Original Article

Blockade of Pancreatic Islet–Derived Ghrelin Enhances Insulin Secretion to Prevent High-Fat Diet–Induced Glucose Intolerance

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The gastric hormone ghrelin and its receptor, growth hormone secretagogue receptor (GHSR), are expressed in pancreas. Here, we report that ghrelin is released from pancreatic islets to regulate glucose-induced insulin release. Plasma concentrations of ghrelin, as well as insulin, were higher in pancreatic veins than in arteries. GHSR antagonist and immunoneutralization of endogenous ghrelin enhanced glucose-induced insulin release from perfused pancreas, whereas exogenous ghrelin suppressed it. GHSR antagonist increased plasma insulin levels in gastrectomized and normal rats to a similar extent. Ghrelin knockout mice displayed enhanced glucose-induced insulin release from isolated islets, whereas islet density, size, insulin content, and insulin mRNA levels were unaltered. Glucose tolerance tests (GTTs) in ghrelin knockout mice showed increased insulin and decreased glucose responses. Treatment with high-fat diet produced glucose intolerance in GTTs in wild-type mice. In ghrelin knockout mice, the high-fat diet–induced glucose intolerance was largely prevented, whereas insulin responses to GTTs were markedly enhanced. These findings demonstrate that ghrelin originating from pancreatic islets is a physiological regulator of glucose-induced insulin release. Antagonism of the ghrelin function can enhance insulin release to meet increased demand for insulin in high-fat diet–induced obesity and thereby normalize glycemic control, which may provide a potential therapeutic application to counteract the progression of type 2 diabetes. *Diabetes* 55:3486–3493, 2006

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ELISA, enzyme-linked immunosorbent assay; GHRP, growth hormone releasing peptide; GHSR, growth hormone secretagogue receptor; GTT, glucose tolerance test; HKRB, HEPES-added Krebs-Ringer bicarbonate buffer; ITT, insulin tolerance test.

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Ghrelin, an acylated 28–amino acid peptide, was isolated from the stomach as the endogenous ligand (1) for the growth hormone secretagogue receptor (GHSR) (2). Circulating ghrelin is produced predominantly in the stomach (3). Ghrelin potently stimulates growth hormone release and feeding and exhibits positive cardiovascular effects, suggesting a possible clinical application of ghrelin (4). Ghrelin inhibits insulin release in mice, rats, and humans (5–8). Low plasma ghrelin levels are associated with elevated fasting insulin levels and insulin resistance (9,10). These findings suggest both physiological and pathophysiological roles for ghrelin in insulin release.

Although the nutritional, endocrine, and neural regulation of insulin release has been well characterized, much less is known about its autoregulation within islets. Ghrelin and GHSR are also located in pancreatic islets (8,11–15). We previously reported that in isolated islets, GHSR blockade and antiserum against acylated ghrelin markedly enhanced glucose-induced increases in insulin release and cytosolic Ca^{2+} concentration in islets (8). Although exogenous ghrelin suppressed insulin release, this effect required a concentration of 10 nmol/l, which is higher than the circulating ghrelin levels (16,17). These findings suggest that ghrelin at relatively high concentrations achieved within islets, rather than the circulating ghrelin, may regulate insulin secretion. The current study examined whether ghrelin originating from pancreatic islets could regulate insulin release and whether manipulation of ghrelin could affect glucose intolerance associated with obesity. We show here that ghrelin is released from pancreatic islets to downregulate glucose-induced insulin release and that ghrelin knockout mice escape high-fat diet–induced glucose intolerance because of enhanced insulin release.

RESEARCH DESIGN AND METHODS

Male Wistar rats (Japan SLC, Hamamatsu, Japan), ghrelin knockout mice, and wild-type C57BL/6J mice (Charles River Laboratories Japan, Yokohama, Japan) were housed in accordance with our institutional guidelines and with the Japanese Physiological Society's guidelines for animal care. Ghrelin knockout mice were the kind gift of Drs. T. Sato and M. Kojima (Kurume University). In these mice, the whole ghrelin gene sequence has been deleted. Animals were backcrossed with the C57B6/J strain for at least six generations. Proper deletion of the ghrelin gene was confirmed by Southern and Northern blot analysis. Total gastrectomy in 6-week-old male Wistar rats was carried out by resecting the stomach, followed by anastomosis of the cut edge of the

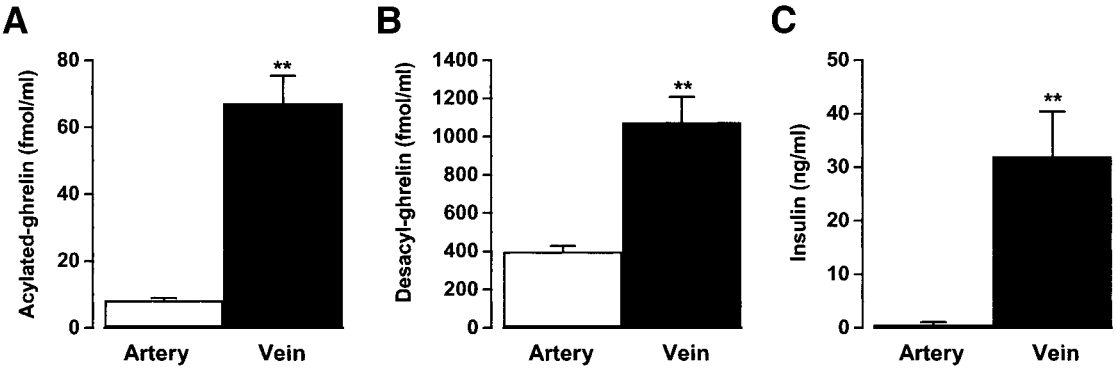

FIG. 1. Acylated ghrelin (*A*) and desacyl-ghrelin (*B*), as well as insulin (*C*), were present at higher concentrations in the pancreatic vein (splenic vein) than in the pancreatic artery (celiac artery) in rats ($n = 8$). **$P < 0.01$ vs. artery.

esophagus and the upper jejunum 4 cm distal to the Treitz ligament. At 2 months after surgery, gastrectomized rats were used for experiments.

