Hepatocyte Growth Factor Plays a Key Role in Insulin Resistance-Associated Compensatory Mechanisms

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Insulin resistance is present in obesity and in type 2 diabetes and is associated with islet cell hyperplasia and hyperinsulinemia, but the driving forces behind this compensatory mechanism are incompletely understood. Previous data have suggested the involvement of an unknown circulating insulin resistance-related β-cell growth factor. In this context, looking for candidates to be a circulating factor, we realized that hepatocyte growth factor (HGF) is a strong candidate as a link between insulin resistance and increased mass of islets/hyperinsulinemia. Our approach aimed to show a possible cause-effect relationship between increase in circulating HGF levels and compensatory islet hyperplasia/hyperinsulinemia by showing the strength of the association, whether or not is a dose-dependent response, the temporality, consistency, plausibility, and reversibility of the association. In this regard, our data showed: 1) a strong and consistent correlation between HGF and the compensatory mechanism in three animal models of insulin resistance; 2) HGF increases β-cell mass in a dose-dependent manner; 3) blocking HGF shuts down the compensatory mechanisms; and 4) an increase in HGF levels seems to precede the compensatory response associated with insulin resistance, indicating that these events occur in a sequential mode. Additionally, blockages of HGF receptor (Met) worsen the impaired insulin-induced insulin signaling in liver of diet-induced obesity rats. Overall, our data indicate that HGF is a growth factor playing a key role in islet mass increase and hyperinsulinemia in diet-induced obesity rats and suggest that the HGF-Met axis may have a role on insulin signaling in the liver. **(*Endocrinology* 153: 5760–5769, 2012)**

In individuals with insulin resistance, there is an increase in pancreatic β-cell mass as a compensatory response (1). This compensatory response of β-cells is the first step in developing diabetes by inducing hyperinsulinemia. In fact, insulin-resistant animals and human subjects present islet hyperplasia and/or hyperinsulinemia before the onset of detectable hyperglycemia, suggesting the existence of as yet unknown mechanisms enhancing compensatory expansion of β-cell mass in response to obesity-related insulin resistance. Although some studies have indicated that a neural component is involved in triggering this response (2–4), others have also suggested the involvement of an unknown circulating insulin resistance-related β-cell growth factor (1, 5).

In this regard, some data have indicated (1, 2, 5, 6) that this hypothetical circulating growth factor is produced by the liver. In addition, it has been demonstrated that the ERK pathway in the liver is involved in this compensatory response (2). In this sense, considering candidates for a circulating factor that could have such features, hepatocyte growth factor (HGF) presents a strong candidate, having at least four characteristics that mark the pathophysiological link between insulin resistance and islet hyperplasia/hyperinsulinemia: 1) HGF is mainly produced by the liver (7); 2) it is under regulation by the ERK pathway (8); 3) HGF stimulates insulin secretion and increased islet mass both *in vitro* and *in vivo* (9–13); and 4) levels of

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Abbreviations: Akt^{ser473}, Protein kinase B serine 473; DIO, diet-induced obesity; DIO-30D, DIO for 30 d; GTT, glucose tolerance test; HFD, high-fat diet; HGF, hepatocyte growth factor; IRS, insulin receptor substrate; Met, HGF receptor; rHGF, recombinant HGF; TUNEL, terminal deoxynucleotidyl transferase 2′-deoxyuridine, 5′-triphosphate nick end labeling.

this factor are elevated in obesity associated-insulin resistance (14, 15).

Taken together, HGF fulfills at least some of the criteria of a good candidate for a circulating factor that connects insulin resistance with increased β-cell mass. Therefore, the aim of this study was to evaluate whether HGF is the driving force behind this compensatory mechanism.

Research Design and Methods

Animal studies

Diet-induced obese (DIO) rats

First, male Wistar rats (9-wk-old; obtained from the State University of Campinas Central Breeding Center) were housed in grouped cages under a 12-h light, 12-h dark cycle in a controlled environment (room temperature, 22 ± 3 C; humidity, 55 ± 5%) and also were randomly assigned to two diet groups: either standard rodent chow and water *ad libitum* or a cafeteria diet, for 30 d [DIO for 30 d (DIO-30D)] or DIO-60D. The cafeteria diet group received soft drinks *ad libitum*, instead of water, alternated daily (Coca-Cola and Guaraná Antarctica), and were fed a pellet made of 37.5% standard rodent chow, 25% peanuts, 25% chocolate, and 12.5% cookies, offered together with palatable food items comprising wafer, snacks, cakes, and biscuits, totaling 4.41 kcal/g of gross energy (43.1% from carbohydrates, 12.1% from proteins, and 46.9% from fats) as opposed to the 2.63 kcal/g of gross energy of the standard chow diet Nuvilab CR-1 (Nuvital, Colombo, Paraná, Brazil) (5, 16). Second, beginning the sixth week of the cafeteria and chow diet, the rats were treated with a pharmacological inhibitor of HGF receptor (SU11274; Tocris Bioscience, Bristol, UK) at a concentration of 0.5 mg/kg·d, ip, for 16 d. On the other hand, for treatment of obese animals with recombinant HGF (rHGF), it was administered to rats from 6 wk after the start of the cafeteria diet. The protein was injected into the tail vein at a dose of 0.5 μg/kg·d diluted in saline for 16 d; the control received only saline. Six rats were used per group (n = 6).

ob/ob *and* Swiss *mice*

Six-week-old *Swiss* and *ob/ob* mice were obtained from the State University of Campinas Central Breeding Center. Animals were housed in individual cages with free access to water and rodent chow or high-fat diet (HFD) under a 12-h light, 12-h dark cycle in a controlled environment (room temperature, 22 ± 3 C; humidity, 55 ± 5%). The *Swiss* mice were fed with HFD (consisted of 55% of calories derived from fat, 29% from carbohydrates, and 16% from protein) for 2 months, as previously described (17, 18). The *ob/ob* mice were fed on a chow diet for 2 months.

