Leptin restores the insulinotropic effect of exenatide in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and high-fat diet

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1Department of Diabetes, Endocrinology, and Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan; 2Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan; 3Institute for Advancement of Clinical and Translational Science, Kyoto University Hospital, Kyoto, Japan; and 4Department of Human Health Science, Kyoto University Graduate School of Medicine, Kyoto, Japan

Submitted 11 June 2014; accepted in final form 25 August 2014

Sakai T, Kusakabe T, Ebihara K, Aotani D, Yamamoto-Kataoka S, Zhao M, Gumbilai VM, Ebihara C, Aizawa-Abe M, Yamamoto Y, Noguchi M, Fujikura J, Hosoda K, Inagaki N, Nakao K. Leptin restores the insulintropic effect of exenatide in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and high-fat diet. Am J Physiol Endocrinol Metab 307: E712–E719, 2014. First published August 26, 2014; doi:10.1152/ajpendo.00272.2014.—Leptin may reduce pancreatic lipid deposition, which increases with progression of obesity and can impair β-cell function. The insulintropic effect of glucagon-like peptide-1 (GLP-1) and the efficacy of GLP-1 receptor agonist are reduced associated with impaired β-cell function. In this study, we examined whether leptin could restore the efficacy of exenatide, a GLP-1 receptor agonist, in type 2 diabetes with increased adiposity. We chronically administered leptin (500 μg·kg−1·day−1) and/or exenatide (20 μg·kg−1·day−1) for 2 wk in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and high-fat diet (STZ/HFD mice). The STZ/HFD mice exhibited hyperglycemia, overweight, increased pancreatic triglyceride level, and reduced glucose-stimulated insulin secretion (GSIS); moreover, the insulintropic effect of exenatide was reduced. However, leptin significantly reduced pancreatic triglyceride level, and adding leptin to exenatide (LEP/EX) remarkably enhanced GSIS. These results suggested that the leptin treatment restored the insulintropic effect of exenatide in the mice. In addition, LEP/EX reduced food intake, body weight, and triglyceride levels in the skeletal muscle and liver, and corrected hyperglycemia to a greater extent than either monotherapy. The pair-feeding experiment indicated that the marked reduction of pancreatic triglyceride level and enhancement of GSIS by LEP/EX occurred via mechanisms other than calorie restriction. These results suggest that leptin treatment may restore the insulintropic effect of exenatide associated with the reduction of pancreatic lipid deposition in type 2 diabetes with increased adiposity. Combination therapy with leptin and exenatide could be an effective treatment for patients with type 2 diabetes with increased adiposity.

LEPTIN, AN ADIPOCYTE-DERIVED hormone, has therapeutic potential for treating diabetes and obesity (7, 13, 19, 27, 32, 34). In our previous clinical trial in patients with lipodystrophy (6), we confirmed the therapeutic usefulness of leptin as a glucose-lowering agent, and it was first approved for the treatment of lipodystrophy in Japan in March 2013. Given these glucoregulatory effects of leptin, we and others have reported the therapeutic usefulness of leptin for various forms of diabetes, including type 2 diabetes, in rodent models (20, 23, 26, 28, 47). The glucoregulatory effects of leptin are associated with the reduction of ectopic lipid deposition, which increases with progression of obesity (36, 39, 46). The reduction of ectopic lipid deposition in the liver and skeletal muscle could improve insulin sensitivity (42). In the pancreas, the reduction of ectopic lipid deposition could improve β-cell function such as glucose-stimulated insulin secretion (GSIS) in rodents and humans (22, 39, 46).

