Original Article

Glucagon-Like Peptide-1 Gene Therapy in Obese Diabetic Mice Results in Long-Term Cure of Diabetes by Improving Insulin Sensitivity and Reducing Hepatic Gluconeogenesis

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Long-term treatment with glucagon-like peptide (GLP)-1 or its analog can improve insulin sensitivity. However, continuous administration is required due to its short half-life. We hypothesized that continuous production of therapeutic levels of GLP-1 in vivo by a gene therapy strategy may remit hyperglycemia and maintain prolonged normoglycemia. We produced a recombinant adenovirus expressing GLP-1 (rAd-GLP-1) under the cytomegalovirus promoter, intravenously injected it into diabetic *ob/ob* mice, and investigated the effect of this treatment on remission of diabetes, as well as the mechanisms involved. rAd-GLP-1–treated diabetic *ob/ob* mice became normoglycemic 4 days after treatment, remained normoglycemic over 60 days, and had reduced body weight gain. Glucose tolerance tests found that exogenous glucose was cleared normally. rAd-GLP-1–treated diabetic *ob/ob* mice showed improved β-cell function, evidenced by glucose-responsive insulin release, and increased insulin sensitivity, evidenced by improved insulin tolerance and increased insulin-stimulated glucose uptake in adipocytes. rAd-GLP-1 treatment increased basal levels of insulin receptor substrate (IRS)-1 in the liver and activation of IRS-1 and protein kinase C by insulin in liver and muscle; increased Akt activation was only observed in muscle. rAd-GLP-1 treatment reduced hepatic glucose production and hepatic expression of phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fatty acid synthase in *ob/ob* mice. Taken together, these results show that a single administration of rAd-GLP-1 results in the long-term remission of diabetes in *ob/ob* mice by improving insulin sensitivity through restoration of insulin signaling and reducing hepatic gluconeogenesis. *Diabetes* 56:1671–1679, 2007

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FAS, fatty acid synthase; FFA, free fatty acid; G6Pase, glucose-6-phosphatase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLP, glucagon-like peptide; IRS, insulin receptor substrate; PEPCK, phosphoenolpyruvate carboxykinase; PKC, protein kinase C; rAd-βgal, recombinant adenovirus expressing β-galactosidase; rAd-GLP-1, recombinant adenovirus expressing GLP-1.

Glucagon-like peptide (GLP)-1 is produced through posttranslational processing of proglucagon and is secreted by intestinal L-cells in response to nutrient ingestion. Studies have shown that GLP-1 acts as a potent insulin secretagogue; enhances β-cell function; stimulates β-cell growth, survival, differentiation, and proliferation; and promotes satiety and delaying gastric emptying (1,2). Furthermore, impaired GLP-1 secretion was observed in patients with type 2 diabetes (3). Therefore, GLP-1 has been proposed as a treatment for type 2 diabetes. Treatment with GLP-1 or its analog, exendin-4, improved insulin sensitivity and glucose tolerance and reduced hyperinsulinemia in animal models of type 2 diabetes (4,5). In type 2 diabetic patients, subcutaneous infusion of GLP-1 for 6 weeks resulted in improved insulin sensitivity and β-cell function (6). However, the precise mechanisms by which insulin sensitivity and glucose tolerance are improved are not known.

Although subcutaneous injections or intravenous or subcutaneous infusions of GLP-1 showed therapeutic effects on lowering blood glucose levels, the short half-life (~2 min) and rapid clearance of GLP-1 limits the maintenance of therapeutic levels by exogenous administration. GLP-1 is degraded by the enzyme dipeptidyl peptidase IV (7,8); therefore, GLP-1 agonists that are resistant to dipeptidyl peptidase IV degradation and inhibitors of dipeptidyl peptidase IV have been investigated for the treatment of type 2 diabetes (9). We hypothesized that continuous expression of GLP-1 in vivo by a gene therapy strategy may remit hyperglycemia and maintain normoglycemia. In this study, we produced a recombinant adenovirus that expresses and secretes GLP-1 under the control of the cytomegalovirus promoter (recombinant adenovirus expressing GLP-1 [rAd-GLP-1]) and examined its therapeutic effect in diabetic *ob/ob* mice, an animal model of human type 2 diabetes, as well as the mechanisms involved in the improvement of insulin sensitivity.

RESEARCH DESIGN AND METHODS

Heterozygous *OB/ob* mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred and maintained at the animal facility at Rosalind Franklin University of Medicine and Science. Animals were fed ad libitum on a standard rodent diet. Pair-fed diabetic *ob/ob* mice were given the same daily amount of food as that eaten by the corresponding rAd-GLP-1–treated group during the previous day. The wild, lean (*OB/OB*), and mutant obese (*ob/ob*) genotypes were screened by PCR. Mutant *ob/ob* mice were monitored for the development of hyperglycemia using a glucometer. All animal experiments

were approved by the institutional animal care and use committee at the Rosalind Franklin University of Medicine and Science.

Production of rAd-GLP-1 and treatment of *ob/ob* mice with rAd-GLP-1. Recombinant adenoviral vectors expressing GLP-1 and β-galactosidase (recombinant adenovirus expressing β-galactosidase [rAd-βgal]), as a control, were constructed as previously described (10). The recombinant adenoviruses were produced and amplified in human embryonic kidney cell line (HEK-293). After purification of viruses by CsCl-gradient ultracentrifugation, viral titer was determined by optical particle units and 50% tissue culture infectious dose. Six- to 8-week-old diabetic male and female *ob/ob* mice (blood glucose levels >250 mg/dl for 3 consecutive days) were injected via the tail vein with rAd-GLP-1 or rAd-βgal (4×10^9 plaque-forming units [pfu]), and body weights were measured weekly. Blood glucose levels were determined every 2 days using a glucometer.

Intraperitoneal glucose tolerance tests. Mice were not fed for 4 h, and a glucose solution (2 g/kg body wt) was injected intraperitoneally. Blood glucose levels were measured at 0, 30, 60, 90, 120, 150, and 180 min after glucose injection.

RT-PCR analysis of insulin mRNA expression in islets. Mice were not fed for 4 h, and islets were isolated as described previously (11). Total RNA was isolated from the islets, and expression of insulin mRNA was analyzed by RT-PCR using the following primers: sense 5′-TCAGAGACCATCAGCAAG CAG-3′ and antisense 5′-GTCTGAAGGTCCCCGGGGCT-3′. Expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was analyzed as an internal control using the following primers: sense 5′-AGTGCCAGC CTCGTCCGTA-3′ and antisense 5′-TGAGCCCTTCCACAATGCAA-3′.

Quantitative real-time RT-PCR analysis. Total RNA was isolated from the liver, and cDNA was synthesized using a Superscript III Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA). PCR was carried out in a LightCycler (Roche Applied Science, Indianapolis, IN) at 95°C for 15 min, followed by 45 cycles of 95°C for 15 s, 55°C for 20 s, and 72°C for 35 s. The primer sequences used were fatty acid synthase (FAS): sense 5′CAAGACTGACTCGGCT-3′, antisense 5′-GATGCAATCTATGTAGTAGGC-3′; phosphoenolpyruvate carboxykinase (PEPCK): sense 5′-CTCCGTAGCTGGTTCG-3′, antisense 5′-CGATCCGCAA CGCAAA-3′; and glucose-6-phosphatase (G6Pase): sense 5′-GTGTTGA CATCGGCCC-3′, antisense 5′-AACTGAAGCCGGTTAG-3′. As an internal control, GAPDH mRNA was amplified. Relative copy number was calculated using the threshold crossing point (C_t) as calculated by the LightCycler software combined with the $\Delta\Delta$ C_t calculations.

Serum insulin, triglyceride, free fatty acid, glucagon, and GLP-1 measurements. Mice were not fed for 4 h, and blood samples were collected. The concentration of serum insulin was measured by a rat ultrasensitive insulin enzyme immunosorbent assay kit, which is cross-reactive with mouse insulin (Crystal Chem, Dowers Grove, IL). Serum triglycerides and free fatty acids (FFAs) were measured using a colorimetric kit (Sigma, St. Louis, MO) and FFA assay kit (Wako Chemicals, Neuss, Germany), respectively. Serum glucagon and GLP-1 levels were measured using a glucagon radioimmunoassay kit (Linco Research, St. Charles, MO) and GLP-1 radioimmunoassay kit (Phoenix Pharmaceuticals, Inc., Belmont, CA), respectively.