Measurements of plasma insulin and ghrelin concentrations. Ghrelin (Peptide Institute, Osaka, Japan) and [D-Lys^3]-growth hormone releasing peptide-6 ([D-Lys^3]GHRP-6; Sigma-Aldrich, St. Louis, MO) were administered intraperitoneally to male Wistar rats (8 weeks old) or gastrectomized rats (3 months old) after overnight fasting. Blood was obtained from tails, and plasma insulin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Yokohama, Japan). To measure plasma ghrelin concentrations, blood samples were collected from the pancreatic arteries (celiac artery) and veins (splenic vein) and portal veins of anesthetized rats or mice. To avoid inflow of ghrelin from intestine and stomach to the splenic vein, the inferior mesenteric vein and spleen side of the splenic vein—including the short gastric and left gastroomental veins—were ligated. Plasma concentrations of acylated ghrelin and desacyl-ghrelin were measured using ELISA kits (Mitsubishi Kagaku Iatron, Tokyo, Japan).

Morphological analysis of pancreatic islets in wild-type and ghrelin knockout mice. Pancreata from male wild-type and ghrelin knockout mice were fixed in 4% paraformaldehyde, and two to three random sections were generated per mouse pancreas. Three mice were studied in each genotype. The sections were incubated overnight with mouse monoclonal anti-insulin antibodies (Sigma-Aldrich) at dilutions of 1:1,000 at 4°C. Samples were then incubated in Alexa Fluor 488–labeled goat anti-mouse IgG (Molecular Probes, Eugene, OR). Immunofluorescence for insulin was observed with photomultipliers of a multiphoton laser–scanning microscope (FluoView FV300-TP; Olympus, Tokyo, Japan). Islet number per unit area of pancreas and islet size were measured.

Measurements of insulin release in islets. Islets of Langerhans were isolated by collagenase digestion from male ghrelin knockout and wild-type (C57BL/6J) mice as previously reported (8,18), with slight modifications. Animals were anesthetized by intraperitoneal injection of pentobarbitone at 80 mg/kg, and collagenase at 1.05 mg/ml (Sigma-Aldrich) was injected into the common bile duct. Collagenase was dissolved in 5 mmol/l Ca^{2+}-containing HEPES-added Krebs-Ringer bicarbonate buffer (HKRB) solution (in mmol/l: 129 NaCl, 5.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 2.0 $CaCl_2$, 1.2 $MgSO_4$, and 10 HEPES, pH 7.4, with 0.1% BSA). Pancreata were dissected and incubated at 37°C for 16 min. Islets were collected and used for insulin release experiments. Groups of 12–15 islets were incubated for 1 h in HKRB at 37°C with 2.8 mmol/l glucose for stabilization, followed by test incubation for 1 h in HKRB with 2.8, 8.3, or 16.7 mmol/l glucose. Insulin concentrations were determined by an ELISA kit (Morinaga Institute of Biological Science).

Real-time RT-PCR analysis. Total RNA of islets was isolated using TRIzol (Invitrogen, Carlsbad, CA) and treated with RQ1-DNase (Promega, Madison, WI) to remove residual contaminations with DNA. First-strand cDNA synthesis was completed using ReverTra Ace (Toyobo, Osaka, Japan). Primers for real-time PCR were first examined by HotStarTaq DNA polymerase (94°C for 15 s, 55°C for 20 s, and 72°C for 20 s × 30 cycles; Qiagen, Hilden, Germany) and agarose gel electrophoresis for correct product size and absence of primer-dimer formation. Using a QuantiTect SYBR Green PCR kit, real-time PCRs (95°C for 15 s, 55°C for 20 s, and 72°C for 20 s × 35 cycles) were performed in an ABI-Prism 7700 sequence detector (Applied Biosystems, Foster City, CA). Product accumulation was measured in real time, and the mean cycle threshold (Ct; the cycle during which product is first detected) was determined for replicate samples ($n = 5$ independent reactions per primer pair and cDNA sample) run on the same plate. Different cDNA samples were normalized using primer sets to the housekeeping gene β-actin. Primers were as follows: β-actin, 5′-TTCCCCTCCATCGTGGGCCGC-3′ and 5′-GATGGCTACGTACATGGCTGG-3′; Insulin 1, 5′TAGTGACCAGCTATAATCAGAG-3′ and 5′-ACGCCAAGGTCTGAAGGTCC-3′; and Insulin 2, 5′-CCCTGCTGGCCCTGCTCTT-3′ and 5′-AGGTCTGAAGGTCACCTGCT-3′.