On the other hand, glucagon-like peptide-1 (GLP-1), a hormone released from the L cells of the intestine, improves glucose metabolism by enhancing GSIS (18). However, in patients with type 2 diabetes, the insulintropic effect of GLP-1 is substantially reduced (15, 29). This reduction may be a consequence of the diabetic state rather than a contributor to it (30). Chronic hyperglycemia and hyperlipidemia could reduce β-cell function and could reduce the insulintropic effect of GLP-1 (10, 15). The correction of both these abnormalities may restore β-cell function and may restore the insulintropic effects of GLP-1 (11, 14, 49). Pancreatic lipid deposition can also reduce β-cell function (41, 45), but its effect on the insulintropic effect of GLP-1 remains unknown.

In patients with type 2 diabetes, pancreatic lipid deposition increases with progression of obesity, and GSIS is reduced (17, 36, 41, 45). Therefore, we speculated that leptin could restore the insulintropic effect of GLP-1 associated with the reduction of pancreatic lipid deposition and enhance the efficacy of GLP-1 receptor agonists. If this hypothesis is confirmed, we might be able to manage type 2 diabetes more effectively.

In the present study, we examined whether leptin could reduce pancreatic lipid deposition and enhance the insulintropic effect of exenatide, a GLP-1 receptor agonist, in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin (STZ) and high-fat diet (HFD) (STZ/HFD mice) (20).

MATERIALS AND METHODS

Animals. Seven-week old male C57BL/6J mice were purchased from Japan SLC (Shizuoka, Japan). The mice were individually caged and kept at a constant room temperature (25°C) under a 12:12-h light-dark cycle with ad libitum access to water and a standard diet.

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(SD; NMF, 3.5 kcal/g, and 13% of energy as fat; Oriental Yeast, Tokyo, Japan). Animal care and all experiments were conducted in accordance with the Guidelines for Animal Experiments of Kyoto University and were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

Generation of the mouse model of type 2 diabetes with increased adiposity. We generated a mouse model of type 2 diabetes with increased adiposity, as described previously (20). Eight-week-old male C57BL/6J mice were intraperitoneally injected one time with STZ (120 μg/g body wt) to induce partial loss of pancreatic β-cells. Three weeks after the STZ injection, the mice that exhibited hyperglycemia (over 250 mg/dl, ad libitum) were fed with HFD (D12451, 4.7 kcal/g, and 45% of energy as fat; Research Diets, New Brunswick, NJ) for 5 wk and used for the infusion experiments from 16 wk of age. The mice continued to receive HFD during the infusion and pair-feeding (PF) experiments. Age-matched male C57BL/6J mice fed SD without an STZ injection were used as normal controls (NCs).

Leptin and/or exenatide infusion experiment. The STZ/HFD mice were divided into four infusion groups [saline alone (SAL), leptin alone (LEP), exenatide alone (EX), and leptin plus exenatide (LEP/EX)] to counterbalance their starting body weights and blood glucose levels. On day 0, all of the mice were implanted with two miniosmotic pumps subcutaneously in the midscapular region (Alzet model 2002; Alza, Palo Alto, CA). Each pump chronically delivered either saline, recombinant mouse leptin (500 μg·kg⁻¹·day⁻¹; Amgen, Thousand Oaks, CA), or exenatide (20 μg·kg⁻¹·day⁻¹; Bachem, Bubendorf, Switzerland) for 14 days. To examine the insulinotropic effect of exenatide in NCs, we also chronically administered exenatide (20 μg·kg⁻¹·day⁻¹) for 14 days as described above.

Food intake and body weight. The food intake and body weight of the mice were measured every day between 1500 and 1700 for 14 days.

Indirect calorimetry. The measurement of oxygen consumption (V\(\text{O}_2\)) and carbon dioxide production (V\(\text{CO}_2\)) was performed 48 h between day 9 and 10 after >72 h of acclimation using an Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH). The respiratory exchange ratio [RER, ratio of CO\(\text{O}_2\) production to O\(\text{O}_2\) consumption (V\(\text{CO}_2\)/V\(\text{O}_2\))] was calculated and averaged across the measurement session.