Dose-response evaluation

To perform the dose-response evaluation of treatment with purified recombinant active mouse HGF (rHGF) (Peprotech, Inc., Rocky Hill, NJ), *Swiss* mice at 6 wk were treated for 5 d with rHGF at concentrations of 0.05, 0.5, or 5.0 μg/kg·d. Mice were randomly distributed into four groups, each group having six mice. The rHGF was injected into the tail vein and diluted in saline (50 μl).

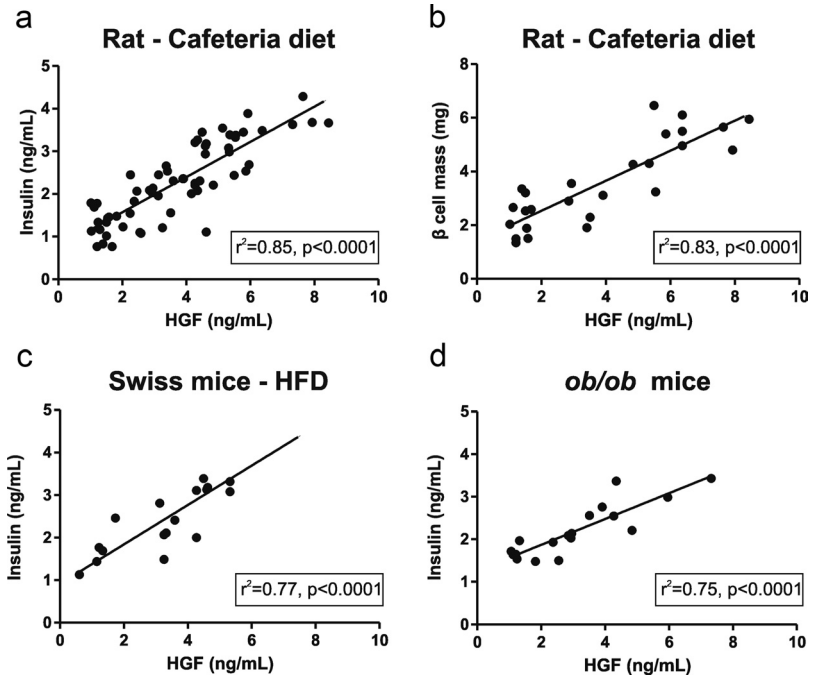

FIG. 1. Strong correlation between circulating HGF and insulin levels in three different models of insulin resistance. Correlation between fasting plasma HGF and insulin levels (A) and between fasting plasma HGF levels and β-cell mass (B) in DIO (DIO-60D) rats. Correlation between fasting plasma HGF and insulin levels in *Swiss* mice fed on HFD (C) and in *ob/ob* mice (D). Correlations between parameters were tested by linear regression analysis.

70% Partial hepatectomy (Higgins procedure)

Additionally, to conduct a study of another model that shows an increase of endogenous HGF (19), we used the technique of partial hepatectomy 70%. Wistar rats were anesthetized with ketamin 5% (30 mg/kg) and xylazine 2% (30 mg/kg) ip. Rats were divided into two groups (n = 6). Under strict sterile conditions, 70% (two thirds) partial hepatectomy was performed according to the method of Higgins and Anderson (20). In brief, the left lateral and median hepatic lobes, constituting approximately 70% of the total liver weight, were ligated and resected. In sham-operated controls, the livers were briefly removed from the peritoneal cavity but not tied or excised.

During the surgery, animals were warmed by a halogen light (45 W, 127 V), and corporeal temperature was monitorized by a rectal digital thermometer (YSI Precision 4000A Thermometer; YSI Temperature, Dayton, OH) and kept around 37 C. Animals were allowed to spontaneous ventilation with an oxygen-enriched mixture (40%) during all the procedure

(21). All animal studies were approved by the Animal Care and Use Committee at the State University of Campinas and are in accordance with the guidelines for the Care and Use of Laboratory Animals.

Assays

Rats and mice were fasted overnight, and the blood samples were withdrawn from the retrobulbar intraorbital capillary plexus. The plasma was separated by centrifugation at 2500 × *g*/5 min, in tubes containing EDTA (1 mg/ml, disodium salt). Glucose was measured from whole venous blood with a glucose monitor (glucometer; Bayer Diagnostics, New York, NY). Readouts of fasting blood insulin, C-peptide, and HGF levels were determined by ELISA [insulin and C-peptide were from Millipore (Bedford, MA) and HGF was from Abcam, Inc. (Cambridge, MA)]. The glucose tolerance test (GTT) was performed as follows. After a 6-h fasting, rats were anesthetized by an ip injection of sodium amobarbital (15 mg/kg body weight), and the experiments were initiated after the loss of corneal and pedal reflexes. After collection of an unchallenged sample (time 0), a bolus of 2.0 g/kg body weight of glucose was administered into the peritoneal cavity. Blood samples were collected from the tail tip for determination of glucose and insulin concentrations (17, 18, 22).