In vitro perfusion of the pancreas. Pancreata were perfused using a modification of the method of Grodsky and Fanska (19). Pancreata were isolated with segments of the duodenum and spleen. An arterial cannula was introduced into the celiac artery, and a venous cannula was inserted into the portal vein. The baseline perfusate consisted of HKRB (pH 7.4) containing 2.8 mmol/l glucose, 0.5% BSA, and 4% Dextran T70. The perfusate, maintained at 37°C, was continually oxygenated in an atmosphere of 95% O_2/5% CO_2. After a 20-min preincubation period, each pancreas was perfused for 10 min with the baseline perfusate. Pancreata were then perfused for 30 min with 8.3 mmol/l glucose or 8.3 mmol/l glucose with test reagents. The flow rate was maintained at 2.5 ml/min throughout measurements. Fractions, collected in chilled tubes at 1-min intervals, were stored at −20°C until assayed for immunoreactive insulin.

Glucose tolerance tests and insulin tolerance tests. In glucose tolerance test (GTT) studies, 2 g/kg glucose was injected intraperitoneally into mice, followed by blood sampling from the tail vein. In insulin tolerance test (ITT) studies, insulin (0.75 units/kg) was injected intraperitoneally, followed by collection of blood samples from the tail vein. Blood glucose concentrations were measured using a GlucoCard DIA meter (Arkray, Kyoto, Japan), while insulin concentrations were determined using an ELISA kit (Morinaga Institute of Biological Science).

Statistical analysis. Data are the means ± SE. Statistical analyses were performed using Student's *t* test or one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Release of ghrelin from pancreas. Ghrelin is expressed in the pancreatic islets (8,11–15). Release of ghrelin from pancreatic islets was assessed by comparing the ghrelin level in the pancreatic vein (splenic vein) with that in the pancreatic artery (celiac artery) in anesthetized rats. The concentrations of both acylated ghrelin and desacyl-ghrelin in the pancreatic vein were significantly higher (about eight times and three times, respectively) than those in the pancreatic artery in rats (Fig. 1*A* and *B*). The concentration of insulin was significantly higher in the pancreatic vein than in the pancreatic artery (Fig. 1*C*).

Endogenous ghrelin inhibits insulin release in perfused pancreas. Our previous in vitro study showed that GHSR antagonists and anti-ghrelin antiserum enhanced glucose-induced insulin release in isolated rat islets (8), suggesting an insulinostatic action of islet-originated ghrelin. To establish a physiological function of endogenous ghrelin in islets, we studied insulin release using perfused rat pancreas, which retains well intact islet circulation. A rise in the perfusate glucose concentration from 2.8 to 8.3 mmol/l evoked insulin release in a biphasic manner (Fig. 2*A*). The first and second phases of glucose-induced