Metabolic variables. Right before the infusion experiments, blood samples were obtained after 4 h of fasting. During the infusion experiments, ad libitum blood glucose levels were determined after tail bleeds using a reflectance glucometer by the glucose oxidase method between 1500 and 1700. At the end of the infusion experiment, blood was obtained from the inferior vena cava after 4 h of fasting. The plasma levels of insulin, leptin, triglyceride, total cholesterol, and nonesterified fatty acid (NEFA) were measured as described previously (20). The plasma exenatide levels were measured using ELISA kits specific for exenatide (Phoenix Pharmaceuticals, Burlingame, CA).

Insulin tolerance test and intraperitoneal glucose tolerance test. Either an insulin tolerance test (ITT) or intraperitoneal glucose tolerance test (IPGTT) was performed in each mouse on day 11. For ITTs, the mice were intraperitoneally injected with 0.4 mU/g human regular insulin (Humulin R; Eli Lilly Japan, Kobe, Japan) after 4 h of fasting. For IPGTTs, the mice were intraperitoneally injected with 1.0 mg/g glucose after overnight fasting. Blood samples were obtained from the tail vein at the indicated time points after insulin or glucose injection. GSIS was assessed by dividing the incremental insulin response by the incremental glucose response from 0 to 15 min ([Δinsulin]/[Δglucose] (0–15 min) mg/ml/μg/ml × 10^3) during IPGTTs.

Pancreatic, liver, and skeletal muscle triglyceride levels and pancreatic insulin level. The pancreas, liver, and gastrocnemius muscles were isolated at the end of the experiments after 4 h of fasting. Tissue triglyceride and pancreatic insulin levels were measured as described previously (20, 28).

PF experiment. The STZ/HFD mice were randomly divided into three groups (SAL, LEP/EX, and PF) to counterbalance the starting body weights and blood glucose levels. The PF group was fed daily the same amount of HFD as that consumed by the LEP/EX group once at the end of the light phase for 14 days. Saline, leptin (500 μg·kg⁻¹·day⁻¹), and exenatide (20 μg·kg⁻¹·day⁻¹) were chronically infused in each group as described above for 14 days.

Data analyses. Data were expressed as means ± SE. In Figs. 1A, 1D, 1E, 1F, 3A, 3B, 4C, 4D, and 4L, comparisons were made using two-way repeated-measure ANOVA models for all the data. For within-group and between-group comparisons, corresponding contrasts were tested within the model. Between-group comparisons were made at all time points. In Table 1, comparisons were made using Student’s t-test in each parameter. In the other figures and Table 2, comparisons were made using one-way ANOVA followed by Tukey’s multiple-comparison test. A P value <0.05 was considered statistically significant.

RESULTS

Generation of the mouse model of type 2 diabetes with increased adiposity. As shown in Table 1, the STZ/HFD mice manifested hyperphagia and increased body weight. Hyperglycemia was exacerbated, although the plasma insulin levels were similar to those in NCs, suggesting the development of insulin resistance and impaired β-cell ability to secrete insulin adequately. In these mice, the plasma cholesterol and tissue triglyceride levels were also increased. Reduced pancreatic insulin levels in the STZ/HFD mice suggested substantial loss of pancreatic β-cells. GSIS was also reduced. These charac-