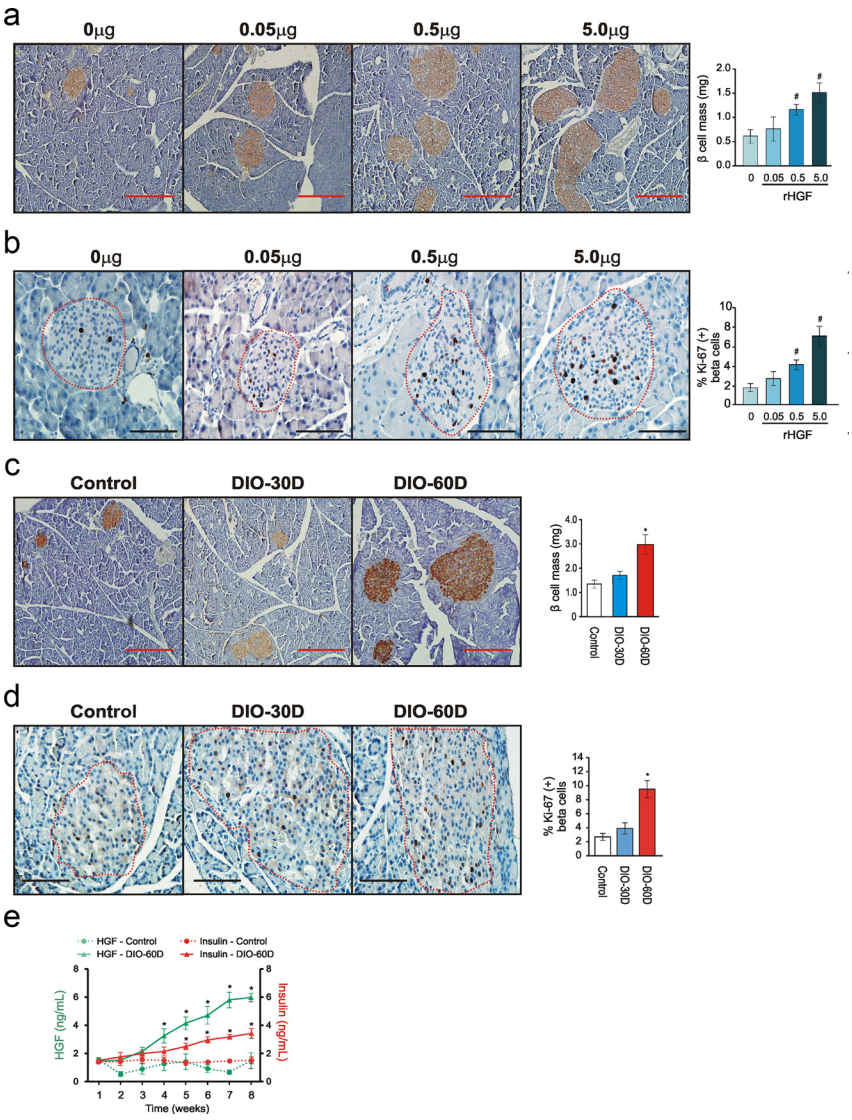

FIG. 2. Effect of HGF on insulin resistance-associated compensatory mechanisms. A, β-Cell mass and representative images of pancreatic islets stained for insulin, as well as the percentage of Ki-67-positive cell in β-cells and representative images of islets stained for Ki-67 (B) from mice after receiving different concentrations of rHGF (0.05, 0.5, and 5.0 μg/kg·d) for 5 d. C, β-Cell mass determinations and their respective representative images, plus the percentage of Ki-67-positive cell in β-cells and representative images of islets stained for Ki-67 (D) in control, DIO-30D, and DIO-60D rats. E, Temporal evolution of circulating HGF and insulin levels in control and DIO-60D rats for 8 wk. *Scale bars*, 100 μm (*black*) and 400 μm (*red*). Data are presented as means ± SEM from six mice or six rats per group in experiments that were repeated at least three times. *, $P < 0.05$ *vs.* control; #, $P < 0.05$ *vs.* dose 0.

Stereologic estimation of β-cell

To study the morphometric parameters of endocrine pancreas, six pancreases from each group were excised and processed according to a previous description (23). The cellular distribution of insulin was analyzed as described previously (23). β-Cell mass was determined by point-counting morphometry on each pancreas section immunostained for insulin according to previous descriptions (23). Each section was systematically scored with a grid of 100 points. The β-cell relative volume was calculated by dividing the intercepts over β-cells by the intercepts over the total pancreatic tissue; the β-cell mass was then estimated by multiplying the β-cell relative volume by the total pancreas weight. A minimum of 500 fields/pancreas was counted.

Average β-cell proliferation was obtained by counting total islet cell nuclei stained for insulin and Ki-67, a well-established proliferation marker, using the image analysis software ImageJ (http://rsbweb.nih.gov/ij/). At least 50 islets per group were sampled. The β-cell proliferation was estimated by the percentage of Ki-67-positive cells from the total of insulin-positive cells (24). On the other hand, average β-cell death was obtained by counting total islet β-cell nuclei stained for insulin and terminal deoxynucleotidyl transferase 2′-deoxyuridine, 5′-triphosphate nick end labeling (TUNEL), an *in situ* DNA end-labeling method. The TUNEL staining was done by a commercial apoptosis detection kit (Roche Diagnostics, Indianapolis, IN), according to the recommendations of the manufacturer. At least 50 islets per group were sampled. Using the same software aforementioned, the β-cell death was estimated by the percentage of TUNEL-positive cells from the total of insulin-positive cells (25).