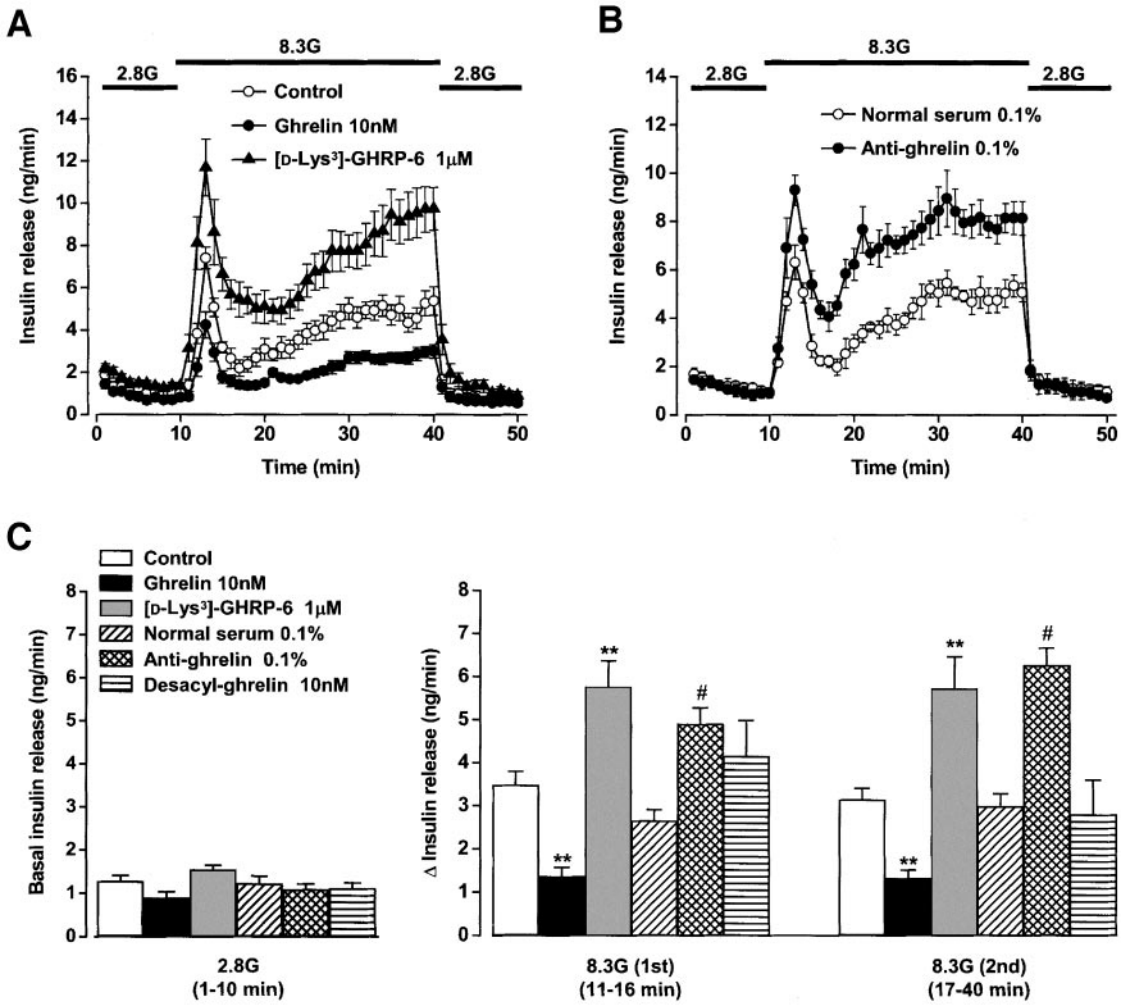

FIG. 2. Insulinostatic effects of endogenous ghrelin in perfused pancreas. *A*: Blockade of GHSR by [D-Lys^3]GHRP-6 (1 μmol/l) enhanced glucose (8.3 mmol/l)-induced insulin release in perfused rat pancreas, whereas exogenous ghrelin (10 nmol/l) administration inhibited it (n = 6–9). *B*: Immunoneutralization of endogenous ghrelin using an anti-ghrelin antiserum (0.1%) enhanced glucose (8.3 mmol/l)-induced insulin release in perfused rat pancreas (n = 3–4). *C*: [D-Lys^3]GHRP-6 (1 μmol/l) and anti-ghrelin antiserum (0.1%) increased, whereas exogenous ghrelin (10 nmol/l) inhibited, both the first and second phases of insulin release. Control nonimmune serum (0.1%) had no effect on insulin release. Desacyl-ghrelin (10 nmol/l), an inactive form of ghrelin incapable of activating GHSR, did not alter insulin release (n = 3–9). None of these treatments affected basal levels of insulin release at 2.8 mmol/l glucose. *P < 0.05; **P < 0.01 vs. control; $^{\#}P$ < 0.05 vs. nonimmune normal serum (0.1%). G, glucose.

insulin release were significantly enhanced both by blockade of GHSR with the GHSR antagonist [D-Lys^3]GHRP-6 (1 μmol/l) (Fig. 2*A* and *C*) and by immunoneutralization of endogenous ghrelin with anti-ghrelin antiserum (0.1%) (Fig. 2*B* and *C*). Conversely, administration of exogenous ghrelin (10 nmol/l) suppressed both phases of glucose-induced insulin release (Fig. 2*A* and *C*). Desacyl-ghrelin, which cannot activate GHSR (1,20), did not significantly alter glucose-induced insulin release (Fig. 2*C*). None of these treatments affected basal levels of insulin release at 2.8 mmol/l glucose.

Endogenous ghrelin downregulates plasma insulin concentrations in both normal and gastrectomized rats. Our findings that glucose-induced insulin release from perfused pancreas was enhanced by ghrelin immunoneutralization and GHSR antagonist suggest that ghrelin originating from pancreatic islets suppresses insulin release. GHSR antagonists also increase systemic insulin responses to GTTs (8), which could be attributable to blockade of ghrelin originated from stomach and/or from other tissues, including islets. To examine the contribution of ghrelin from the stomach and other sources, we produced gastrectomized rats lacking stomach-derived ghrelin and examined the effect of intraperitoneal administration of the GHSR antagonist [D-Lys^3]GHRP-6 (10 μmol/kg) on plasma insulin concentrations in gastrectomized and normal rats fasted overnight. In gastrectomized rats, plasma concentrations of acylated ghrelin were markedly reduced (5.2 ± 0.7 vs. 32.5 ± 9.7 fmol/ml in gastrectomized rats vs. normal rats, respectively; $P < 0.01$, $n = 15$) (Fig. 3*C*), indicative of a lack of stomach-derived ghrelin. The remaining levels of acylated ghrelin may be derived substantially from the intestine, the second largest source of ghrelin (12,21). Although the remaining circulating acylated ghrelin was dramatically reduced in gastrectomized rats, intraperitoneal injection of GHSR antagonist increased plasma insulin concentrations at 30 min in gastrectomized rats to a similar extent as that in normal rats ($P < 0.05$, $n = 15$) (Fig. 3*B* vs. *A*). These results suggest that the effect of GHSR antagonist is not attributable to antagonism of circulating ghrelin but primarily to blockade of local ghrelin, including that in islets. It was con-