Table 1. Metabolic characteristics of the mouse model of type 2 diabetes with increased adiposity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mouse Group</th>
<th>NC</th>
<th>STZ/HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake, kcal/wk</td>
<td></td>
<td>82.1 ± 2.1</td>
<td>98.6 ± 3.4**</td>
</tr>
<tr>
<td>Body wt, g</td>
<td></td>
<td>25.9 ± 0.4</td>
<td>28.2 ± 0.5**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4 ± 0.1</td>
<td>1.5 ± 0.3**</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td></td>
<td>124.9 ± 4.5</td>
<td>407.5 ± 30.6**</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td></td>
<td>0.97 ± 0.07</td>
<td>1.03 ± 0.06</td>
</tr>
<tr>
<td>Triglyceride, mg/dl</td>
<td></td>
<td>56.4 ± 2.4</td>
<td>57.3 ± 5.2</td>
</tr>
<tr>
<td>NEFA, meq/l</td>
<td></td>
<td>0.47 ± 0.03</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td></td>
<td>43.7 ± 3.8</td>
<td>93.6 ± 4.3**</td>
</tr>
<tr>
<td>Muscle triglyceride level, mg/g</td>
<td></td>
<td>4.9 ± 1.5</td>
<td>11.1 ± 2.2*</td>
</tr>
<tr>
<td>Liver triglyceride level, mg/g</td>
<td></td>
<td>10.0 ± 1.1</td>
<td>30.7 ± 3.3**</td>
</tr>
<tr>
<td>Pancreatic triglyceride level, mg/g</td>
<td></td>
<td>5.7 ± 1.0</td>
<td>24.1 ± 5.2**</td>
</tr>
<tr>
<td>Pancreatic insulin level, mg/g</td>
<td></td>
<td>519.4 ± 22.4</td>
<td>28.4 ± 4.2**</td>
</tr>
<tr>
<td>GSIS [Δinsulin]/[Δglucose] (0–15 min), mg/ml/μg/ml × 10^3</td>
<td></td>
<td>1.2 ± 0.2</td>
<td>-0.1 ± 0.2**</td>
</tr>
<tr>
<td>GSIS under exendatide infusion [Δinsulin]/[Δglucose] (0–15 min), mg/ml/μg/ml × 10^3</td>
<td></td>
<td>3.2 ± 0.9</td>
<td>0.4 ± 0.5*</td>
</tr>
</tbody>
</table>

Data are reported as means ± SE. NC, normal chow; STZ, streptozotocin; HFD, high-fat diet; NEFA, nonesterified fatty acid. Parameters except for food intake, tissue triglyceride levels, pancreatic insulin levels, and glucose-stimulated insulin secretion (GSIS) were measured right before the infusion experiment. Food intake was measured during 14 days of the saline infusion and halved; tissue triglyceride levels and pancreatic insulin levels were measured after the 14 days of saline infusion (n = 8 mice in each group). GSIS and GSIS under exendatide infusion were assessed as described in detail in MATERIALS AND METHODS (n = 5–6 mice in each group). *P < 0.05 and **P < 0.01 vs. NC.
teristics were compatible with human type 2 diabetes with increased adiposity.

Effects of leptin and/or exenatide on glucose metabolism in the STZ/HFD mice. Continuous administration of leptin (500 μg·kg⁻¹·day⁻¹) and exenatide (20 μg·kg⁻¹·day⁻¹) elevated plasma leptin levels to almost 20–30 ng/ml above baseline and plasma exenatide levels to around 250 pmol/l, respectively, in the exenatide (20 μg·kg⁻¹·day⁻¹)-infused mice, were markedly reduced in the STZ/HFD mice compared with those in the NC group (Fig. 1D). The plasma insulin levels in the exenatide (20 μg·kg⁻¹·day⁻¹)-infused mice, were markedly reduced in the STZ/HFD mice compared with those in the other groups (Fig. 1D). Next, we performed IPGTT to evaluate the effects on insulin secretion, the results of which showed significant improvement of glucose tolerance in the LEP/EX group than in the other three infusion groups (Fig. 1E). The plasma insulin levels in the SAL group at 15 min did not increase from those at 0 min (Fig. 1F), and Δinsulin/Δglucose (0–15 min) values were severally reduced compared with those in the NC group (Fig. 1G), indicating reduced GSIS in the STZ/HFD mice (Table 1). In addition, GSIS under exenatide infusion, the Δinsulin/Δglucose (0–15 min) values in the exenatide (20 μg·kg⁻¹·day⁻¹)-infused mice, were markedly reduced in the STZ/HFD mice compared with those in the NC group (Table 1), suggesting a reduced insulinotropic effect following this, we performed ITT to evaluate the effects on insulin sensitivity, the results of which showed a marked decrease in blood glucose levels in the LEP/EX group than in the other groups (Fig. 1D). Next, we performed IPGTT to evaluate the effects on insulin secretion, the results of which showed significant improvement of glucose tolerance in the LEP/EX group than in the other three infusion groups (Fig. 1E). The plasma insulin levels in the SAL group at 15 min did not increase from those at 0 min (Fig. 1F), and Δinsulin/Δglucose (0–15 min) values were severally reduced compared with those in the NC group (Fig. 1G), indicating reduced GSIS in the STZ/HFD mice (Table 1). In addition, GSIS under exenatide infusion, the Δinsulin/Δglucose (0–15 min) values in the exenatide (20 μg·kg⁻¹·day⁻¹)-infused mice, were markedly reduced in the STZ/HFD mice compared with those in the NC group (Table 1), suggesting a reduced insulinotropic effect.