Islet isolation and culture of pancreatic islets

Islets were isolated from rats after injection of collagenase through the pancreatic duct, then selected with a micropipette un-

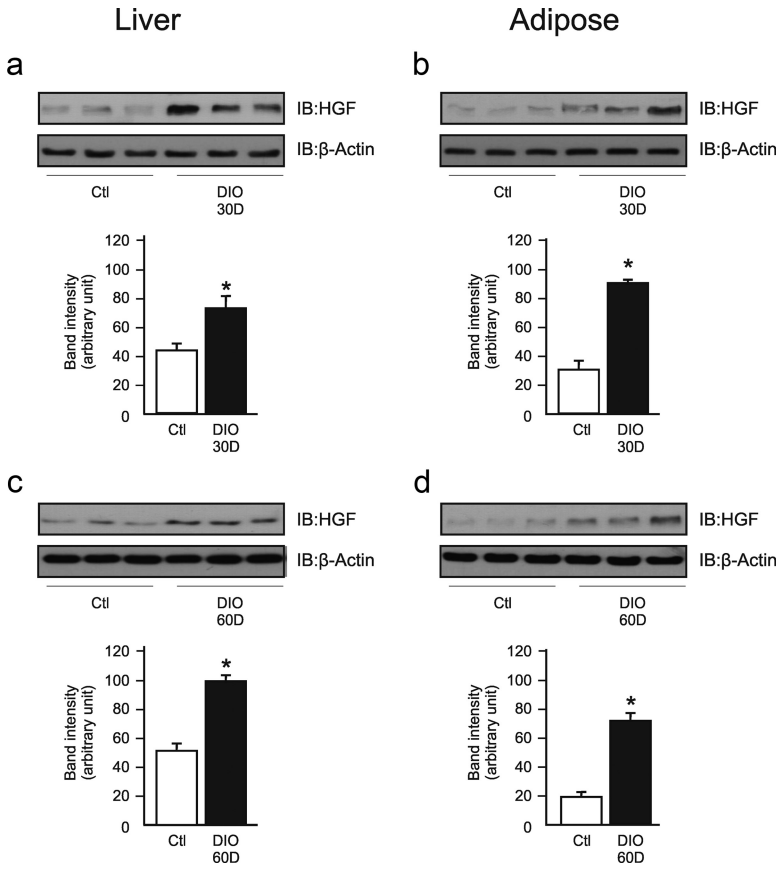

FIG. 3. DIO rats showed an increase in HGF protein content in the liver and adipose tissue. A–D, Representative blots show HGF protein expression in liver and epididymal adipose tissue of control (Ctl), DIO-30D, and DIO-60D rats. Data are presented as means ± SEM from six rats per group in experiments that were repeated at least three times. *, $P < 0.05$ *vs.* control. IB, Immunoblot.

der a microscope to exclude any contaminating tissues. The selected 300- to 400-rat islets were first incubated in Krebs-Ringer bicarbonate buffer equilibrated with 95% O_2-5% CO_2 (pH 7.4) and afterward treated with rHGF for 15 and 30 min (Peprotech, Inc.). Then, at least 300 clean islets from each experimental group were transferred to an Eppendorf and homogenized (Eppendorf, Hamburg, Germany), by sonication 15 sec, in 200 μl of solubilization buffer. The samples (total islets extracts) were treated with Laemmli buffer containing 100 mmol/liter dithiothreitol, heated in a boiling water bath for 4 min, and subjected to SDS-PAGE and immunoblotted, as previously described (26).

Tissue extraction and immunoblotting

Rats were anesthetized (anesthesia was ensured by the loss of pedal and corneal reflexes). The abdominal cavity was opened, the portal vein was exposed, and 0.1 ml of normal saline was injected with or without insulin (10^{-6} mol/liter). At 30 and 90 sec after insulin injection, the liver and epididymal adipose tissue were removed, minced coarsely, and homogenized immediately in extraction buffer, as previously described (18). The whole-tissue extracts were subjected to SDS-PAGE and immunoblotted, as previously described (18, 22). All antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), with the exception of β-actin and antiphospho-Met, which were obtained from Cell Signaling Technology (Beverly, MA).

Statistical analysis

The results of the experiments are displayed as mean ± SEM of a least three independent experiments. The results of blots are presented as direct comparisons of bands or spots in autoradiographs, quantified by optical densitometry (UN SCAN IT gel; Silk Scientific, Inc., Orem, UT). Multiple comparisons were tested by one-way ANOVA, followed by Tukey's *post hoc* test, with the significance level set at $P < .05$ using SPSS software (SPSS for Windows, version 16.0; SPSS, Chicago, IL). Correlations between parameters were tested by linear regression analysis using the GraphPad Prism software program (GraphPad, San Diego, CA).

Results

Increasing levels of HGF correlate with increased islet mass/hyperinsulinemia in DIO animals

In DIO-60D rats, there is a strong correlation between circulating HGF levels and hyperinsulinemia and islet mass increase, suggesting that an association exists between these events (Fig. 1, A and B). Investigating the nature of this correlation, we observed that in different models of insulin resistance (three different animal models of obesity), a strong and significant correlation between HGF and insulin levels exists. The others models used were *Swiss* mice on a HFD (Fig. 1C) and *ob/ob* mice (Fig. 1D).

HGF increases β-cell mass in a dose-dependent manner

Treating *Swiss* mice for 5 d with three different doses of HGF revealed that a strong correlation exists between the dose of infused HGF and islet cell hyperplasia and hyperinsulinemia, indicating that the compensatory mechanism responds to HGF in a dose-dependent manner (Fig. 2A). In addition, this mechanism was confirmed by the marked β-cell proliferation as judged by distribution of Ki-67-positive nuclei (Fig. 2B).