Insulin tolerance tests. Mice were injected with insulin (2 units/kg body wt i.p.; Eli Lilly, Indianapolis, IN), and blood glucose levels were measured at 0, 30, 60, and 90 min after insulin injection.

Measurement of the β-cell area and mass. Quantitative evaluation of the β-cell area was performed on insulin-stained sections using the UTHSCSA Image Tool program. More than 400 serial sections (5-μm thickness) were prepared from each mouse, and every 10th section was stained with anti-insulin antibody. The ratio of the β-cell area was calculated by dividing the area of all insulin-positive cells by the total area of the pancreas. The β-cell mass was calculated by multiplying the pancreas weight by the ratio of the β-cell area.

Extraction and measurement of pancreatic insulin. Insulin was extracted from the pancreas as previously described (12) and measured using an insulin enzyme immunosorbent assay kit (Crystal Chem).

Glucose transport assay. Epididymal adipose tissue was removed, and adipocytes were prepared as previously described (13). Adipocytes ($3–4 \times 10^5$/ml) were suspended in Krebs-Ringer HEPES buffer and treated with 10 nmol/l insulin for 30 min. After incubation with 2-deoxy-D-[1-^{3}H]glucose (0.5 μCi, 0.125 mmol/l; Amersham Pharmacia Biotech, Buckinghamshire, U.K.) for 3 min, glucose uptake was measured as previously described (14).

Immunoprecipitation. Mice were injected with insulin (2 units/kg, i.m.), and liver and muscle samples from the hind limb were harvested 15 min later. Tissue lysates (1.5 mg protein) were incubated overnight with anti-insulin receptor substrate (IRS)-1 (Upstate Biotech, Lake Placid, NY) at 4°C. Twenty microliters of agarose conjugate suspension (Santa Cruz Biotechnologies, Santa Cruz, CA) was added to the samples, and samples were incubated at 4°C on a rotating device for 3 h. After centrifugation, pellets were washed with radioimmunoprecipitation assay buffer (50 mmol/l Tris-HCl, pH 7.4; 1% NP-40; 0.25% Na-deoxycholate; 150 mmol/l NaCl; 1 mmol/l EDTA; 1 mmol/l PMSF; 1 μg/ml aprotinin, leupeptin, and pepstatin; 1 mmol/l Na_3VO_4; and 1 mmol/l NaF). Pellets were resuspended in 2× Laemmlie sample buffer and boiled for 5 min. These immunoprecipitated samples were used for SDS-PAGE and immunoblotting.

Western blot analysis. Immunoprecipated samples or whole lysates were prepared as described above and subjected to SDS-PAGE. Proteins were electrotransferred to nitrocellulose membranes. Membranes were incubated with anti–IRS-1, anti-phosphotyrosine (Upstate Biotech), anti-Akt, or anti–phospho-Akt (Ser 473) antibodies (Cell Signaling Technology, Danvers, MA) overnight at 4°C. After washing, membranes were incubated with a horseradish peroxidase–conjugated secondary antibody (goat anti-rabbit or goat anti-mouse IgG; Chemicon International, Temecula, CA) for 1 h at room temperature. Reactive bands were detected by enhanced chemiluminescence (Pierce Chemical, Rockford, IL) and quantified by scanning densitometry.

Protein kinase C activity assay. Protein kinase C (PKC) activity was measured using a PKC assay kit (Upstate Biotech). Lysates were incubated with the substrate (peptide sequence: QKRPSQRSKYL) and [γ-^{32}P]ATP, then phosphorylated peptide was separated using p81 phosphocellulose paper. Radioactivity on the disks was counted using a scintillation counter. Specific PKC activity was determined after subtraction of the activity without substrates.

Measurement of in vivo hepatic glucose production by clamp studies. Hepatic glucose production in vivo was determined as described previously (15). Briefly, mice were anesthetized, catheterized in the left jugular vein, and allowed 3–5 days of recovery. Mice were not fed for 18 h and infused with [3-^{3}H]glucose (0.05 μCi/min; DuPont NEN Research Products, Boston, MA). Blood samples (35 μl) were taken from the tip of the tail at 90 and 120 min after the initiation of glucose infusion. Plasma glucose concentration was determined by a glucose assay kit (BioVision Research Products, Mountain View, CA). To determine [3-^{3}H]glucose, 10 μl of plasma was mixed with an equal volume of 20% trichloroacetic acid to precipitate protein. The supernatant was heated at 65°C to dryness to evaporate [^{3}H]H_2O, and the samples were reconstituted in 100 μl H_2O. Radioactivity was measured using a scintillation counter. Mean steady-state hepatic glucose production was calculated by dividing the [3-^{3}H]glucose infusion rate by the mean plasma glucose specific activity at 90 and 120 min.

Statistical analyses. Data are presented as means ± SD. Statistical significance of differences was analyzed by unpaired Student's *t* test for comparison of two groups or ANOVA followed by Tukey honestly significant difference test for multiple comparisons. $P < 0.05$ was accepted as significant.

RESULTS

Remission of diabetes and control of body weight gain in rAd-GLP-1–treated diabetic *ob/ob* mice. To examine whether rAd-GLP-1 treatment efficiently produces GLP-1 in vivo, we injected rAd-GLP-1 (4×10^9 pfu) into diabetic *ob/ob* mice and measured serum GLP-1 levels after 4 h without food at 1, 2, and 4 weeks after rAd-GLP-1 treatment. Serum GLP-1 levels in rAd-GLP-1–treated mice were high at 1 week and gradually decreased at 2 and 4 weeks after treatment, whereas serum GLP-1 levels were very low in wild-type mice and rAd-βgal–treated diabetic mice (Fig. 1*A*). Blood glucose levels gradually decreased in rAd-GLP-1–treated diabetic *ob/ob* mice and reached normoglycemia within 4 days after treatment. Mice then became slightly hypoglycemic for a week and then returned to normoglycemia, which was maintained for 60 days when the experiment was terminated. In contrast, rAd-βgal–treated mice remained hyperglycemic, as did untreated diabetic *ob/ob* mice (Fig. 1*B*). Food intake in rAd-GLP-1–treated mice rapidly decreased over the first 4 days after treatment, continued to decrease over days 6–8, and then gradually increased to an amount similar to that ingested by rAd-βgal–treated and untreated control groups by 20 days after treatment (Fig. 1*C*). Changes in food intake appeared to be inversely correlated with the amount of circulating GLP-1. Gain of body weight was significantly lower in rAd-GLP-1–treated as compared with rAd-βgal–treated mice over the 8 weeks of the experiment (Fig. 1*D*).