### Table 2. Plasma leptin and exenatide levels in leptin- and/or exenatide-infused STZ/HFD mice

<table>
<thead>
<tr>
<th>Variables</th>
<th>Infusion Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAL</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>8.6 ± 1.3</td>
</tr>
<tr>
<td>Exenatide, pmol/l</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are reported as means ± SE; n = 12–14 mice in each group for leptin and n = 4–5 mice in each group for exenatide. SAL, saline alone; LEP, leptin alone; EX, exenatide alone. Plasma samples were obtained on day 14 of the infusion experiment. *P < 0.05 and ‡‡‡ P < 0.01 vs. SAL. #P < 0.05 vs. LEP. † † † P < 0.01 vs. EX. ND, not detected.
of exenatide in the STZ/HFD mice. However, LEP/EX significantly increased plasma insulin levels at 15 min from those at 0 min (Fig. 1F) and markedly augmented the Δinsulin/Δglucose (0–15 min) values than the other three infusion groups (Fig. 1G). In IPGTT, at 0 min, the plasma glucose levels were similar among the LEP, EX, and LEP/EX groups. These results suggested that adding leptin to exenatide restored the insulinotropic effect of exenatide in the STZ/HFD mice.

Effects of leptin and/or exenatide on plasma lipid levels and tissue triglyceride levels in the STZ/HFD mice. Because both plasma lipid levels and lipid deposition in the pancreas affect GSIS (22, 41, 45, 50), we measured plasma lipid levels and triglyceride levels in the pancreas.

After the infusion experiment, EX and LEP/EX significantly reduced plasma triglyceride levels, and LEP also tended to reduce triglyceride levels in the liver and skeletal muscle (Fig. 2, E and F). In addition, LEP/EX markedly reduced and almost normalized pancreatic triglyceride levels. Pancreatic triglyceride levels in the LEP/EX group also significantly reduced compared with those in the EX group (Fig. 2D). In addition, LEP/EX markedly reduced and almost normalized and LEP tended to reduce triglyceride levels in the liver and skeletal muscle (Fig. 2, E and F).

Effects of leptin and/or exenatide on food intake, body weight, and energy expenditure in the STZ/HFD mice. Body weight reduction itself may reduce pancreatic triglyceride deposition and improve β-cell function (22, 39). Thus, we examined the effects of leptin and/or exenatide on food intake and body weight in the STZ/HFD mice. Although the body weight was increased in the SAL group during the experiment period, LEP and EX significantly reduced food intake and body weight, and, furthermore, LEP/EX reduced them to a greater extent than either monotherapy (Fig. 3, A and B). VO₂ tended to increase in the LEP and LEP/EX groups (Fig. 3C). LEP/EX significantly decreased RER, indicating increased utilization of fat as a fuel source (Fig. 3D).