The rise in HGF is an early event in the compensatory response of insulin resistance and also to hepatectomy

We observed that weekly analysis of islet mass, insulin, and circulating HGF levels in DIO-60D rats revealed that

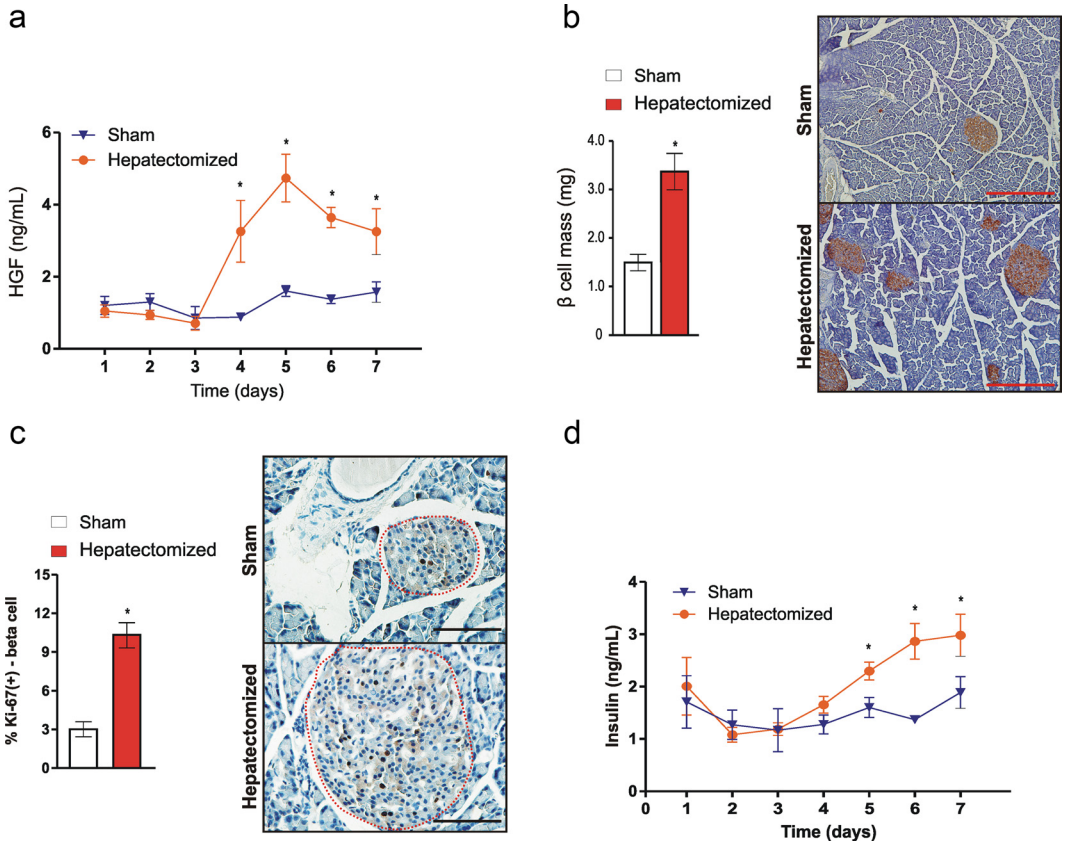

FIG. 4. Partial-hepatectomized (70%) model shows a correlation between HGF levels and β-cell mass. A, Temporal evolution of fasting plasma HGF levels in partial-hepatectomized (70%) and sham-operated rats during 7 d of experiment. B, β-Cell mass and representative images of pancreatic islets stained for insulin, besides the percentage of Ki-67-positive cell in β-cells and representative images of islets stained for Ki-67 (C) from partial-hepatectomized (70%) and sham-operated rats at seventh day. D, Temporal evolution of fasting plasma insulin levels in partial-hepatectomized (70%) and sham-operated rats during 7 d of experiment. *Scale bars*, 100 μm (*black*) and 400 μm (*red*). Data are presented as means ± SEM from six rats per group in experiments that were repeated at least three times. *, $P < 0.05$ *vs.* sham operated.

incremental increases in islet mass, as well as β-cells proliferation, and insulin levels followed an increase in HGF levels (Fig. 2, C–E). Additionally, the DIO-60D rats showed an increase in HGF protein content in the liver and adipose tissue from the fourth week on with the diet (Fig. 3, A–D). These data indicate that a sequential mode occurs, in which an increase in HGF levels seems to precede the compensatory response associated to insulin resistance.

Next, we investigated hepatectomized rats, which carry higher levels of endogenous HGF, and observed the temporal evolution of plasma HGF and insulin levels and β-cell mass, as well as β-cell proliferation, for 7 d. In this study, we noted that from the fourth day after partial hepatectomy (70%), the HGF levels rise and remain high until the seventh day of the experiment (Fig. 4A). This change was followed by incremental changes in islet mass along with augment on Ki-67-positive nuclei of β-cells at seventh day (Fig. 4, B and C). Consequently, we also observed an increase in plasma insulin level from the fifth day after the surgery (Fig. 4D).

Pharmacologic inhibitor of HGF receptor blunts the compensatory mechanism response associated with insulin resistance

SU11274 is a pyrrole indolinone compound, which acts as a HGF receptor (Met) kinase inhibitor thought to specifically block HGF-dependent Met activation. The Met blockage has been associated with inhibition of downstream signaling and biological events typical to Met activity. Indeed, this compound presented low IC_{50} values for Met inhibition; thus, suggesting its high specificity (27). In this regard, when we blocked the action of HGF in DIO-60D rats by using SU11274 for 2 wk (d 45–60), a reduction in islet mass was observed (Fig. 5A). We further hypothesized that a decrease in β-cell proliferation accompanied by increase in β-cell death would likely explain the diminished β-cell mass in DIO-60D rats treated with SU11274. In accordance, here, it was detected a decrease in β-cell proliferation (accessed by Ki-67-stained β-cell). Indeed, we stained pancreas section with TUNEL to detect fragmented DNA and observed that islets from obese rats treated with SU11274 showed increased levels of β-cell