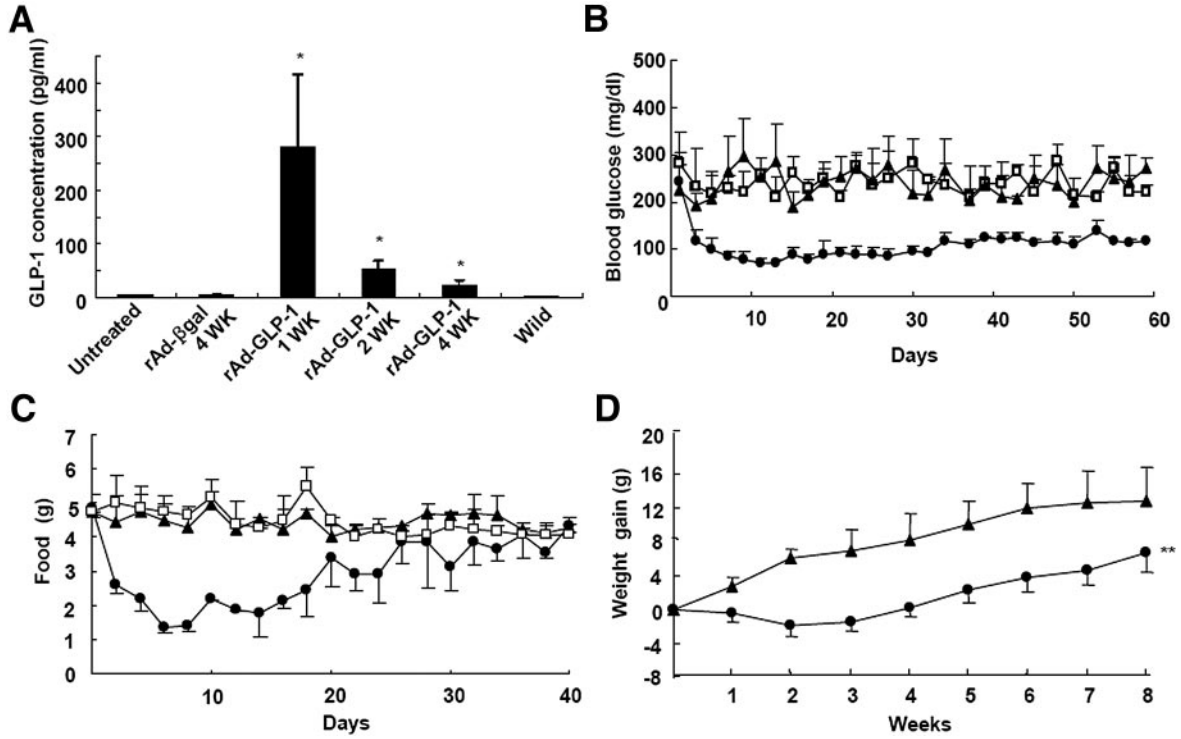

FIG. 1. Reduction in blood glucose and body weight in rAd-GLP-1–treated diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. *A*: Serum GLP-1 levels were measured after 4 h without food at the indicated times after treatment (n = 3–10 per group). Untreated diabetic *ob/ob* and wild-type, lean mice served as controls. Blood glucose concentrations (n = 9 per group) (*B*) and food intake (n = 7 per group) (*C*) were measured. □, untreated; ▲, rAd-βgal; ●, rAd-GLP-1. Untreated diabetic *ob/ob* mice served as a control (n = 6). *D*: Body weights were measured weekly in male mice (n = 5 per group). ▲, rAd-βgal; ●, rAd-GLP-1. Data are means ± SD. *P < 0.01; ** P < 0.001 compared with rAd-βgal–treated mice.

To determine whether rAd-GLP-1 treatment affects triglyceride and FFA production, we measured serum triglyceride and FFA levels after 4 h without food at 2 weeks after rAd-GLP-1 treatment. Both serum triglyceride (Fig. 2*A*) and FFA levels (Fig. 2*B*) were significantly decreased compared with rAd-βgal–treated mice. Serum triglyceride and FFA levels in the pair-fed group were not significantly different from those of untreated or rAd-βgal–treated mice.

Improvement of glucose tolerance and β-cell function in rAd-GLP-1–treated *ob/ob* mice. To determine whether blood glucose levels are properly controlled in rAd-GLP-1–treated *ob/ob* mice, we performed intraperitoneal glucose tolerance tests in normoglycemic *ob/ob* mice at 2 weeks after rAd-GLP-1 treatment. Blood glucose levels in rAd-GLP-1–treated mice were significantly lower at all time points following glucose injection compared with rAd-βgal–treated mice, and the kinetics of exogenous glucose clearance were similar to that in lean wild-type mice. In contrast, untreated and rAd-βgal–treated *ob/ob* mice did not reach normal glucose levels after glucose loading. Blood glucose levels in the pair-fed group were not significantly different at all time points compared with the untreated and rAd-βgal–treated groups, except at 60 min after glucose loading (Fig. 3).

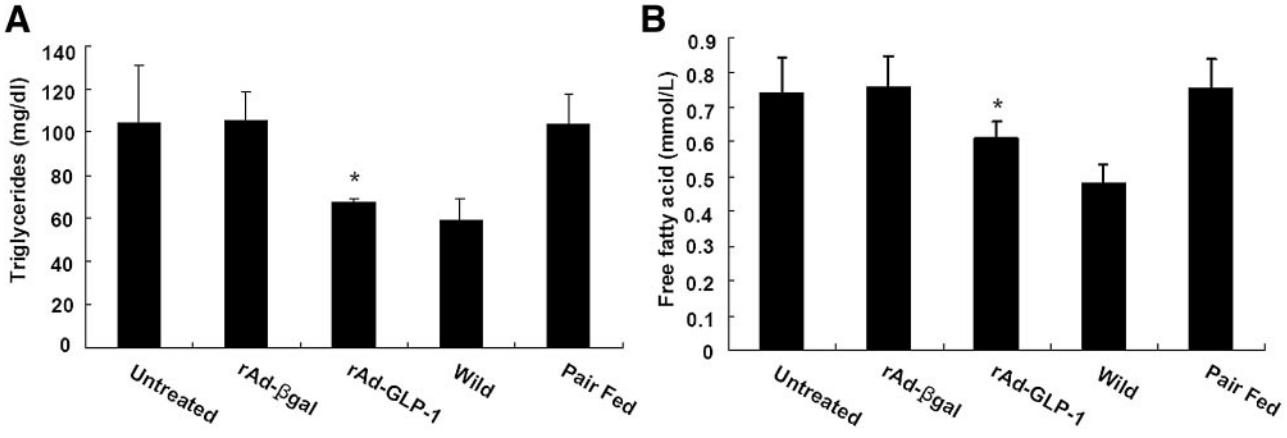

FIG. 2. Serum triglyceride and FFA levels in rAd-GLP-1–treated *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 (n = 3) or rAd-βgal (n = 4). Two weeks later, mice were not fed for 4 h. Serum triglyceride (*A*) and serum FFA (*B*) levels were measured. Untreated diabetic *ob/ob*, wild-type, lean, and pair-fed diabetic *ob/ob* mice (n = 3–5 per group) served as controls. Data are means ± SD. *P < 0.05 compared with rAd-βgal–treated mice.

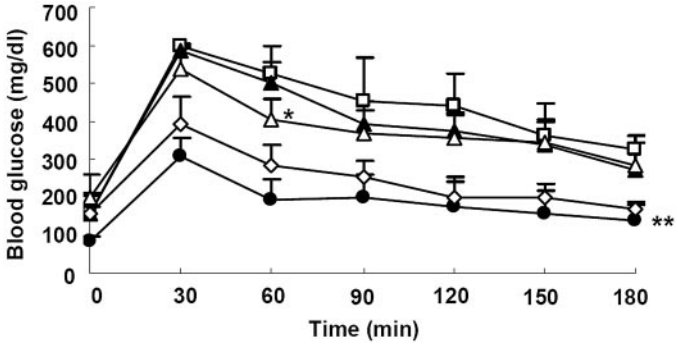

FIG. 3. Glucose tolerance tests in rAd-GLP-1–treated *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 (n = 6) or rAd-βgal (n = 5). Two weeks later, mice were not fed for 4 h and injected with glucose, and blood glucose levels were measured. Untreated diabetic *ob/ob* mice, wild-type lean mice, and pair-fed diabetic *ob/ob* mice (n = 3–8 per group) served as controls. Data are means ± SD. *P < 0.05; **P < 0.01 compared with rAd-βgal–treated mice. □, untreated; ▲, rAd-βgal; ●, rAd-GLP-1; ◇, wild type; △, pair fed.

As GLP-1 is known to have proliferative, differentiating, and antiapoptotic effects on β-cells (2), we measured β-cell mass. The β-cell mass was significantly increased in rAd-GLP-1–treated mice compared with rAd-βgal–treated mice (Fig. 4*A*). We then measured the pancreatic insulin content and insulin mRNA expression in islets and found that pancreatic insulin content was significantly decreased in rAd-GLP-1–treated mice (Fig. 4*B*), which is consistent with the decrease in insulin mRNA expression in pancreatic islets compared with rAd-βgal–treated mice (Fig. 4*C*). When we examined serum insulin levels before and at 30 min after glucose injection, we found that basal serum insulin levels were significantly reduced in rAd-GLP-1–treated *ob/ob* mice compared with rAd-βgal–treated mice. However, glucose-stimulated insulin secretion was increased over basal levels in rAd-GLP-1–treated mice, whereas there was no increase in rAd-βgal–treated mice (Fig. 4*D*). These results indicate that rAd-GLP-1 treatment improved β-cell function.