PF experiment. Next, we performed a PF experiment to examine the effects of anorexic and weight-reducing effects by LEP/EX on pancreatic triglyceride levels and GSIS. In the PF group, we fed daily the same amount of HFD that was consumed by the LEP/EX group for 14 days to the STZ/HFD mice (Fig. 4A). The PF group showed a significant reduction in body weight, and, furthermore, LEP/EX reduced them to a greater extent than either monotherapy (Fig. 3, A and B). VO₂ tended to increase in the LEP and LEP/EX groups (Fig. 3C). LEP/EX significantly decreased RER, indicating increased utilization of fat as a fuel source (Fig. 3D).
Improvement of insulin sensitivity was lower in the PF group to those in the LEP/EX group (Fig. 4, levels were not reduced; plasma lipid levels were comparable), and skeletal muscle (Fig. 4), liver (Fig. 4), triglyceride (E). The pancreatic (Fig. 4, did not improve in the PF group (Fig. 4), and GSIS IPGTT, glucose tolerance did not improve (Fig. 4).

Adding leptin to exenatide enhanced GSIS to a greater extent

In the present study, we used the STZ/HFD mice as a mouse model of type 2 diabetes with increased adiposity. In the mice, adding leptin to exenatide enhanced GSIS to a greater extent than either monotherapy, which was associated with the reduction of pancreatic triglyceride levels. In addition, LEP/EX reduced tissue triglyceride levels in the liver and skeletal muscle, improved insulin sensitivity, and corrected hyperglycemia to a greater extent than either monotherapy. Furthermore, LEP/EX reduced food intake and body weight to a greater extent than either monotherapy. However, the PF experiment indicated that mechanisms other than calorie restriction were involved in the reduction of the pancreatic triglyceride level and the enhancement of GSIS by LEP/EX.

The STZ/HFD mice showed defects in the pancreatic β-cell function (Table 1 and Fig. 1, F and G) and insulin resistance (Fig. 1D). These characteristics are compatible with type 2 diabetes. In addition, plasma leptin levels in the mice (Table 1) suggested that they increased body weight to overweight range because it was reported that twofold increase in plasma leptin levels correspond to a body mass index in the range of 25–30.

**DISCUSSION**

In the present study, we used the STZ/HFD mice as a mouse model of type 2 diabetes with increased adiposity. In the mice, adding leptin to exenatide enhanced GSIS to a greater extent than either monotherapy, which was associated with the reduction of pancreatic triglyceride levels. In addition, LEP/EX reduced tissue triglyceride levels in the liver and skeletal muscle, improved insulin sensitivity, and corrected hyperglycemia to a greater extent than either monotherapy. Furthermore, LEP/EX reduced food intake and body weight to a greater extent than either monotherapy. However, the PF experiment indicated that mechanisms other than calorie restriction were involved in the reduction of the pancreatic triglyceride level and the enhancement of GSIS by LEP/EX.

The STZ/HFD mice showed defects in the pancreatic β-cell function (Table 1 and Fig. 1, F and G) and insulin resistance (Fig. 1D). These characteristics are compatible with type 2 diabetes. In addition, plasma leptin levels in the mice (Table 1) suggested that they increased body weight to overweight range because it was reported that twofold increase in plasma leptin levels correspond to a body mass index in the range of 25–30.
kg/m² in humans (33). Impaired insulin secretion due to STZ injection would have reduced the effect of HFD to increase body weight. Adiposity was also increased in the mice. Therefore, we used the STZ/HFD mice as a mouse model of type 2 diabetes with increased adiposity. Substantially reduced pancreatic insulin levels in the mice (Table 1) also suggested that they were in a late stage of type 2 diabetes. In the mice, LEP/EX markedly improved glucose metabolism.