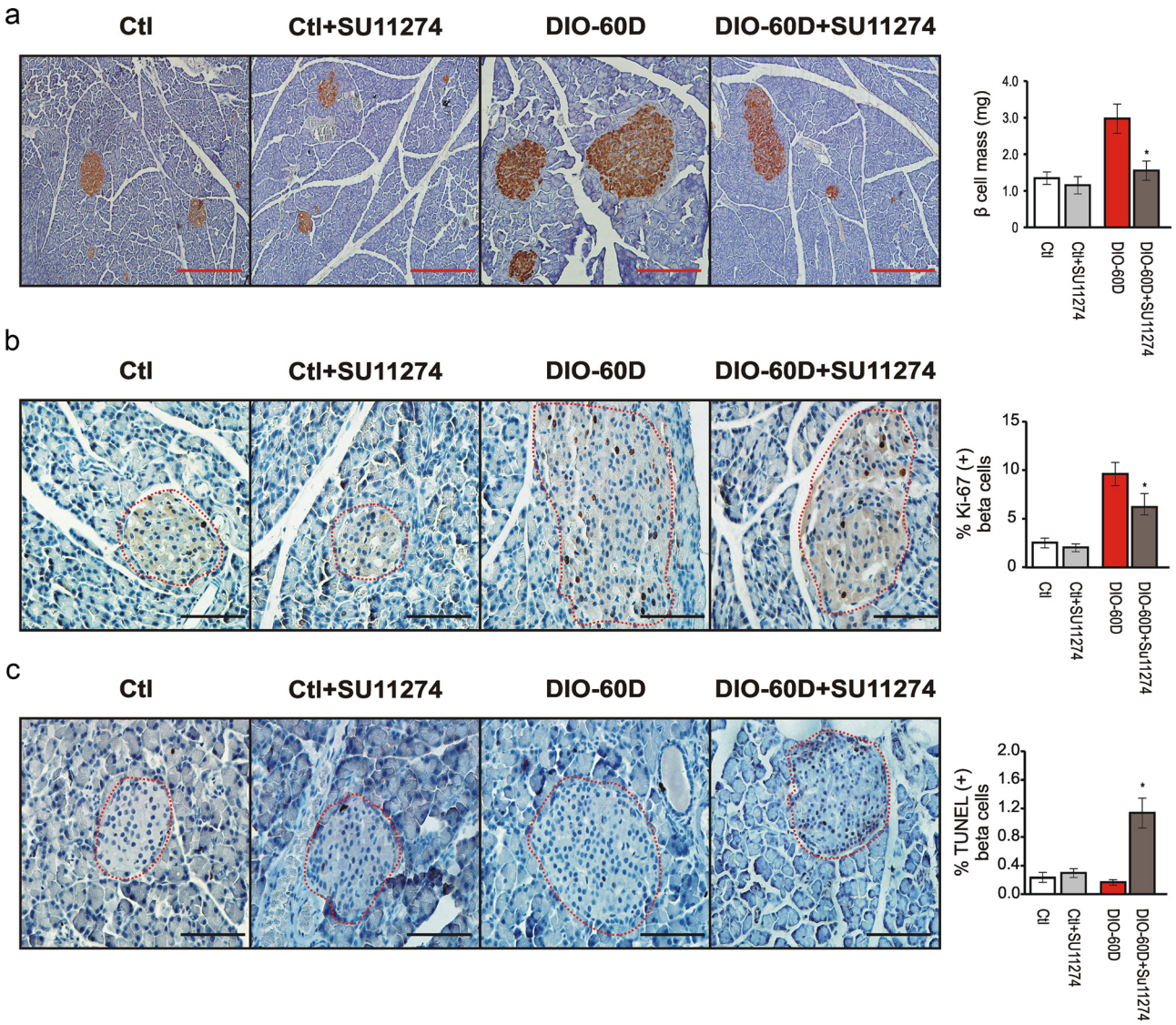

FIG. 5. Pharmacologic inhibitor of HGF receptor blunts the link between insulin resistance and increased mass of islets. A, Effects of the HGF receptor pharmacological inhibitor (SU11274) on β-cell mass, accompanied by representative images of pancreatic islets stained for insulin from DIO rats. B, Percentage of Ki-67-positive cell in β-cells and representative images of islets stained for Ki-67, in addition to (C) percentage of TUNEL-positive cell in β-cells and representative images of islets stained for TUNEL in DIO-60D rats treated with SU11274. *Scale bars*, 100 μm (*black*) and 400 μm (*red*). All experiments were conducted in triplicate (six rats each), and results are expressed as mean ± SEM. *, $P < 0.05$ *vs.* DIO-60D. Ctl, Control.

death (Fig. 5, B and C). Along with these results, an incremental change on insulin levels was no longer observed, suggesting that the compensatory mechanism was shut down (Fig. 6A).

We also measured plasma C-peptide levels to investigate *de novo* insulin production. In accordance with reduction in plasma insulin levels, obese animals treated with SU11274 also showed a decrease in plasma C-peptide concentrations, therefore demonstrating a reduction in *de novo* synthesis of insulin in these animals (Fig. 6B). Additionally, DIO-60D rats treated with SU11274 were more glucose intolerant (Fig. 6C) than untreated DIO-60D rats. This effect in the treated rats was also accompanied by a reduction in insulin levels during the GTT (Fig. 6D).

The animals under a chow diet and treated with SU11274 for the same time showed no change in fasting insulin, glucose, or C-peptide levels or in glucose tolerance. It is important to mention that SU11274 did not change the body weight of the rats on cafeteria or chow diet (Fig. 6E), as well as fat mass (epididymal fat pad content) (data not shown).