Improvement in insulin sensitivity in rAd-GLP-1–treated *ob/ob* mice. To address whether rAd-GLP-1 treatment improves insulin sensitivity, we performed insulin tolerance tests. rAd-GLP-1–treated mice showed an enhanced reduction in glucose levels in response to exogenous insulin at 30 min following insulin injection compared with untreated and rAd-βgal–treated *ob/ob* mice, and this reduction was comparable with lean wild-type mice. Glucose reduction in the pair-fed group was not different from untreated and rAd-βgal–treated mice (Fig. 5*A*). Because insulin induces glucose uptake in peripheral tissues, resulting in reduction of glucose levels, we measured the insulin-stimulated glucose transport in adipocytes of rAd-GLP-1–treated *ob/ob* mice. Glucose transport was significantly lower in untreated and rAd-βgal–treated diabetic *ob/ob* mice, whereas rAd-GLP-1 treatment restored glucose transport to the levels seen in lean wild-type mice (Fig. 5*B*). These results indicate that improved insulin sensitivity contributes to increased glucose uptake, resulting in improved glucose homeostasis.

Improvement of insulin signaling in rAd-GLP-1–treated *ob/ob mice*. To determine whether the improvement of insulin sensitivity in rAd-GLP-1–treated *ob/ob* mice

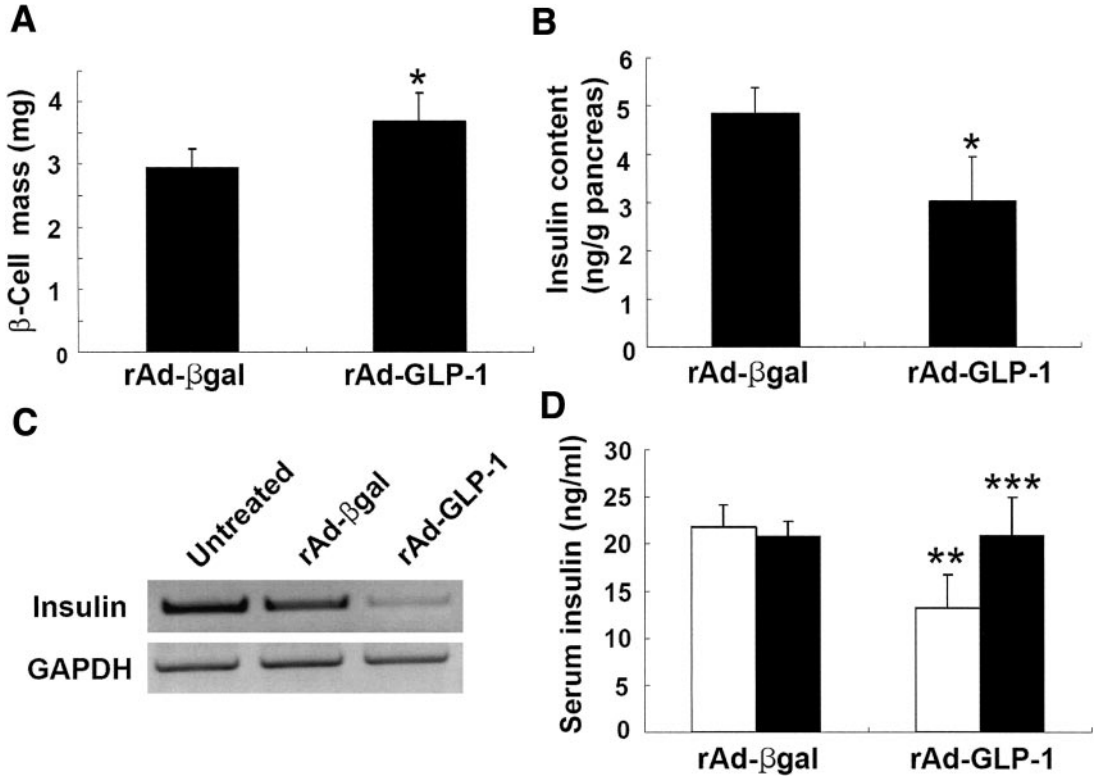

FIG. 4. rAd-GLP-1 treatment improved β-cell function in diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. Two weeks later, β-cell mass (*A*) and insulin content (n = 4–6 per group) (*B*) were measured. *C*: Mice were not fed for 4 h, and the expression of insulin mRNA in islets was determined by RT-PCR. Untreated diabetic *ob/ob* mice served as controls. Expression of GAPDH served as an internal control. A gel picture representative of three different experiments is shown. *D*: Serum insulin levels were measured at 0 and 30 min after glucose injection (2 g/kg body wt i.p.) by enzyme immunosorbent assay (n = 3–6 per group). □, 0 min; ■, 30 min. Data are means ± SD. *P < 0.05; ** P < 0.01 compared with rAd-βgal–treated mice. ***P < 0.01 compared with insulin secretion at 0 min.

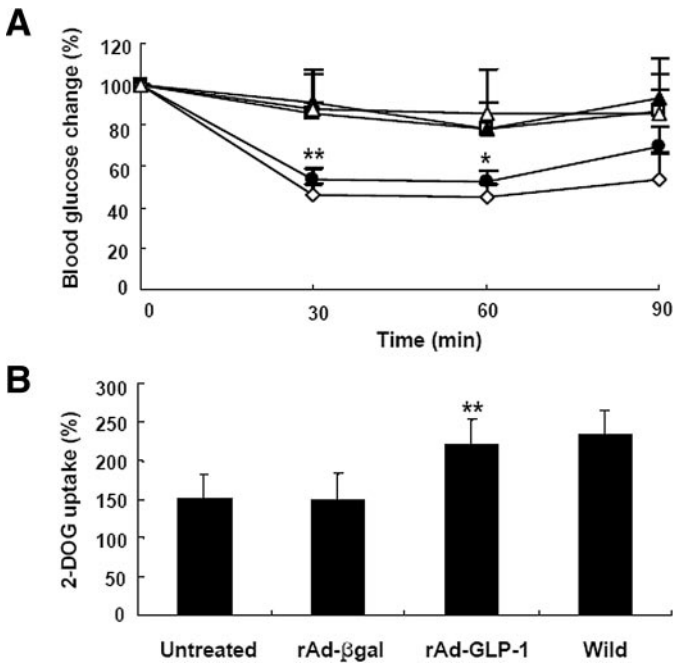

FIG. 5. rAd-GLP-1 treatment improved insulin sensitivity in diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. *A*: Two weeks later, insulin (2 units/kg body wt) was injected and blood glucose levels were measured (n = 4–7 per group). Data are expressed as a percentage of the initial blood glucose level before insulin injection. □, untreated; ▲, rAd-βgal; △, pair fed; ●, rAd-GLP-1; ◇, wild type. *B*: Four weeks later, adipocytes were removed and incubated in the presence of insulin and the uptake of 2-deoxy-D-[1-^{3}H]glucose was measured. Data are expressed as the percentage change from 2-deoxy-D-[1-^{3}H]glucose uptake in the absence of insulin (n = 5–9 mice per group). Untreated diabetic *ob/ob* mice, wild-type lean mice, and pair-fed diabetic *ob/ob* mice served as controls. Data are means ± SD. *P < 0.05; ** P < 0.01 compared with rAd-βgal–treated mice.

is accompanied by restoration of insulin signal transduction, we first examined the levels of total and phosphorylated IRS-1 protein in muscle of rAd-GLP-1–treated mice. The total amount of IRS-1 protein was not changed, but insulin-stimulated phosphorylation of IRS-1 was upregulated in the muscle of rAd-GLP-1–treated mice compared with untreated and rAd-βgal–treated mice (Fig. 6*A*). We then examined the activation of downstream molecules of insulin signaling in the muscle of rAd-GLP-1–treated *ob/ob* mice. Insulin-stimulated phosphorylation of Akt was significantly increased in rAd-GLP-1–treated mice compared with untreated and rAd-βgal–treated *ob/ob* mice, whereas the total Akt and basal level of phosphorylated Akt were not altered (Fig. 6*B*). Insulin-stimulated PKC activity was also increased in the muscle of rAd-GLP-1–treated *ob/ob* mice compared with untreated and rAd-βgal–treated mice (Fig. 6*C*).

We then examined the insulin-stimulated signaling molecules in the liver of rAd-GLP-1–treated *ob/ob* mice. The basal level of IRS-1 protein was decreased in diabetic *ob/ob* mice, and rAd-GLP-1 treatment significantly increased this level comparable with that of wild-type lean mice. In addition, insulin-stimulated phosphorylation of IRS-1 was significantly increased in rAd-GLP-1–treated mice compared with untreated and rAd-βgal–treated mice (Fig. 7*A*). However, rAd-GLP-1 treatment did not affect either insulin-stimulated activation of Akt or total Akt protein levels in the liver (Fig. 7*B*). PKC activity was significantly increased after stimulation with insulin, but the basal level of PKC activity was unaffected compared with untreated and rAd-βgal–treated *ob/ob* mice (Fig. 7*C*).

