play key roles in the control of metabolism (35). GLP-1 receptors are expressed abundantly in these nuclei (9, 27), and recent evidence indicates that activation of arcuate GLP-1 receptors sensitizes the liver to insulin, at least as far as its impact on glucose production is concerned (34). Despite compelling evidence that GLP-1 can cross the blood-brain barrier (17), peripheral and central GLP-1 signaling systems are generally considered as separate regulatory circuits. To date, GLP-1 effects on insulin action have been reported only as a result of central GLP-1 receptor activation (19, 34). We have shown recently that peripheral administration of a novel GLP-1 agonist ameliorates insulin resistance of glucose and VLDL-triglyceride (VLDL-TG) metabolism in high-fat-fed C57Bl/6J mice (29). It is currently unknown to what extent the (metabolic) effects of GLP-1 treatment are mediated by central GLP-1 receptors. Here, we aimed to evaluate the impact of blocking central GLP-1 receptors on the metabolic effects of peripherally administered GLP-1. Our data show beneficial effects of GLP-1 treatment on glucose and lipid metabolism in insulin-resistant high-fat-fed mice. GLP-1 enhanced insulin’s action to inhibit glucose production via activation of central GLP-1 receptors.

MATERIALS AND METHODS

Animals and diet. Male C57Bl/6J mice (12 wk old; Charles River, Maastricht, The Netherlands) were housed in a temperature- and humidity-controlled environment on a 12:12-h light-dark cycle (lights on from 700 to 1900) and fed a high-fat diet (44 energy% fat derived from bovine fat; Hope Farms, Woerden, The Netherlands) with free access to water for 16 wk to induce insulin resistance (20, 38). All animal experiments were approved by the Animal Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Drugs. GLP-1 (7–36) (human, bovine, guinea pig, mouse, and rat; molecular weight: 3,297.68 g/mol) and exendin-9 (EX-9; molecular weight: 3,369.80 g/mol) were purchased from Bachem (Weil am Rhein, Germany).

Surgical procedures. For intracerebroventricular (icv) cannula implantation, mice were anesthetized with 0.5 mg/kg medetomidine (Orion, Espoo, Finland), 5.0 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 0.05 mg/kg fentanyl (Bipharma, Weesp, The Netherlands). A 30-gauge guide cannula (brain infusion kit 3, Alzet; Durect, Cupertino, CA) was stereotactically implanted in the left lateral ventricle using the following coordinates from Bregma: 0.46 mm posterior, 1.0 mm lateral, and 2.2 mm ventral. The guide cannula was connected to an osmotic minipump (model 1004, Alzet) via a catheter. This catheter was filled with artificial cerebral spine fluid (CSF; Harvard Apparatus, Natick, MA) to delay the start of delivery of the drug by 5 days. A small air bubble was introduced to separate the drug from the CSF. The minipump was placed subcutaneously in the right back region for the continuous delivery of 0.5 pmol·kg⁻¹·min⁻¹·EX-9 (dissolved in CSF) or CSF at a rate of 0.11 μl/h as a control. This dose of EX-9 is known to specifically block the central (but not peripheral) GLP-1 receptors (19). The anesthesia was

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were euthanized by cervical dislocation. After 5 days of recovery (on the day the icv EX-9/CSF treatment started), mice were matched for body weight and nonfasting glucose levels, and a second minipump (model 1002, Alzet) was implanted subcutaneously in the left back region under light isoflurane anesthesia for the continuous subcutaneous delivery of 3 pmol·kg⁻¹·min⁻¹ GLP-1 (dissolved in PBS) to increase plasma GLP-1 levels (47) or PBS as a control. We chose a two times higher dose of GLP-1 than referred to, since that dose did not result in a significant decrease in nonfasting glucose levels throughout the treatment. After the surgical procedures, we ended up with four groups, from now on referred to by the routes of administration (icv/cs) and names of the compounds: CSF/PBS, CSF/GLP-1, EX-9/PBS, and EX-9/GLP-1. After 2 wk of intervention, experiments were performed as described below. At the end of the experiment, dye (0.5% Evans blue) was injected through the cannula to confirm its position in the ventricle. Only mice with correct placement of cannulas were included in the final analysis.