HGF signaling in isolated β-cells and in liver

HGF treatment of isolated β-cells from rats for 15 and 30 min resulted in increased levels of insulin receptor sub-

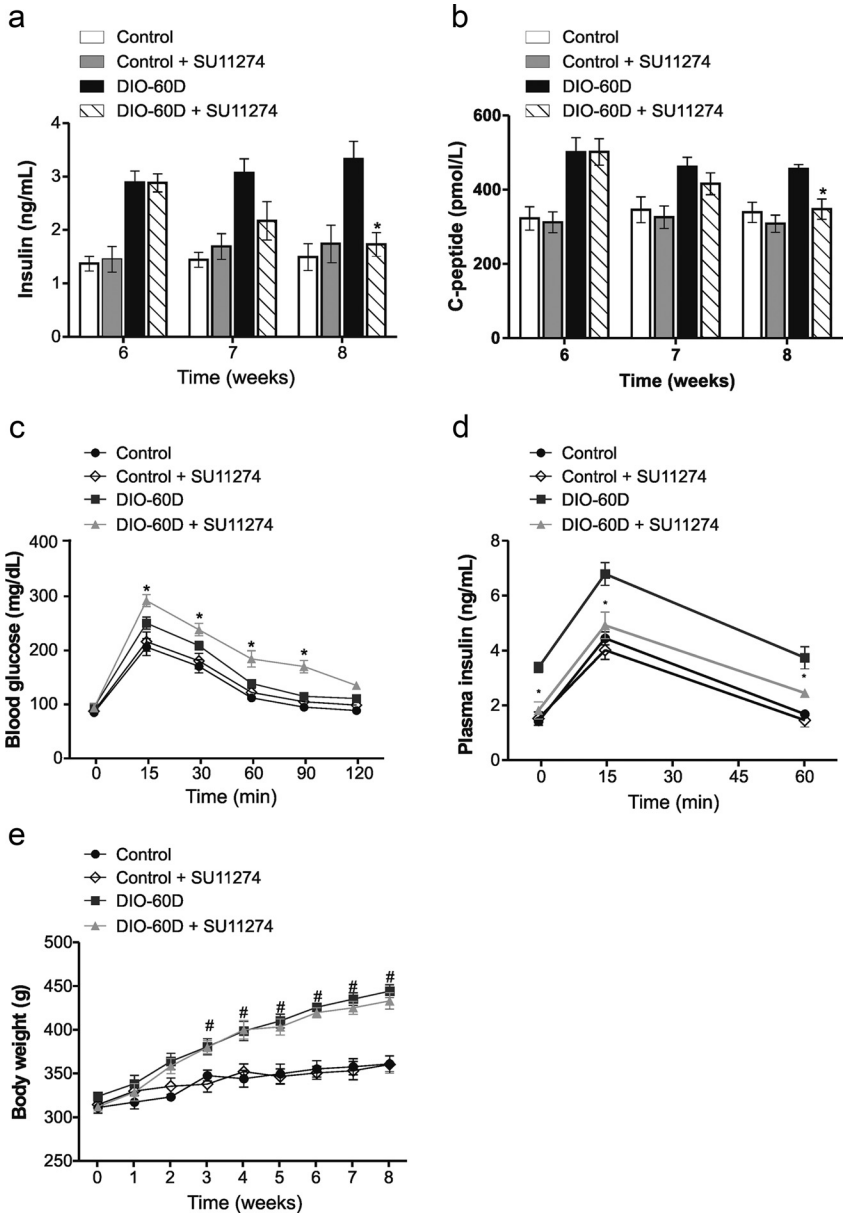

FIG. 6. SU11274 treatment and metabolic parameters of obese and nonobese rats. Fasting plasma insulin (A) and C-peptide levels (B) of obese rats treated with SU11274. The treatment with SU11274 started in the sixth week of the cafeteria or chow diet and lasted until the eighth week. All groups of animals received the respective diet throughout the treatment (*i.e.* from wk 6 to 8). Blood glucose (C) and plasma insulin (D) during ip GTT from control, control + SU11274, DIO-60D, and DIO-60D + SU11274 groups in the eighth week of the experiment. E, Temporal evolution of body weight from control, control + SU11274, DIO-60D, and DIO-60D + SU11274 groups for 8 wk. All experiments were conducted in triplicate (six rats each), and results are expressed as mean ± SEM. *, $P < 0.05$ *vs.* DIO-60D; #, $P < 0.05$ *vs.* control.

strate IRS-2 tyrosine phosphorylation, and also protein kinase B serine 473 (AKT^{ser473}) phosphorylation and ERK phosphorylation (Fig. 7A). In liver, as previously described (5, 17), insulin-induced IR and AKT^{ser473} phosphorylation were reduced in DIO-60D rats, but the infusion of HGF together with insulin significantly induce IR and AKT^{ser473} phosphorylation. On the other side, the blockage of HGF signaling was able to decrease even more the already reduced insulin-induced IR and AKT^{ser473} phosphorylation (Fig. 7B).

Discussion

In most situations of insulin resistance, β-cells compensate for the insulin resistance with an increase in β-cell mass. However, the driving forces behind this compensatory mechanism are incompletely understood. Although it has been demonstrated that there is a neuronal component in pancreatic β-cell proliferation in obesity (2–4), there is also clear evidence that circulating growth factors have a determinant role in this pathophysiological mechanism (1, 28, 29).

It has been assumed that glucose, independent of other factors, can induce an increase in islet mass, and it is known that glucose stimulates β-cell replication (29). However, in most situations of insulin resistance, the increase in islet mass precedes the increase in blood glucose, and also, there is no correlation between the blood glucose levels and islet hyperplasia (1, 30), indicating that other circulating factors independent of glucose likely contribute to the islet growth. Among circulating growth factors, GH, IGF-I, prolactin, placental lactogen, leptin, and HGF can induce islet mass hyperplasia (1, 9, 10, 31, 32). Prolactin and placental lactogen are candidates that can contribute to explain the compensatory response to insulin resistance in pregnancy. Leptin has also been suggested to promote β-cell growth (33); but in genetic leptin-deficient mouse, which also has insulin resistance and islet hyperplasia, this could not be a factor (34). Our data, in accordance with previous data, showed that GH and IGF-I are either normal or low in DIO mice or rats, indicating that these growth factors are unlikely candidates (35, 36). In the present study, we focus on HGF, as previously described, on the basis that it is increased in the most prevalent situation of insulin resistance-obesity, it is produced by the liver under the control of ERK (8), and it is able to