Decreased hepatic glucose production and expression of PEPCK, G6Pase, and FAS mRNA in rAd-GLP-1–treated *ob/ob* mice. To determine whether rAd-GLP-1 treatment affects glucose production, we measured hepatic glucose production by clamp studies. rAd-GLP-1 treatment significantly decreased basal hepatic glucose production compared with rAd-βgal–treated *ob/ob* mice (Fig. 8*A*). Glucagon increases gluconeogenesis in the liver (16), and GLP-1 is known to downregulate glucagon secretion (17). Thus, we examined whether decreased hepatic glucose production is due to the reduction of glucagon. We found that 4-h fasting serum glucagon levels at 2 weeks after treatment were not significantly different between rAd-GLP-1– and rAd-βgal–treated groups (Fig. 8*B*).

To determine whether rAd-GLP-1 treatment affects expression of genes involved in glucose metabolism in the liver, we examined the expression of G6Pase and PEPCK mRNA, which are involved in gluconeogenesis. The expression of both G6Pase and PEPCK mRNA was significantly decreased in the liver of rAd-GLP-1–treated *ob/ob* mice compared with that in untreated and rAd-βgal–treated diabetic *ob/ob* mice (Fig. 8*C* and *D*). Because we found that serum triglyceride levels were reduced by rAd-GLP-1 treatment, we examined the expression of FAS mRNA, which is involved in lipogenesis, and found that the expression of FAS mRNA was also significantly decreased in the liver after rAd-GLP-1 treatment (Fig. 8*E*).

DISCUSSION

GLP-1 has been studied as a potential therapy for type 2 diabetes; however, its short duration of action, owing to its short half-life, limits the maintenance of therapeutic levels by administration of GLP-1 peptide (1,2). Various methods have been tried to overcome this problem, including development of a long-acting analog of GLP-1 and an inhibitor of the GLP-1 degrading enzyme (5). Clinical trials using Exenatide, a long-acting, synthetic version of exendin-4, found that it improved glycemic control by stimulating glucose-dependent insulin secretion, suppressing glucagon secretion, slowing gastric emptying, and enhancing β-cell function; however, twice daily injections were required (18). A plasmid construct containing a modified cDNA of GLP-1 (7–37) injected into Zucker diabetic fatty rats lowered blood glucose levels, but not to the normal range (19), probably due to the low expression level of GLP-1.

In this study, we sought to deliver sustained, therapeutic levels of GLP-1 by injecting diabetic *ob/ob* mice with a recombinant adenovirus expressing GLP-1 under the control of the cytomegalovirus promoter (rAd-GLP-1). Circulating GLP-1 was significantly increased for at least 4 weeks compared with rAd-βgal–treated diabetic and untreated normal mice, indicating that a substantial amount of circulating GLP-1 is exogenously produced by rAd-GLP-1 therapy. A recent report showed that intramuscular injection of a plasmid expressing a GLP-1/IgG-Fc fusion construct significantly lowered fasting blood glucose levels in *db/db* mice but not until 3 months after injection (20). In our study, diabetic *ob/ob* mice given a single injection of rAd-GLP-1 showed lowered blood glucose levels within 4 days, and normoglycemia was maintained. The rapid remission of diabetes in our study relative to the recent report is probably because we used *ob/ob* mice, which are less severely hyperglycemic than *db/db* mice,

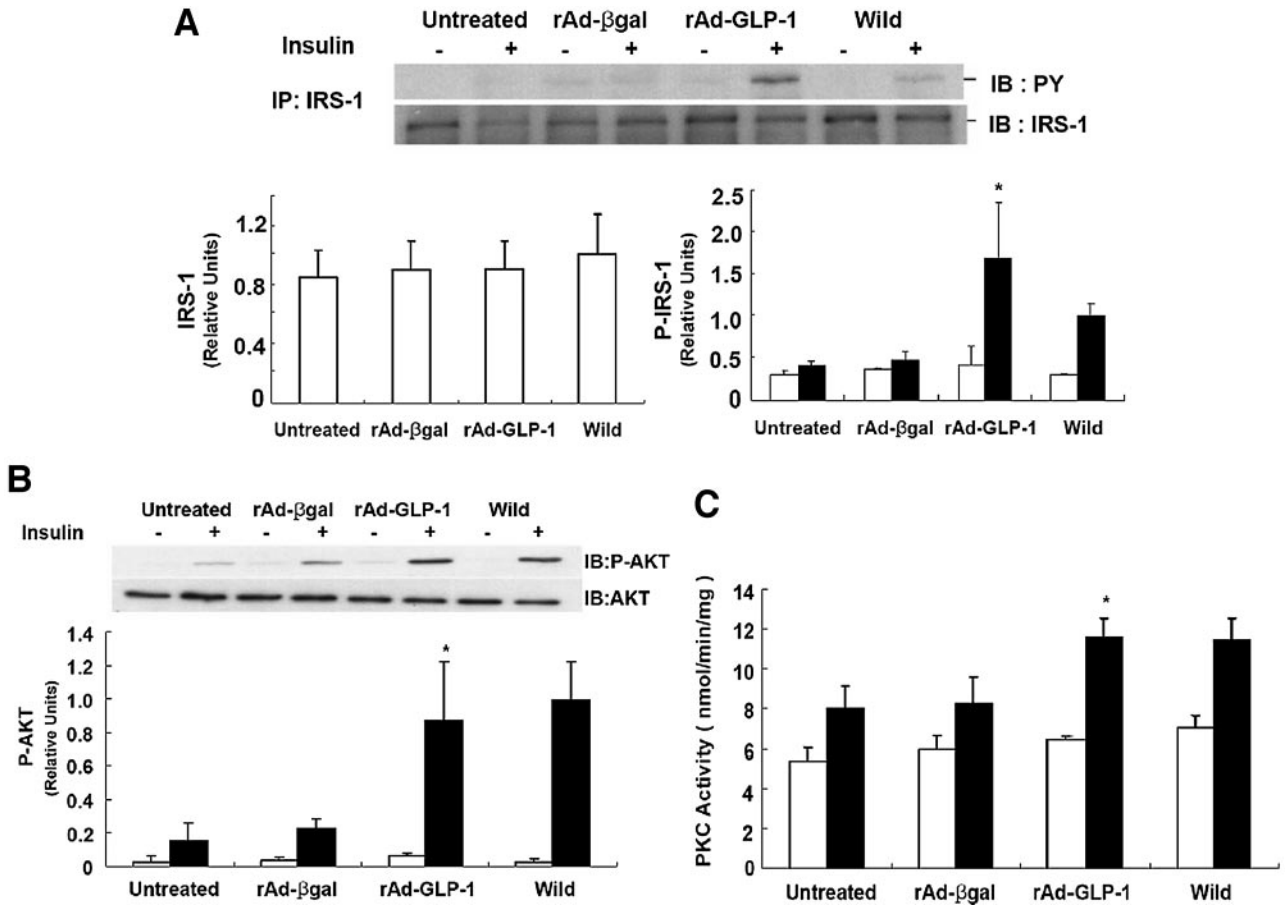

FIG. 6. Expression and phosphorylation of IRS-1 and activity of Akt and PKC in the muscle of rAd-GLP-1–treated diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. Four weeks later, mice were treated without (−) or with (+) insulin, and muscle tissue was sampled 15 min later. *A*: Tissue lysates were immunoprecipitated (IP) with anti–IRS-1 antibody and immunoblotted (IB) with anti–IRS-1 (IRS-1) or anti-phophotyrosine (PY) antibody. Representative immunoblots are shown (*upper panel*). IRS-1 protein in mice without insulin treatment is presented as a ratio of IRS-1 in wild-type lean mice (*lower left panel*). Phosphorylated IRS-1 was normalized with total IRS-1 protein and presented as a ratio of the phosphorylated IRS-1 level in insulin-stimulated wild-type mice (*lower right panel*). *B*: Tissue extracts were immunoblotted (IB) with anti-Akt (AKT) or anti–phospho-Akt (P-AKT) antibody. Representative immunoblots are shown (*upper panel*). Phosphorylated Akt was normalized with total Akt protein and presented as a ratio of the phosphorylated Akt level in insulin-stimulated wild-type mice (*lower panel*). *C*: PKC activity was determined. Untreated diabetic *ob/ob* mice and wild-type lean mice served as controls. Data are means ± SD. n = 3–7 per group. *$P < 0.05$ compared with rAd-βgal–treated mice. □, basal; ■, insulin.

and an adenoviral vector, which permits high transgene expression due to high transduction efficiency.