**Hyperinsulinemic euglycemic clamp.** Mice were fasted prior to the clamp for 16 h overnight, with food withdrawn at 1700 the day before the study. Hyperinsulinemic euglycemic clamp experiment was performed as described before (29, 41). During the experiment, mice were sedated with 6.25 mg/kg acepromazine (Alfasan, Woerden, The Netherlands), 6.25 mg/kg midazolam (Roche), and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands). First, basal rates of glucose and glycerol turnover were determined by isotope dilution methodology using a primed (p) continuous (c) iv infusion of [1-¹⁴C]glucose (p: 0.2 μCi; c: 0.3 μCi/h; GE Healthcare, Little Chalfont, UK) and [1-³H]glycerol (p: 0.6 μCi; c: 0.9 μCi/h; GE Healthcare) for 60 min. The choice of these tracers may not be that common. However, it is important to also recognize that other a fraction of the labeled breakdown products is reincorporated in the mother compound, resulting in an underestimation of the rate of appearance. However, it is important to also recognize that other tracers have their own problems. With [³H]glucose for example, recycling occurs with unlabeled glucose via glucose cycling from fructose 6-phosphate, etc. Also, part of the glucose is lost in the hexose monophosphate pathway (43). After the basal period, insulin (Actrapid, Novo Nordisk, Denmark) was administered in a primed (4.5 mU), continuous (6.8 mU/h), intravenous (iv) infusion for 90 min to attain steady-state circulating insulin levels of ~5 ng/ml. A variable iv infusion of a 12.5% D-glucose solution was used to maintain euglycemia as determined at 10-min intervals via tail bleeding (Fig. 1A). The requirement for constant euglycemia were more than twice as high in GLP-1-treated animals compared with controls (CSF/PBS: 17 ± 10; CSF/GLP-1: 43 ± 9 μmol·min⁻¹·kg⁻¹, P < 0.01), indicating that GLP-1 reinforces whole body insulin action in this experimental condition (Fig. 1B). Blocking the central GLP-1 receptors by iv infusion of EX-9 clearly blunted the effect of GLP-1 on GIR by 62% (EX-9/GLP-1: 27 ± 11 μmol·min⁻¹·kg⁻¹, P < 0.01 vs. CSF/ GLP-1). Central GLP-1 receptor antagonism by itself did not affect GIR (EX-9/PBS: 20 ± 13 μmol·min⁻¹·kg⁻¹).

**RESULTS**

**Body weight and plasma parameters.** Fasting body weights, plasma glucose, insulin, glycerol, and NEFA concentrations in basal and hyperinsulinemic conditions are shown in Table 1. Body weight did not differ, and plasma glucose and insulin levels were similar in all groups in both basal and hyperinsulinemic conditions. Insulin infusion reduced plasma glycerol and NEFA concentrations to a similar extent in all groups. Differences between groups were determined with the Kruskal-Wallis nonparametric test for k-independent samples. When significant differences were found, the Mann-Whitney nonparametric test was used as a followup test to determine differences between two independent groups. Effects on the suppression of glycerol turnover between groups were assessed by repeated-measures ANOVA. A P value of <0.05 was considered statistically significant. Data are presented as means ± SD.

**Analytical procedures.** Commercially available kits were used to determine plasma levels of glucose, NEFA, TG (Instruchemie, DeFijziel, The Netherlands), and free glycerol (Sigma). Plasma insulin concentration was measured by ELISA (Crystal Chem, Downers Grove, IL). Total plasma [¹⁴C]glucose and [¹-³H]glycerol were determined in 8 μl of plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water.

**Calculations.** The turnover rates of glucose and glycerol (μmol·min⁻¹·kg⁻¹) were calculated during the basal period and under steady-state clamp conditions as the rate of tracer infusion (disintegrations/min) divided by the plasma-specific activity of [¹⁴C]glucose or [¹-³H]glycerol (disintegrations·min⁻¹·μmol⁻¹). The ratio was corrected for body weight. EGP was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate (GIR). Hepatic VLDL-TG production rates (μmol·h⁻¹·kg⁻¹) were calculated from the linear increase in plasma TG concentrations in time. All metabolic parameters were expressed per kilogram of body weight.

**Statistical analysis.** Statistical analysis was performed using SPSS. Differences between groups were determined with the Kruskal-Wallis nonparametric test for k-independent samples. When significant differences were found, the Mann-Whitney nonparametric test was used as a followup test to determine differences between two independent groups. Effects on the suppression of glycerol turnover between groups were assessed by repeated-measures ANOVA. A P value of <0.05 was considered statistically significant. Data are presented as means ± SD.
GLP-1, P < 0.05). Intracerebroventricular infusion of EX-9 alone did not affect EGP (EX-9/PBS: 26 ± 11 μmol·min⁻¹·kg⁻¹).