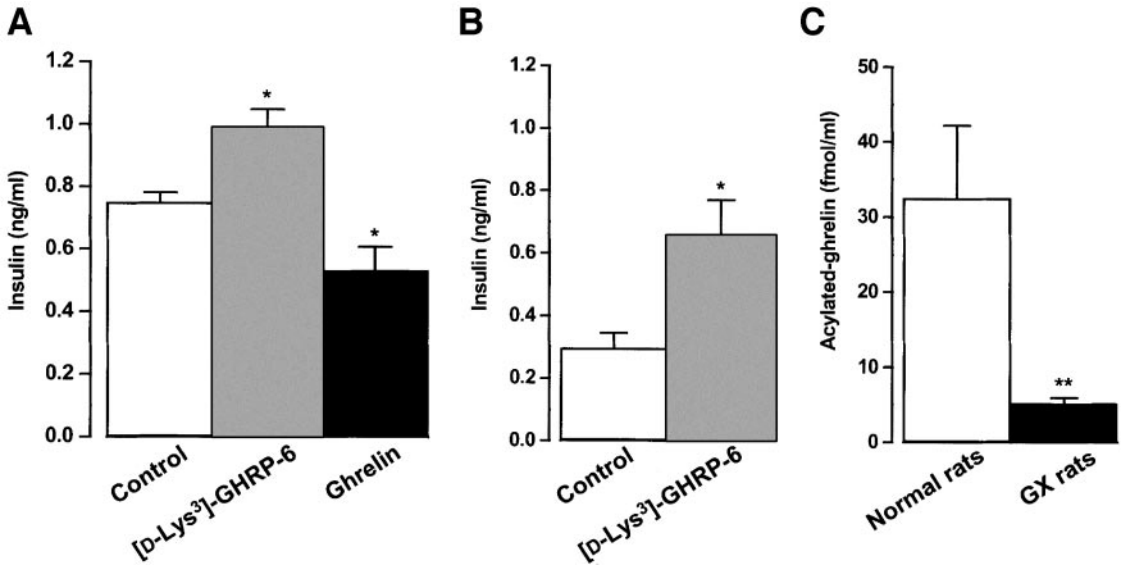

FIG. 3. Endogenous ghrelin decreases plasma insulin concentrations in normal and gastrectomized (GX) rats. *A*: A GHSR antagonist, [D-Lys^3]GHRP-6 (10 μmol/kg i.p.), increased plasma insulin concentrations at 30 min after administration in rats fasted overnight. Conversely, administration of ghrelin (10 nmol/kg i.p.) significantly decreased plasma insulin levels. Data are the means ± SE of 15 rats. *$P < 0.05$ vs. control. *B*: GHSR blockade increased plasma insulin levels in gastrectomized rats ($n = 15$). *$P < 0.05$ vs. control. *C*: Plasma acylated ghrelin levels were reduced in gastrectomized rats ($n = 15$). **$P < 0.01$ vs. normal rats.

firmed that application of exogenous ghrelin (10 nmol/kg i.p.) significantly decreased plasma insulin levels in control rats (Fig. 3*A*).

Ghrelin knockout mice display increased insulin and decreased glucose levels. The effects of GHSR antagonist and anti-ghrelin antiserum in the perfused pancreas and in isolated islets may result from counteraction of the action of endogenous ghrelin in islets. This hypothesis was further examined using ghrelin knockout mice. When fed standard chow, no significant differences between male ghrelin knockout and wild-type (C57BL/6J) mice were observed at 8 weeks of age in body weights (23.4 ± 0.7 vs. 23.5 ± 0.3 g in ghrelin knockout vs. wild-type mice, respectively; $n = 10$), total 24-h food intake (3.51 ± 0.14 vs. 3.54 ± 0.04 g, $n = 10$), and blood glucose levels in fed states (120 ± 3.1 vs. 127 ± 6.0 mg/dl, $n = 10$), confirming previous reports in ghrelin knockout mice (22–25). In ghrelin knockout mice, plasma acylated ghrelin levels were undetectable (Fig. 4*A*). Morphological analysis of pancreatic sections showed that the density and average size of islets were not significantly different between wild-type and ghrelin knockout mice (Fig. 4*B* and *C*). Moreover, the number and size of isolated islets obtained by collagenase digestion were not altered in ghrelin knockout mice (islet number: 138.6 ± 14.3, $n = 5$ mice, vs. 151.2 ± 18.9, $n = 5$, for ghrelin knockout vs. wild-type mice; islet diameter: 165.2 ± 2.3 μm, $n = 714$ islets, vs. 164.7 ± 2.3, $n = 756$). Glucose (8.3 and 16.7 mmol/l)-induced insulin release from isolated islets of ghrelin knockout mice was significantly greater than that of wild-type mice (Fig. 4*D*), whereas basal levels of insulin release at 2.8 mmol/l glucose were not altered. No difference was observed between ghrelin knockout and wild-type mice in insulin content per islet (Fig. 4*E*), mRNA expression of insulin 1, and that of insulin 2 (Fig. 4*F*). These data indicate that the larger amount of insulin release in islets of ghrelin knockout mice results from greater insulin secretory response to glucose, whereas insulin production is unaltered. In GTTs, ghrelin knockout mice exhibited markedly enhanced insulin responses and attenuated glucose responses (Fig. 4*G* and *H*). The profiles of blood glucose levels during ITTs exhibited little differences between ghrelin knockout and wild-type mice (Fig. 4*I*), suggesting that insulin sensitivity was not significantly altered. Thus, the suppressed glycemic responses to GTTs in ghrelin knockout mice may primarily result from enhanced insulin secretion, although possible additional effects of ghrelin on glucose production (26) or insulin sensitivity (27) cannot be disregarded.

High-fat diet–induced glucose intolerance is prevented in ghrelin knockout mice. The enhanced insulin and suppressed glycemic responses to GTTs in ghrelin knockout mice could be beneficial under conditions of increased demand for insulin. We examined this possibility using a model of high-fat diet–induced obesity. Both wild-type and ghrelin knockout mice fed a high-fat diet for 4 weeks displayed moderate increases in body weight (Fig. 5*A*). High-fat diet resulted in moderate increases in blood glucose levels in wild-type mice, whereas this change was not significant in ghrelin knockout mice (Fig. 5*B*). High-fat diet also increased plasma insulin levels, and this change was much greater in ghrelin knockout than wild-type mice (Fig. 5*C*). These results suggest that high-fat diet–induced elevation of blood glucose was corrected by enhanced insulin release in islets of ghrelin knockout mice. A possible impact of this ghrelin knockout mouse islet phenotype on systemic control of glucose and insulin was examined by GTTs. In wild-type mice, increases in blood glucose levels at 15–120 min of the GTT were significantly larger in the high-fat diet group than in the control diet group, exhibiting glucose intolerance (Fig. 5*D*). Insulin response to GTTs at 15 min also tended to be enhanced in the high-fat diet group, although the change was not statistically significant (Fig. 5*E*). In ghrelin knockout mice, by contrast, increases in blood glucose levels at 15–120 min of the GTT in the high-fat diet group were not significantly different from those of the control diet group, and insulin response to GTTs at 15 min was markedly enhanced in the high-fat diet group (Fig. 5*F* and *G*). Thus,