Previous reports showed that GLP-1 or exendin-4 treatment increased β-cell mass (21,22). Consistent with this, our rAd-GLP-1 therapy also increased β-cell mass in *ob/ob* mice. Despite this increase in β-cell mass, we found that pancreatic insulin content was significantly decreased in rAd-GLP-1–treated *ob/ob* mice compared with rAd-βgal–treated mice, which is supported by decreased insulin mRNA expression in the islets and decreased fasting serum insulin levels in rAd-GLP-1–treated mice. Similarly, treatment with exendin-4 or GLP-1 reduced fasting serum insulin levels in *ob/ob* and *db/db* mice (4,21). Our interpretation is that rAd-GLP-1 increases β-cell mass due to its proliferative and antiapoptotic effects on β-cells, but basal insulin content is reduced, probably due to the improvement of insulin sensitivity. Also, the increase in insulin secretion after glucose loading suggests that β-cell function is also improved by rAd-GLP-1 treatment.

It has been reported that long-term treatment with GLP-1 or its analog improved insulin sensitivity in both animal models and human type 2 diabetic patients (4,6,23). Improvement of insulin sensitivity by exendin-4 treatment was found to be independent of body weight (23). However, the precise mechanisms are not fully understood. Insulin tolerance tests showed that exogenous insulin appropriately cleared blood glucose in rAd-GLP-1–treated *ob/ob* mice. In addition, hepatic glucose production and serum FFA and triglyceride levels were significantly decreased compared with rAd-βgal–treated mice. There was no significant difference in insulin tolerance and serum FFA and triglyceride levels between the pair-fed group and the untreated or rAd-βgal–treated groups, suggesting that these effects in rAd-GLP-1–treated mice are not likely to be the result of reduced food intake and body weight. Although all these results showed that rAd-GLP-1 treatment clearly improved insulin sensitivity, hyperinsulinemic-euglycemic clamp studies will be required to directly quantify the improvement of insulin sensitivity.

Impaired glucose transport is one of the major factors contributing to insulin resistance, and insulin-mediated glucose disposal is greatly reduced in type 2 diabetes (24). rAd-GLP-1 treatment significantly improved insulin-stimulated glucose uptake in adipocytes compared with rAd-βgal–treated adipocytes. Insulin stimulates glucose transport through activation of the insulin signaling pathway. The binding of insulin to its receptor results in the activation of the receptor tyrosine kinase, which subse-

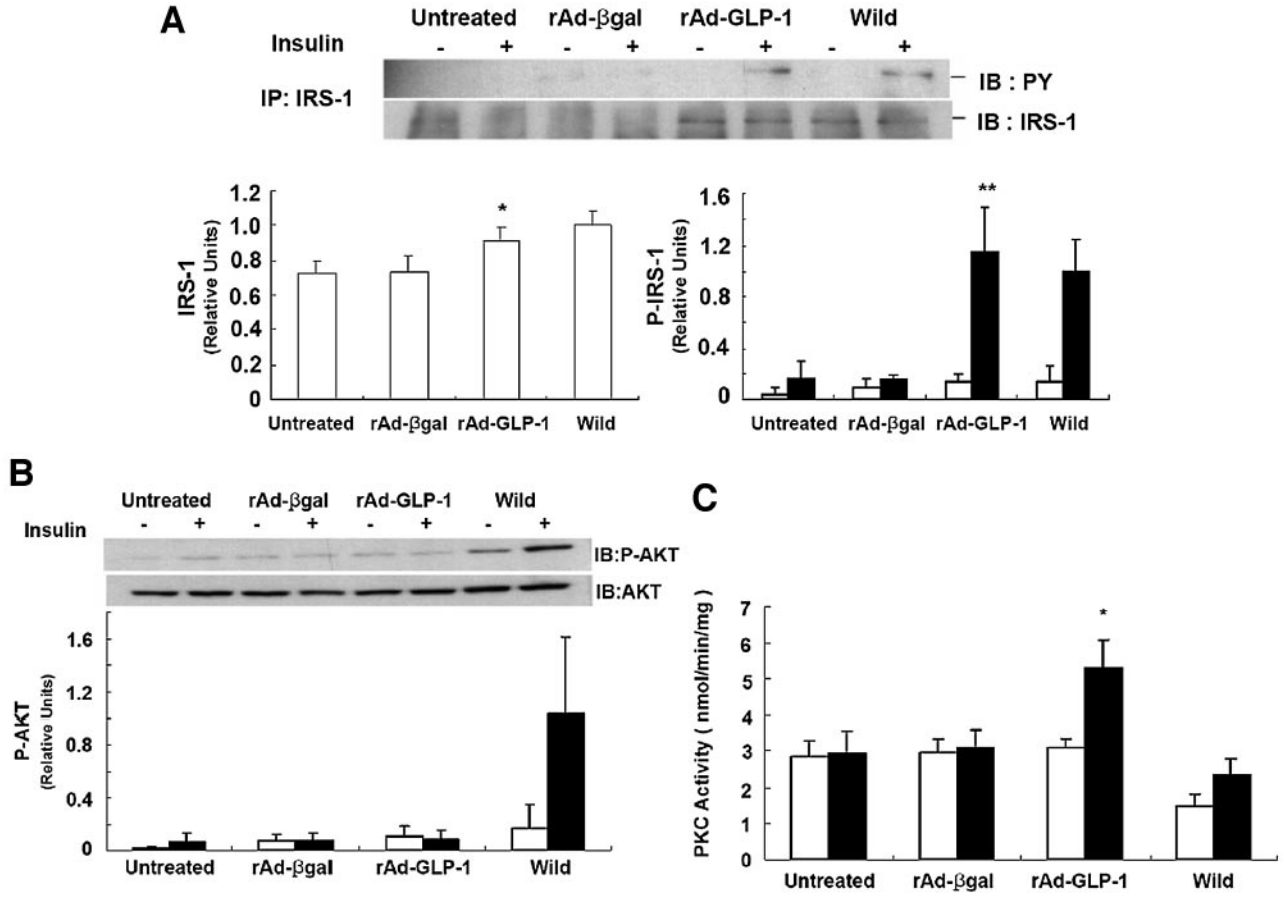

FIG. 7. Expression and phosphorylation of IRS-1 and activity of Akt and PKC in the liver of rAd-GLP-1–treated diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. Four weeks later, mice were treated without or with insulin and liver tissue was sampled 15 min later. *A–C*: As for Fig. 6. Data are means ± SD. $n = 3–7$ per group. *$P < 0.05$; **$P < 0.01$ compared with rAd-βgal–treated mice. □, basal; ■, insulin.

quently phosphorylates IRS-1/2 (25). The phosphorylated IRS-1/2 activates phosphoinositol-3-kinase, and activation of the phosphoinositol-3-kinase signaling pathway activated downstream protein kinases such as protien kinase B (PKB)/Akt and PKC (26,27). Treatment of diabetic *ob/ob* mice with rAd-GLP-1 increased insulin-stimulated tyrosine phosphorylation of IRS-1 in both muscle and liver. PKB/Akt is required for insulin-stimulated glucose transport and glycogen synthesis in muscle and adipocytes (28–30) and is also required for glycogen synthesis and inhibition of gluconeogenesis in the liver (31–33). It is known that *ob/ob* mice have defects in the activation of PKB/Akt in both liver and muscle (34); thus, we examined whether rAd-GLP-1 treatment would reverse this defect. There was a marked increase in the level of insulin-stimulated Akt-Ser 473 phosphorylation in the muscle but not in the liver. This difference might be due to the differential regulation of signaling molecules induced by insulin in different tissues in rAd-GLP-1–treated *ob/ob* mice. PKCs are also involved in insulin-stimulated glucose transport in muscle and adipocytes (35), and atypical PKC activation is impaired in the muscle of *ob/ob* mice (36). We found that insulin-induced PKC activity significantly increased in both muscle and liver after rAd-GLP-1 treatment. Taken together, these results indicate that rAd-GLP-1 therapy restored insulin signaling by increasing the activation of IRS-1, PKB/Akt, and PKC, subsequently improving glucose transport in peripheral tissues such as adipose tissue.