**Glycerol turnover.** Lipolysis, as reflected by basal rates of glycerol turnover, was not different between groups in basal conditions (CSF/PBS: 3.0 ± 0.7; CSF/GLP-1: 3.5 ± 0.9; EX-9/PBS: 3.1 ± 0.8; EX-9/GLP-1: 3.4 ± 1.3 μmol·min⁻¹·kg⁻¹). Glycerol turnover was significantly reduced during hyperinsulinemia only in GLP-1-treated animals (CSF/PBS: 2.9 ± 1.1; CSF/GLP-1: 2.7 ± 0.7; EX-9/PBS: 2.8 ± 1.1; EX-9/GLP-1: 2.9 ± 0.9 μmol·min⁻¹·kg⁻¹; Fig. 3). However, the effect expressed as percentage reduction of baseline values was not significantly different between interventions (CSF/PBS: 18 ± 15; CSF/GLP-1: 20 ± 17; EX-9/PBS: 19 ± 21; EX-9/GLP-1: 19 ± 20% reduction from baseline).

**VLDL-TG production.** Plasma TG levels (Fig. 4) and hence, VLDL-TG production rate in hyperinsulinemic condition were significantly reduced by GLP-1 treatment (CSF/PBS: 252 ± 47; CSF/GLP-1: 205 ± 31 μmol·h⁻¹·kg⁻¹, P < 0.05). Simultaneous icv infusion of EX-9 did not significantly impact this effect (EX-9/GLP-1: 216 ± 39 μmol·h⁻¹·kg⁻¹), and icv infusion of EX-9 alone did not affect VLDL-TG production (EX-9/PBS: 249 ± 40 μmol·h⁻¹·kg⁻¹).

**DISCUSSION**

This study shows that chronic subcutaneous administration of GLP-1 enhances whole body insulin sensitivity of glucose metabolism in high-fat-fed, insulin-resistant, C57Bl/6j mice. This is brought about by the composite stimulatory effects of GLP-1 on the capacity of insulin to suppress EGP and stimulate glucose disposal. Moreover, GLP-1 enhanced insulin’s ability to inhibit VLDL-TG production in this experimental context. Simultaneous icv infusion of EX-9, blocking central GLP-1 receptors, abolished the inhibition of EGP, whereas it did not affect VLDL-TG production. Intracerebroventricular infusion of EX-9 alone did not affect insulin action in any way. These data show that GLP-1 treatment ameliorates insulin resistance in high-fat-fed mice and strongly suggest that it reinforces insulin action to suppress endogenous glucose production via central GLP-1 receptors.

To the best of our knowledge, this is the first study to focus on the role of central GLP-1 receptors in the metabolic effects (pharmacological levels of) circulating GLP-1. All other studies evaluating the behavioral and metabolic effects of central GLP-1 receptor signaling have quantified the impact of central administration of GLP-1 (analogs) or GLP-1 receptor antagonists (e.g., Refs 19, 28, and 34). Our data suggest that peripheral GLP-1 treatment modulates insulin action via central receptors as far as insulin’s ability to inhibit glucose production is concerned. This inference is consistent with data indicating that circulating GLP-1 has access to the brain (9, 12, 17). The fact that icv administration of EX-9 alone did not impact glucose or lipid metabolism in this experimental context suggests that central GLP-1 receptors do not play a major role in the control of (postprandial) fuel flux by endogenous GLP-1 [and thereby suggest that a supraphysiological plasma concentration of GLP-1 is required to activate central receptors; although we did not measure plasma GLP-1 levels, which is a limitation of this study, we assume GLP-1 levels to be increased considerably, as observed in a previous experiment of similar design (47)]. Gut-derived circulating GLP-1 is rapidly degraded, and blood levels rise for only a limited period of time in response to a meal (26). The kinetics of postprandial GLP-1 production may not allow for substantially activation of central receptors in high-fat-fed insulin-resistant mice, whereas continuous GLP-1 infusion might. This issue clearly requires further study. Our data do suggest that GLP-1 analogs, with extended plasma half-lives for therapeutic use, act to improve metabolism in part via the brain.