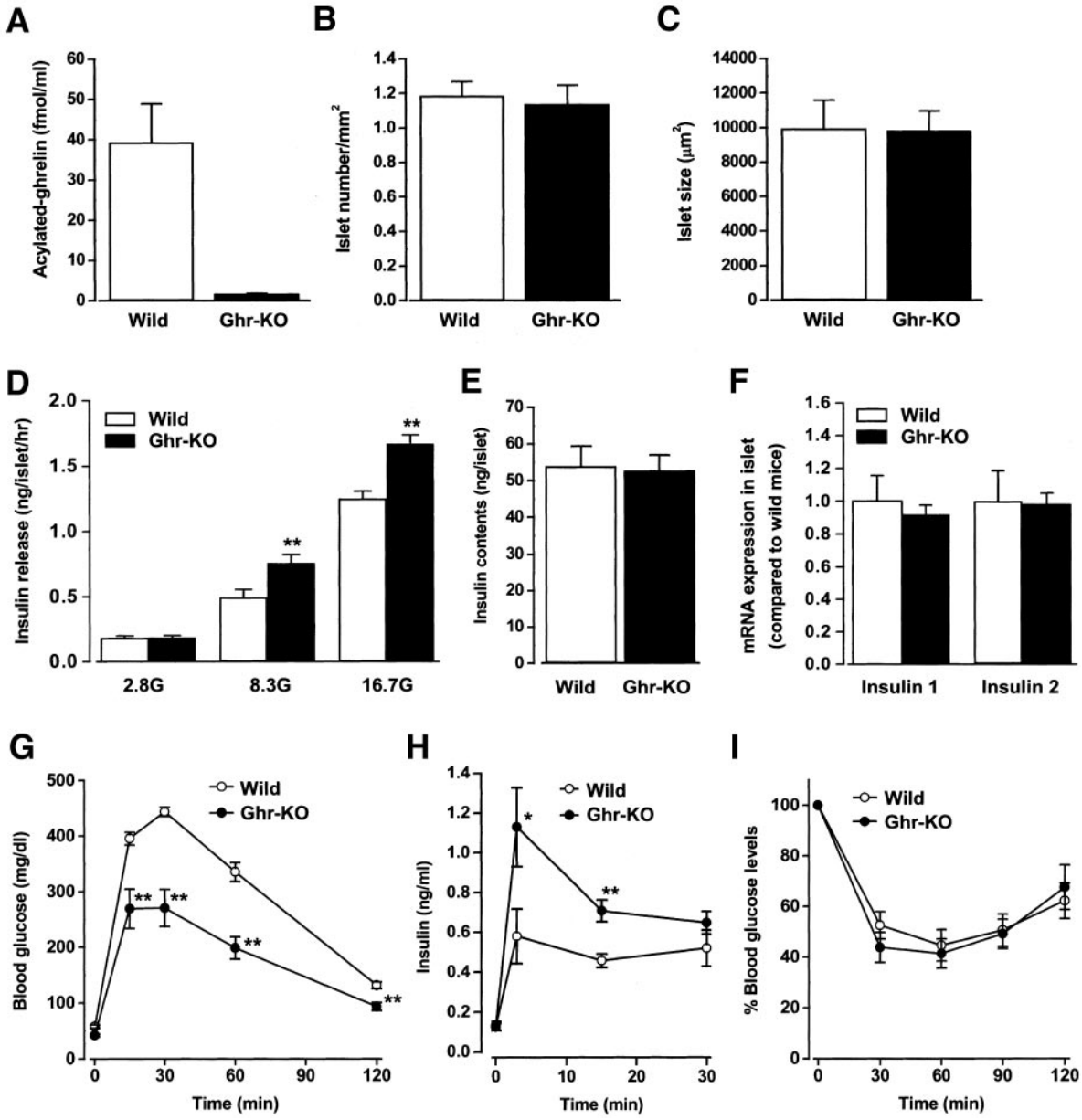

FIG. 4. Ghrelin knockout (Ghr-KO) mice display increased insulin and decreased glucose levels. *A*: Acylated ghrelin was not detected in the plasma of male ghrelin knockout mice ($n = 6$). *B* and *C*: The number of islets (*B*) in an area unit (1 mm², $n = 8$ slices from three mice) and the size of islets (μm², $n = 50–51$ islets) (*C*) on pancreatic sections were not significantly different between wild-type (Wild) and ghrelin knockout mice at 8 weeks of age. *D*: Glucose (8.3 and 16.7 mmol/l)-induced insulin release was enhanced in ghrelin knockout mouse islets ($n = 9–12$). **$P < 0.01$ vs. wild-type mice. *E* and *F*: Islet insulin (*E*) contents ($n = 12$) and mRNAs expressions (*F*) of insulin 1 and 2 ($n = 4–5$) were not different between wild-type and ghrelin knockout mice. In GTTs (glucose 2 g/kg i.p.), male ghrelin knockout mice exhibited attenuated elevations of blood glucose (*G*) and enhanced elevations of insulin levels (*H*) in comparison to wild-type mice ($n = 9–10$). *$P < 0.05$; **$P < 0.01$ vs. wild-type mice. *I*: Profiles of blood glucose levels during the ITT (insulin 0.75 units/kg i.p.) did not differ between ghrelin knockout and wild-type mice ($n = 12–15$). G, glucose.

ghrelin deficiency promoted insulin release and prevented glucose intolerance in a high-fat diet–induced obese model.