Gluconeogenesis plays an important role in glucose homeostasis (37). Elevated hepatic glucose production, due to increased gluconeogenesis, glycogenolysis, or both, is associated with the pathogenesis of type 2 diabetes (38,39). We found that basal hepatic glucose production was significantly decreased by rAd-GLP-1 treatment. The expression of G6Pase, which is involved in gluconeogenesis and glycogenolysis (40), and PEPCK, which is involved in gluconeogenesis (41), was significantly decreased in the liver of rAd-GLP-1–treated *ob/ob* mice, suggesting that the blood glucose–lowering effect of rAd-GLP-1 might be due in part to the reduction of hepatic glucose output. As elevated glucagon levels are correlated with increased hepatic glucose production (42), we examined whether rAd-GLP-1 treatment reduced glucagon secretion. However, we found no significant changes in fasting serum glucagon levels between rAd-GLP-1– and rAd-βgal–treated mice, suggesting that the decrease of hepatic gluconeogenesis may not be a result of reduced glucagon levels. The effect of GLP-1 on inhibition of glucagon secretion is glucose dependent (17). As blood glucose levels are already normalized at 2 weeks after treatment, GLP-1 may not affect fasting glucagon levels at this time. This result is consistent with previous findings, which showed that continuous infusion of GLP-1 or treatment with GLP-1 analog had no effect on fasting glucagon levels (6,43,44). An increase in hepatic triglyceride synthesis and secretion is observed in type 2 diabetes (45). FAS plays a central role in de novo lipogenesis (46,47). In rAd-GLP-1–treated mice, FAS mRNA expression in liver

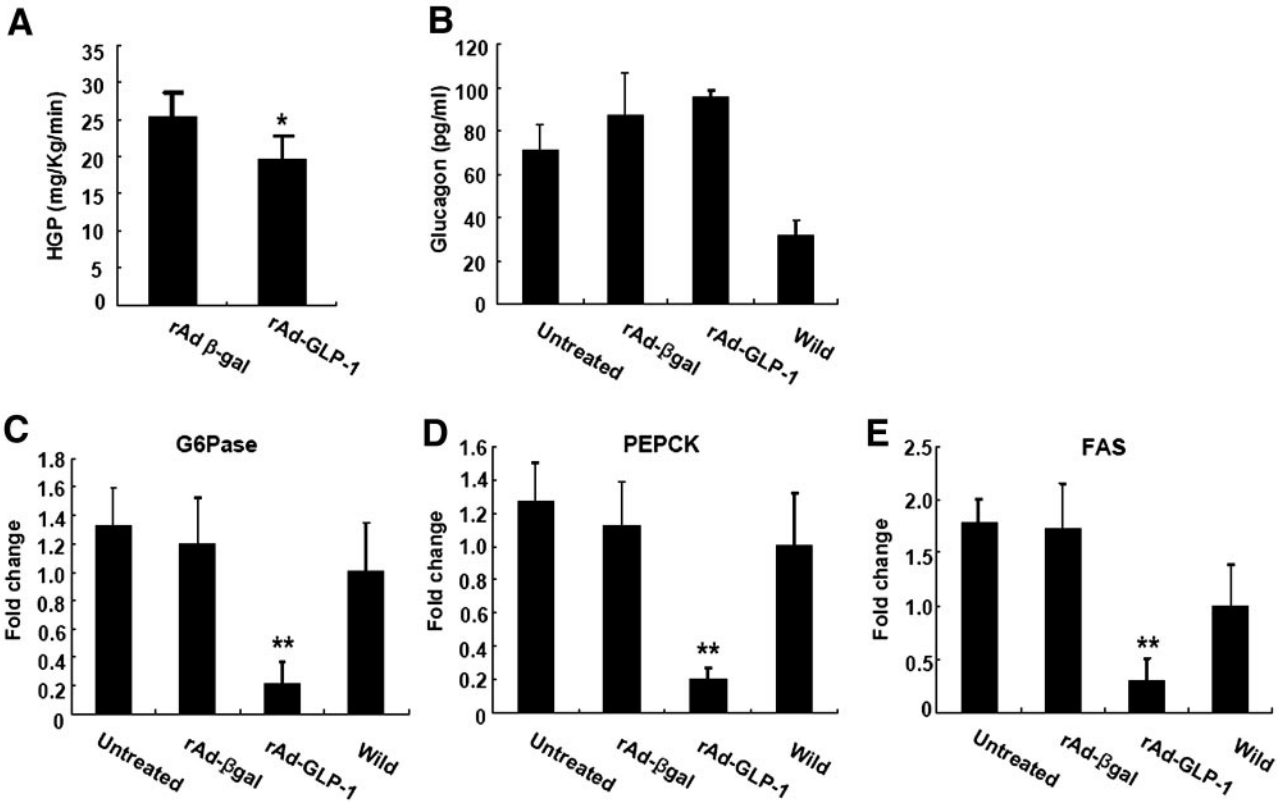

FIG. 8. Hepatic glucose production and expression of G6Pase, PEPCK, and FAS mRNA in the liver of rAd-GLP-1–treated diabetic *ob/ob* mice. Diabetic *ob/ob* mice were treated with rAd-GLP-1 or rAd-βgal. *B*: Two weeks later, basal hepatic glucose production (HGP) was measured (n = 4–5 per group). Mice were not fed for 4 h. Serum glucagon levels were measured (n = 3–7 per group) and liver tissue was removed and the expression of G6Pase (*C*), PEPCK (*D*), and FAS (*E*) mRNA was analyzed by real-time quantitative PCR and normalized by GAPDH expression. The fold change was calculated as a ratio of the expression level in wild-type lean mice. Untreated diabetic *ob/ob* mice and wild-type lean mice were used as controls (n = 3–8 per group). Data are means ± SD. *P < 0.05; **P < 0.01 compared with rAd-βgal–treated mice.

and serum triglycerides were decreased, suggesting that GLP-1 reduces lipid production. Consistent with this result, it was reported that treatment of *ob/ob* mice with exendin-4, an analog of GLP-1, reversed hepatic steatosis (4).

The presence of GLP-1 receptors in peripheral tissues such as muscle, fat, and liver is controversial (4,48,49); therefore, it is unclear whether the improvement in insulin sensitivity in extrapancreatic tissues by rAd-GLP-1 treatment is due to direct effects on these tissues or indirect effects through the regulation of other molecules. Regardless, it is clear that a single administration of rAd-GLP-1 resulted in long-term remission of diabetes in *ob/ob* mice. The effect of rAd-GLP-1 treatment on the long-term remission of diabetes might result from the improvement of β-cell function, reduction of gluconeogenesis, and the improvement of insulin sensitivity.

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REFERENCES

1. Kieffer TJ, Habener JF: The glucagon-like peptides. *Endocr Rev* 20:876–913, 1999
2. Drucker DJ: The biology of incretin hormones. *Cell Metab* 3:153–165, 2006
3. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ: Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 86:3717–3723, 2001
4. Ding X, Saxena NK, Lin S, Gupta N, Anania FA: Exendin-4, a glucagon-like protein-1 (GLP-1) receptor agonist, reverses hepatic steatosis in ob/ob mice. *Hepatology* 43:173–181, 2006
5. Arulmozhi DK, Portha B: GLP-1 based therapy for type 2 diabetes. *Eur J Pharmacol Sci* 28:96–108, 2006
6. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
7. Mentlein R, Gallwitz B, Schmidt WE: Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 214:829–835, 1993
8. Kieffer TJ, McIntosh CH, Pederson RA: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136:3585–3596, 1995
9. Kendall DM, Kim D, Maggs D: Incretin mimetics and dipeptidyl peptidase-IV inhibitors: a review of emerging therapies for type 2 diabetes. *Diabetes Technol Ther* 8:385–396, 2006
10. Liu MJ, Shin S, Li N, Shigihara T, Lee YS, Yoon JW, Jun HS: Prolonged remission of diabetes by regeneration of beta cells in diabetic mice treated with recombinant adenoviral vector expressing glucagon-like peptide-1. *Mol Ther* 15:86–93, 2007
11. Jun HS, Yoon CS, Zbytnuik L, van Rooijen N, Yoon JW: The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 189:347–358, 1999
12. Yoon JW, Lesniak MA, Fussganger R, Notkins AL: Genetic differences in susceptibility of pancreatic beta cells to virus-induced diabetes mellitus. *Nature* 264:178–180, 1976
13. Olefsky JM: Effect of dexamethasone on insulin binding, glucose transport,