The beneficial effects of GLP-1 on glucose metabolism corroborate the results of a previous study investigating the therapeutic potential of a novel GLP-1 analog in insulin-resistant mice (29). They are also in keeping with earlier reports indicating that GLP-1 and its analogs ameliorate whole body insulin resistance in obese animal models (8, 45) and in type 2 diabetes mellitus patients (46). They further extend our knowledge of the precise actions of GLP-1 on distinct components of glucose flux in insulin-resistant animals inasmuch as they show that it enhances insulin action on both glucose disposal and production in the long term. However, in apparent contrast, a recent paper indicates that central administration of GLP-1 hampers insulin-mediated glucose disposal in male Long-Evans rats, albeit not to a major extent (34). Moreover, another paper has consistently reported that icv exendin-4 (a GLP-1 receptor agonist) acutely impairs insulin’s action on...
glucose uptake in normal C57Bl/6J mice (but only in hyperglycemic conditions) (19). The same investigators also reported that chronic (4 wk) infusion of EX-9 (in a dose that was equal to ours) into the lateral ventricle of high-fat-fed male C57Bl/6J mice rescues insulin sensitivity and prevents the onset of glucose intolerance in these animals, again suggesting that central GLP-1 receptor activation impairs insulin action (18). Our data indicate unequivocally that peripheral administration of GLP-1 enhances insulin sensitivity in the same insulin-resistant animal model. In particular, they indicate that activation of central GLP-1 receptors contributes substantially to the effect of GLP-1 treatment on glucose production. This inference agrees with at least one aforementioned study showing that activation of central GLP-1 receptors reinforces insulin’s capacity to suppress EGP (34). It is difficult to reconcile the conflicting results of these studies. One major difference between the design of all other experiments and ours is the fact that we studied the effects of peripherally injected GLP-1, whereas others evaluated the impact of centrally administered compounds (where circulating GLP-1 levels were presumably low). It is conceivable that centrally injected GLP-1 activates neuronal circuits that are distinct from those activated by circulating GLP-1, and therefore, it exerts differential metabolic effects. However, it remains difficult to understand that icv EX-9 alone appears to have no effect on insulin sensitivity in our studies, whereas it reinforced insulin action in virtually the same experiment reported by Knauf et al. (18). Our study design differed from Knauf et al. in that EX-9 was given icv for

Fig. 1. Plasma glucose concentration (A) and glucose infusion rate (B) during a hyperinsulinemic euglycemic clamp in mice that received chronic intracerebroventricular/subcutaneous infusion of cerebrospinal fluid (CSF)/PBS, CSF/glucagon-like peptide-1 (GLP-1), exendin-9 (EX-9)/PBS, or EX-9/GLP-1. Values represent means ± SD for ≥9 mice/group. *P < 0.05 vs. control; **P < 0.01 vs. control.

Fig. 2. Glucose disposal (A) and glucose production (B) during a hyperinsulinemic euglycemic clamp in mice that received chronic intracerebroventricular/subcutaneous infusion of CSF/PBS, CSF/ GLP-1, EX-9/PBS, or EX-9/GLP-1. Values represent means ± SD for ≥9 mice/group. *P < 0.05 vs. control; **P < 0.01 vs. control.
4 wk in their experiment, 2 wk longer than in ours. Thus, the effects observed by Knauf et al. may have been (indirect) effects of longer-term EX-9 treatment. Also, the timing and composition of the dietary intervention were somewhat different in the studies by Knauf et al. (18). Experiments were done after 4 wk of a virtually carbohydrate-free diet, whereas we studied animals after 16 wk on a diet containing ~20% carbohydrate. The longer period of high-fat feeding may have reduced basal GLP-1 levels and diminished the GLP-1 response to food intake (2).

GLP-1 reinforced the capacity of insulin to inhibit VLDL-TG production. This finding adds to our knowledge of the effects of GLP-1 on lipid metabolism. Our observations are fully consistent with those reported in one of our previous papers, delineating the metabolic effects of a novel GLP-1 analog in the same animal model (29). Also, native GLP-1 reduces postprandial plasma TG levels in healthy, normal-weight humans and type 2 diabetes mellitus patients (16, 25).

Fig. 3. Glycerol turnover before (basal) and after (hyperinsulinemic) the initiation of a hyperinsulinemic euglycemic clamp in mice that received chronic intracerebroventricular/subcutaneous infusion of CSF/PBS, CSF/GLP-1, EX-9/PBS, or EX-9/GLP-1. Values represent means ± SD for ≥9 mice/group. *P < 0.05 vs. basal.

Fig. 4. Triglyceride (TG) concentrations during hyperinsulinemia after tyloxapol infusion in mice that received chronic intracerebroventricular/subcutaneous infusion of CSF/PBS, CSF/GLP-1, EX-9/PBS, or EX-9/GLP-1. Values represent means ± SD for ≥9 mice/group. *P < 0.05 vs. control.
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[although this has been debated (4, 24)], and these exceedingly active NPY neurons may drive overproduction of glucose in these models. GLP-1 has been reported to prevent the orexigenic effects of NPY possibly by reducing hypothalamic NPY mRNA expression (37, 39, 40). In analogy, peripherally administered GLP-1 may have inhibited arcuate NPY neurons to explain the findings on glucose turnover presented here.

In conclusion, we show here that GLP-1 treatment ameliorates insulin resistance of glucose and VLDL-TG metabolism in high-fat-fed mice. In particular, peripheral administration of GLP-1 reinforces the capacity of insulin to stimulate glucose disposal and inhibit glucose and VLDL-TG production in this model, where central GLP-1 receptors are required for the effect on glucose production.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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