DISCUSSION

In this study, we demonstrated that plasma ghrelin concentrations were significantly higher in the pancreatic vein than in the artery in rats and that glucose-induced insulin release from the perfused pancreas was markedly enhanced by blockade of GHSR and immunoneutralization of endogenous ghrelin. Furthermore, GHSR blockade increased plasma insulin concentrations in gastrectomized and normal rats to a similar extent. In addition, in ghrelin-deficient (ghrelin knockout) mice, glucose-induced insulin release from isolated islets was enhanced, systemic insulin response was increased, and glucose response was attenuated in the GTT. Furthermore, ghrelin deficiency promoted insulin release and prevented glucose intolerance in a high-fat diet–induced obese model.

This study demonstrated that ghrelin knockout mice exhibit decreased glucose responses and increased insulin responses in GTTs. Similar results have recently been reported in another line of ghrelin-deficient mice (28). The results of GTTs and ITTs in our ghrelin knockout mice are similar to those previously observed with pharmacological blockade of ghrelin action (8), reinforcing the concept that endogenous ghrelin serves as a downward regulator of

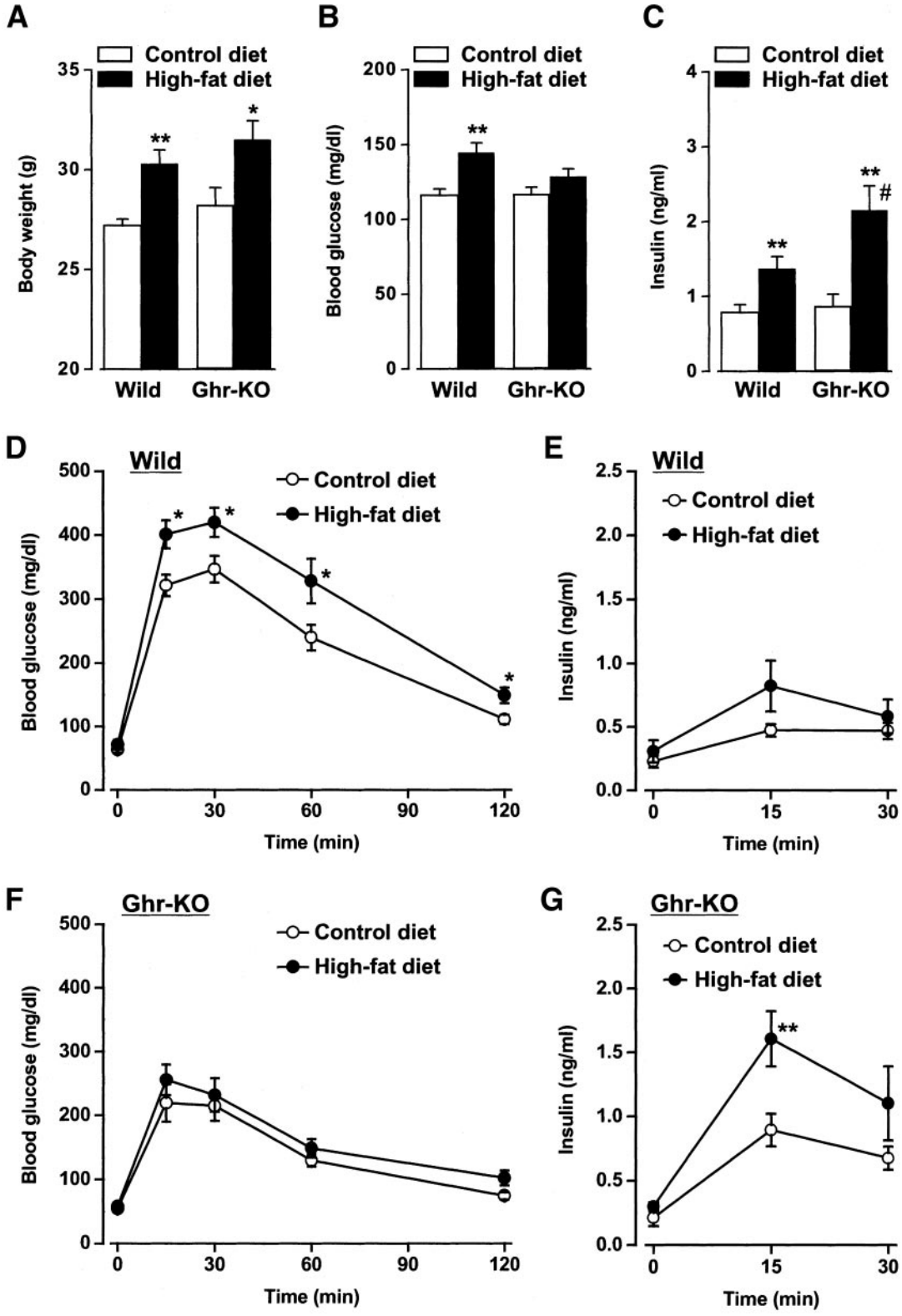

FIG. 5. High-fat diet–induced glucose intolerance is prevented in ghrelin knockout (Ghr-KO) mice. Body weight (*A*), fed blood glucose (*B*), and insulin (*C*) levels in 12-week-old wild-type and ghrelin knockout mice fed a high-fat diet or control diet. The mice were given a high-fat diet or control diet from 8 to 12 weeks old. On a caloric base, high-fat diet consisted of 23% protein, 44.6% carbohydrates, and 32.4% fat (total 17.9 kJ/g), whereas control diet consisted of 29.2% protein, 58.8% carbohydrates, and 12% fat (total 14.5 kJ/g, $n = 9$ per group). In wild-type mice, the high-fat diet group exhibited glucose intolerance (*D*) and slight enhancement of insulin release (*E*) during the GTT (glucose 2 g/kg i.p.). In ghrelin knockout mice, in contrast, glycemic responses to GTTs in the high-fat diet group were not different from those of control diet group (*F*), and insulin response at 15 min was markedly enhanced in the high-fat diet group (*G*). *$P < 0.05$; **$P < 0.01$ vs. control diet mice; $^{\#}P < 0.05$ vs. wild-type high-fat diet mice.

insulin release and consequently upward regulator of glycemia. Furthermore, we found that glucose-induced insulin release from isolated islets of ghrelin knockout mice was greater than that of wild-type mice, whereas insulin content per islet was unaltered in ghrelin knockout mice. Consistent with this observation, glucose-induced insulin release from perfused pancreas in normal rats was augmented by GHSR antagonist and ghrelin immunoneutralization. Thus, pharmacological, immunological, and genetic blockade of ghrelin or ghrelin action in pancreatic islets all markedly enhanced glucose-induced insulin release. These findings reveal the insulinostatic function of endogenous ghrelin within islets. It should be noted that the effect of GHSR antagonist could be partly attributable to blockade of the constitutive activity of GHSR (29,30).