and glucose oxidation of isolated rat adipocytes. *J Clin Invest* 56:1499–1508, 1975
14. Kim W, Khil LY, Clark R, Bok SH, Kim EE, Lee S, Jun HS, Yoon JW: Naphthalenemethyl ester derivative of dihydroxyhydrocinnamic acid, a component of cinnamon, increases glucose disposal by enhancing translocation of glucose transporter 4. *Diabetologia* 49:2437–2448, 2006
15. Liu S, Croniger C, Arizmendi C, Harada-Shiba M, Ren J, Poli V, Hanson RW, Friedman JE: Hypoglycemia and impaired hepatic glucose production in mice with a deletion of the C/EBPbeta gene. *J Clin Invest* 103:207–213, 1999
16. Johnson ME, Das NM, Butcher FR, Fain JN: The regulation of gluconeogenesis in isolated rat liver cells by glucagon, insulin, dibutyryl cyclic adenosine monophosphate, and fatty acids. *J Biol Chem* 247:3229–3235, 1972
17. Dunning BE, Foley JE, Ahren B: Alpha cell function in health and disease: influence of glucagon-like peptide-1. *Diabetologia* 48:1700–1713, 2005
18. Barnett AH: Exenatide. *Drugs Today (Barc)* 41:563–578, 2005
19. Oh S, Lee M, Ko KS, Choi S, Kim SW: GLP-1 gene delivery for the treatment of type 2 diabetes. *Mol Ther* 7:478–483, 2003
20. Kumar M, Hunag Y, Glinka Y, Prud'homme GJ, Wang Q: Gene therapy of diabetes using a novel GLP-1/IgG1-Fc fusion construct normalizes glucose levels in db/db mice. *Gene Ther* 14:162–172, 2007
21. Wang Q, Brubaker PL: Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. *Diabetologia* 45:1263–1273, 2002
22. Parsons GB, Souza DW, Wu H, Yu D, Wadsworth SG, Gregory RJ, Armentano D: Ectopic expression of glucagon-like peptide 1 for gene therapy of type II diabetes. *Gene Ther* 14:38–48, 2007
23. Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, Baron AD, Parkes DG, Young AA: Exenatide (exendin-4) improves insulin sensitivity and beta-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. *Endocrinology* 146:2069–2076, 2005
24. Reaven GM: Insulin resistance in noninsulin-dependent diabetes mellitus: does it exist and can it be measured? *Am J Med* 74:3–17, 1983
25. Lane MD, Flores-Riveros JR, Hresko RC, Kaestner KH, Liao K, Janicot M, Hoffman RD, McLenithan JC, Kastelic T, Christy RJ: Insulin-receptor tyrosine kinase and glucose transport. *Diabetes Care* 13:565–575, 1990
26. Whitehead JP, Clark SF, Urso B, James DE: Signalling through the insulin receptor. *Curr Opin Cell Biol* 12:222–228, 2000
27. Shepherd PR, Withers DJ, Siddle K: Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *Biochem J* 333:471–490, 1998
28. Kohn AD, Summers SA, Birnbaum MJ, Roth RA: Expression of a constitutively active Akt Ser/Thr kinase in 3T3–L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. *J Biol Chem* 271:31372–31378, 1996
29. Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, Klip A: Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol* 19:4008–4018, 1999
30. Bae SS, Cho H, Mu J, Birnbaum MJ: Isoform-specific regulation of insulin-dependent glucose uptake by Akt/protein kinase B. *J Biol Chem* 278:49530–49536, 2003
31. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA: Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378:785–789, 1995
32. Czech MP: Insulin's expanding control of forkheads. *Proc Natl Acad Sci U S A* 100:11198–11200, 2003
33. van Weeren PC, de Bruyn KM, de Vries-Smits AM, van Lint J, Burgering BM: Essential role for protein kinase B (PKB) in insulin-induced glycogen synthase kinase 3 inactivation: characterization of dominant-negative mutant of PKB. *J Biol Chem* 273:13150–13156, 1998
34. Standaert ML, Sajan MP, Miura A, Kanoh Y, Chen HC, Farese RV Jr, Farese RV: Insulin-induced activation of atypical protein kinase C, but not protein kinase B, is maintained in diabetic (ob/ob and Goto-Kakazaki) liver: contrasting insulin signaling patterns in liver versus muscle define phenotypes of type 2 diabetic and high fat-induced insulin-resistant states. *J Biol Chem* 279:24929–24934, 2004
35. Bandyopadhyay G, Standaert ML, Zhao L, Yu B, Avignon A, Galloway L, Karnam P, Moscat J, Farese RV: Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells: transfection studies suggest a role for PKC-zeta in glucose transport. *J Biol Chem* 272:2551–2558, 1997
36. Farese RV, Sajan MP, Standaert ML: Insulin-sensitive protein kinases (atypical protein kinase C and protein kinase B/Akt): actions and defects in obesity and type II diabetes. *Exp Biol Med (Maywood)* 230:593–605, 2005
37. Nordlie RC, Foster JD, Lange AJ: Regulation of glucose production by the liver. *Annu Rev Nutr* 19:379–406, 1999
38. Radziuk J, Pye S: Production and metabolic clearance of glucose under basal conditions in type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 44:983–991, 2001
39. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI: Increased rate of gluconeogenesis in type II diabetes mellitus: a 13C nuclear magnetic resonance study. *J Clin Invest* 90:1323–1327, 1992
40. Seoane J, Trinh K, O'Doherty RM, Gomez-Foix AM, Lange AJ, Newgard CB, Guinovart JJ: Metabolic impact of adenovirus-mediated overexpression of the glucose-6-phosphatase catalytic subunit in hepatocytes. *J Biol Chem* 272:26972–26977, 1997
41. Hanson RW, Reshef L: Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annu Rev Biochem* 66:581–611, 1997
42. Gastaldelli A, Baldi S, Pettiti M, Toschi E, Camastra S, Natali A, Landau BR, Ferrannini E: Influence of obesity and type 2 diabetes on gluconeogenesis and glucose output in humans: a quantitative study. *Diabetes* 49:1367–1373, 2000
43. Meneilly GS, Greig N, Tildesley H, Habener JF, Egan JM, Elahi D: Effects of 3 months of continuous subcutaneous administration of glucagon-like peptide 1 in elderly patients with type 2 diabetes. *Diabetes Care* 26:2835–2841, 2003
44. Degn KB, Juhl CB, Sturis J, Jakobsen G, Brock B, Chandramouli V, Rungby J, Landau BR, Schmitz O: One week's treatment with the long-acting glucagon-like peptide 1 derivative liraglutide (NN2211) markedly improves 24-h glycemia and α- and β-cell function and reduces endogenous glucose release in patients with type 2 diabetes. *Diabetes* 53:1187–1194, 2004
45. Memon RA, Grunfeld C, Moser AH, Feingold KR: Fatty acid synthesis in obese insulin resistant diabetic mice. *Horm Metab Res* 26:85–87, 1994
46. Griffin MJ, Sul HS: Insulin regulation of fatty acid synthase gene transcription: roles of USF and SREBP-1c. *IUBMB Life* 56:595–600, 2004
47. Lavau M, Bazin R, Karaoghlanian Z, Guichard C: Evidence for a high fatty acid synthesis activity in interscapular brown adipose tissue of genetically obese Zucker rats. *Biochem J* 204:503–507, 1982
48. Marquez L, Gonzalez N, Puente J, Valverde L, Villanueva-Penacarrillo ML: GLP-1 effect upon the GPI/IPG system in adipocytes and hepatocytes from diabetic rats. *Diabetes Nutr Metab* 14:239–244, 2001
49. Sandhu H, Wiesenthal SR, MacDonald PE, McCall RH, Tchipashvili V, Rashid S, Satkunarajah M, Irwin DM, Shi ZQ, Brubaker PL, Wheeler MB, Vranic M, Efendic S, Giacca A: Glucagon-like peptide 1 increases insulin sensitivity in depancreatized dogs. *Diabetes* 48:1045–1053, 1999