In healthy individuals, oral glucose ingestion enhances insulin secretion to a greater extent than intravenous glucose infusion (24), which is called the "incretin effect." This effect is elicited by gut hormones such as GLP-1 and glucose-dependent insulinoactive polypeptide (GIP) called "incretin," which are released from the enteroendocrine cells of the intestine in response to meal ingestion (5, 18). However, in patients with type 2 diabetes, the insulinoactive effect of GIP is largely disappeared, and the insulinoactive effect of GLP-1 is also reduced (15, 31). Recent studies have indicated that consequences of the diabetic state, such as reduced β-cell function and mass, could largely contribute to the reduction of the incretin effect in type 2 diabetes, whereas genetic defects (i.e., allelic variation of TCF7L2 and WFS1) and reduced incretin secretion may also contribute (35, 38). Hyperglycemia and hyperlipidemia, which impair β-cell function, can also reduce the incretin effect (15, 50). In this context, Hojberg et al. recently reported that correcting hyperglycemia could restore the insulinoactive effect of GLP-1 (11), and Kang et al. also reported that correcting hyperlipidemia using bezafibrate could restore the insulinoactive effect of exenatide (14). However, in the present study, another factor, besides hyperglycemia and hyperlipidemia, was suspected to play a role in the reduction of the insulinoactive effect of exenatide. Before IPGTT, the blood glucose levels were similar among the LEP, EX, and LEP/EX groups (Fig. 1E). Plasma lipid levels were also similar among the three groups (Fig. 2, A, B, and C). However, LEP/EX enhanced GSIS to a greater extent than either monotherapy (Fig. 1G).

Pancreatic lipid deposition, which increases with progression of obesity, reportedly causes β-cell dysfunction (41, 45). Improvement of β-cell function associated with the reduction of pancreatic lipid deposition has been found in rodents and humans (22, 39, 46). Even in obese patients with normal glucose tolerance, the pancreatic lipid levels are increased and the incretin effect is reduced (17, 36). These results have suggested that pancreatic lipid deposition could also affect the incretin effect; however, this has not been confirmed. In the present study, we reported for the first time the restoration of the insulinoactive effect of exenatide by the coadministration of leptin, and this effect was associated with the reduction of pancreatic triglyceride levels in the STZ/HFD mice (Figs. 1F, 1G, and 2D).

Leptin itself could not be expected to produce direct insulinoactive effects; however, it may improve β-cell functions such as GSIS associated with the reduction of pancreatic lipid deposition in rodents (39, 46). This ectopic lipid-lowering effect of leptin has been reported to be far beyond its effect on food intake and body weight and was attained by mechanisms such as sympathetic nerve activation and increasing lipid oxidation (20, 25, 43). Although a GLP-1 receptor agonist could also reduce ectopic lipid deposition, a substantial effect was associated with body weight reduction (3, 40). The results of the PF experiment also suggested that the reduction of pancreatic triglyceride levels by LEP/EX was achieved by mechanisms other than weight reduction (Fig. 4). Therefore, the marked reduction of pancreatic triglyceride levels by LEP/EX (Fig. 2D) could have been achieved by leptin rather than exenatide, at least during the 2 wk of the experimental period. The pancreatic lipid-reducing effect of leptin may have restored β-cell function and the insulinoactive effect of exenatide in the STZ/HFD mice.

There may be some reasons that leptin alone did not restore GSIS, although it did significantly reduce the pancreatic triglyceride levels (Figs. 1F, 1G, and 2D). First, the intrinsic incretin levels may have not been high enough to exert the incretin effect in the LEP group. We performed IPGTT rather than an oral challenge because it enabled the investigation of insulin secretion without any confounding effects from intrinsic incretins. Therefore, incretin levels should have been low in the fasting condition of the LEP group during IPGTT. On the other hand, plasma exenatide levels in the LEP/EX group were 235.7 ± 31.4 pM (Table 2), which was comparable to the plasma GLP-1 level reported in obese patients after undergoing bariatric surgery, and could markedly improve glucose and energy metabolism (8, 21). In addition, in patients with type 2 diabetes, the physiological concentration of GLP-1 could not sufficiently induce GSIS, but a supraphysiological dose of a GLP-1 receptor agonist could; however, compared with healthy controls, the insulinoactive effect of the supraphysiological dose of GLP-1 was still reduced (15, 31). Thus the exenatide treatment may have been necessary to produce a marked restoration of GSIS by LEP/EX in the STZ/HFD mice. Second, the substantial loss of pancreatic β-cells in the STZ/HFD mice, suggested by the reduction of pancreatic insulin levels to less than 1/10th of those of the NC group (Table 1), may have masked the improvement of β-cell function with leptin. Hosokawa et al. reported that GSIS and the insulinoactive effect of GLP-1 were substantially reduced in diabetic rats after a 90% pancreatectomy (12). Thus, leptin alone could not have restored GSIS in the STZ/HFD mice.