This study demonstrated that the ghrelin level is higher in the pancreatic vein than in the artery. Moreover, blockade of ghrelin action with antagonist and antiserum enhanced glucose-induced insulin release from perfused pancreas, which retains well physiologic circulation. Insulin release from isolated islets was similarly enhanced with ghrelin antagonist and antiserum in our previous report (8). These findings may indicate that ghrelin is released from and acting on pancreatic islets, thus serving as an intraislet regulator of insulin release. The importance of islet-originated ghrelin in the regulation of insulin release is supported by the current finding that GHSR antagonist increased plasma insulin concentrations in gastrectomized and normal rats to a similar extent. Regarding the islet cell species that could release ghrelin, multiple experimental systems have shown ghrelin immunoreactivity in α-cells (8,11), β-cells (13), and islet ghrelin cells (14,31,32), including those named ε-cells (15). It was also reported that ghrelin is expressed together with glucagon or pancreatic polypeptide in immature islet cells in rats (31). mRNAs encoding ghrelin and GHSR are expressed in the pancreas of rats and humans (1,11–13) as well as in β-cell lines (31). Thus, ghrelin appears to be expressed by multiple islet cell types. Further studies are required to identify the cell types that produce ghrelin, which could depend on specific conditions and ages of animals/humans.

We have not yet examined the effect of ghrelin on δ-cells, and therefore a possibility that the observed effects require the participation of δ-cells cannot be excluded. Ghrelin reportedly suppresses the release of somatostatin (6). However, the reduction in this insulinostatic hormone does not appear to mediate the action of ghrelin to inhibit insulin release. Moreover, the direction of microcirculation previously reported was against the physiological role of somatostatin in the regulation of insulin release (33). Because ghrelin directly inhibits β-cells (8), the insulinostatic effect of ghrelin is produced, at least partly, via its direct effect on β-cells. In addition to the regulation of insulin release, ghrelin could also serve as a novel medium of communication between β- and non-β-cells: anterograde versus retrograde perfusion with antisera against ghrelin and conventional islet hormones appears to be a promising approach. However, further studies are definitely needed to address this issue.

Low plasma ghrelin levels are associated with elevated insulin levels (9,10). The inverse relationship between plasma levels of ghrelin and insulin may be explained, at least in part, by the inhibition of insulin release by ghrelin. The current study, by using blockade of ghrelin in pancreas and islets, as well as in vivo in gastrectomized rats, demonstrated that ghrelin originating from pancreatic islets plays an essential role in suppression of insulin release. This study also suggested a pathophysiological role for ghrelin. High-fat diet produced glucose intolerance in wild-type mice. By contrast, ghrelin knockout mice fed a high-fat diet showed close to normal glucose responses and markedly enhanced insulin responses to GTTs compared with control ghrelin knockout mice fed a normal diet. As a possible underlying mechanism, lack of ghrelin and its insulinostatic activity may raise the maximal capacity of glucose-induced insulin release and enable islets to secrete more insulin to meet an increased demand associated with high-fat diet–induced obesity, thereby achieving normoglycemia. It has recently been reported that in *ob/ob* mice, a genetic model of obesity attributable to leptin deficiency, ablation of ghrelin in *ob/ob* mice augmented insulin release and thereby markedly reduced hyperglycemia (28). We hypothesize that the ghrelin system in islets, by altering its insulinostatic efficacy, optimizes the amount of insulin release to meet the systemic demand. In early stages of obesity, antagonization of ghrelin function can prevent glucose intolerance, providing a potential therapeutic application to counteract the progression of type 2 diabetes.

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