Plasma leptin levels in the LEP group (Table 2) were comparable to the peak plasma leptin levels observed in our clinical trial of leptin replacement therapy in patients with lipodystrophy (6), and it could be clinically applicable in humans.

The anorexic and weight-reducing effects of leptin and GLP-1 are reduced in obesity (1, 4, 9, 44). However, LEP/EX enhanced these effects to a greater extent than either monotherapy (Fig. 3, A and B). These results were similar to those recent reports by Williams and others using lean and obese rodents (27, 48). As for mechanisms, Williams et al. reported that leptin could potentiate the anorexic effect of GLP-1 via central nervous system (CNS) mechanisms (48). On the other hand, the marked reduction of tissue triglyceride levels (Fig. 2, D, E, and F) and RER (Fig. 3D) by LEP/EX also suggested the action of leptin being restored because these effects were expected with leptin rather than exenatide (2, 3). GLP-1 could also regulate glucose metabolism via CNS mechanisms such as the arcuate nucleus in the hypothalamus, which plays an essential role in the glucoregulatory action of leptin (16, 37). Thus, leptin and exenatide may have interacted to restore each other’s energy balance regulating and glucoregulatory effects via CNS mechanisms. This issue may also have a therapeutic
potential but requires further investigation. Furthermore, the STZ/HFD mice would not be obese but overweight as mentioned above, and the glucagon-lowering effect of leptin monotherapy was partially preserved in the present study. Whether LEP/EEX could exhibit marked glucagon-lowering effect to a greater extent than either monotherapy even in leptin-resistant (9) obese type 2 diabetes will deserve specific attention in the future.

In conclusion, our findings suggest that leptin treatment may restore the insulinoimetic effect of exenatide associated with the reduction of the pancreatic lipid deposition in type 2 diabetes with increased adiposity. In addition, the coadministration of leptin and exenatide reduced food intake and body weight to a greater extent than either monotherapy. Thus, combination therapy with leptin and exenatide could be an effective treatment for patients with type 2 diabetes with increased adiposity.

ACKNOWLEDGMENTS

We thank Mayumi Nagamoto for technical assistance and Yoko Koyama for secretarial assistance.

GRANTS

This work was supported in part by research grants from JSPS KAKENHI (Grant No. 23791054); the Ministry of Education, Culture, Sports, Science and Technology of Japan, including a Grant-in-Aid for Scientific Research on Innovative Areas (Research in a proposed research area) “Molecular Basis and Disorders of Control of Appetite and Fat Accumulation”; the Ministry of Health, Labour and Welfare of Japan; Japan Foundation for Applied Enzymology; the Takeda Medical Research Foundation; the Smoking Research Foundation; Suzuken Memorial Foundation; Novo Nordisk Insulin Research Foundation; Suzuken Memorial Foundation; Novo Nordisk Insulin Research award; and Lilly Education and Research Grant Office.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: T.S., T.K., K.E., and K.N. edited and revised manuscript. T.S. performed experiments; T.S. analyzed data; T.S., T.K., K.E., N.I., and K.N. interpreted results of experiments; T.S. prepared figures; T.S. drafted manuscript; T.S., T.K., and K.N. approved final version of manuscript; T.K., K.E., N.I., and K.N. edited and revised manuscript.

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