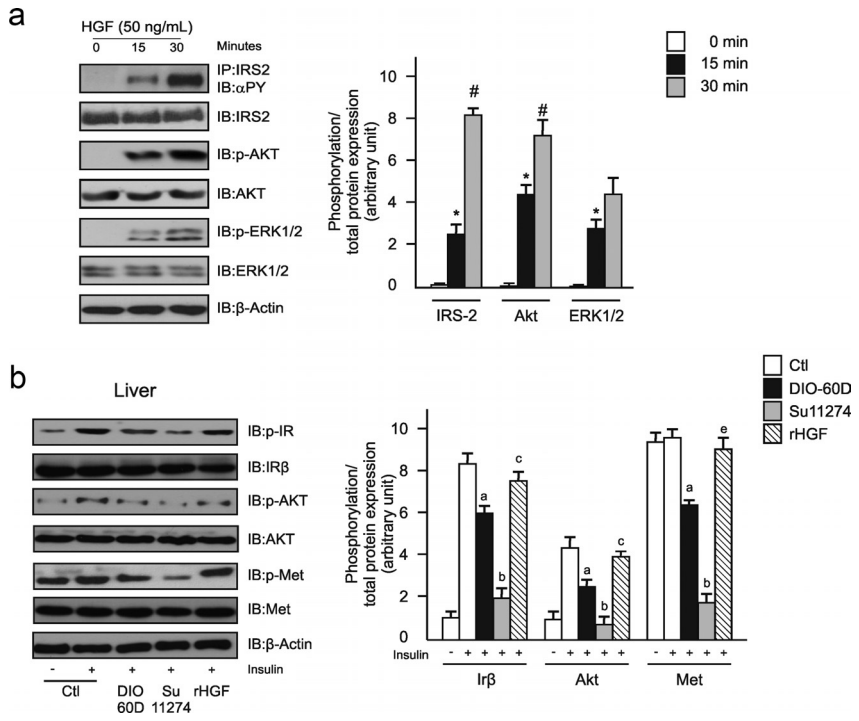

FIG. 7. HGF induces insulin signaling proteins in pancreatic islets and liver of rats. A, The effects of rHGF treatment for 15 or 30 min on phosphorylation levels of IRS-2, AKT^{ser473}, and ERK1/2 in isolated rat islet. B, Insulin-induced phosphorylation of IRβ, AKT^{ser473}, and Met in liver of control (Ctl), DIO-60D, SU11274-treated, and rHGF-treated obese rats. With the exception of the control group, all groups of animals received the cafeteria diet throughout the treatment (*i.e.* from wk 6 to 8). All experiments were conducted in triplicate (six rats each), and results are expressed as mean ± SEM. *, $P < 0.05$ *vs.* 0 min; #, $P < 0.05$ *vs.* 15 min; a, $P < 0.05$ *vs.* control; b, $P < 0.01$ *vs.* DIO-60D; c, $P < 0.05$ *vs.* SU11274-treated group; and e, $P < 0.05$ *vs.* DIO-60D and SU11274-treated group. IB, Immunoblot; IP, immunoprecipitation.

induce islet hyperplasia. Our approach aimed to show a possible cause-effect relationship between increase in circulating HGF levels and compensatory islet hyperplasia/hyperinsulinemia by showing the strength of the association, whether or not is a dose-dependent response, and the temporality, consistency, plausibility, and reversibility of the association.

Our data showed that in different models of insulin resistance, a strong and significant correlation between HGF and islet hyperplasia/insulin levels exists, indicating that this association is consistent. In addition, we observed that an increase in HGF levels seemed to precede the compensatory response associated with insulin resistance and also after partial hepatectomy and that there was a clear dose response for the effect of HGF on increased islet mass.

It is important to mention that in an animal model of insulin resistance, we observed an increase in HGF protein expression in liver and in adipose tissue, which are two tissues very well characterized in insulin-resistant situations. We can then hypothesize that somehow insulin resistance in these tissues might induce an increase in HGF, which will contribute to trigger a compensatory response.

HGF exerts its effects through Met, which is the tyrosine kinase cell surface receptor for HGF. It is important to mention that this receptor is structurally related to the insulin receptor, which is also a tyrosine kinase. Very recently, Fafalios *et al.* (37) demonstrated that Met activation induces a Met-insulin receptor complex that uses substrates of the insulin receptor and amplifies the signal. It is well established that the insulin signaling pathway has an important role in islet cell hyperplasia and increases in islet mass. Our data, in accordance with very recent data obtained in mice liver (37), showed that HGF treatment of isolated β-cells from rats resulted in increased levels of IRS-2 tyrosine phosphorylation and also of AKT^{ser473} and ERK phosphorylation. This indicates that the correlation between HGF and β-cell mass increase may involve these proteins from insulin signaling pathways, which may represent the molecular mechanism by which HGF induces the compensatory response associated with insulin resistance.

Taken together, these data suggest the association between HGF and islet hyperplasia may be a cause-effect phenomenon. This is supported by the importance and consistency of the association, as well as by its dose responsiveness and its temporal component. There is also a molecular mechanism through the insulin signaling pathway that can contribute to explaining this association, and equally important, by blocking the Met receptor we can completely reverse islet hyperplasia and in this way impair glucose tolerance, confirming the reversibility of the phenomenon. In addition, several studies have demonstrated the antiapoptotic effect of HGF (38–40), and our data suggest that such effect was blunted by blockage of the Met receptor.

Another important point in our study is the demonstration that the compensatory effect of HGF is not only related to islets. At least in liver, blocking Met impaired the already reduced insulin-induced insulin signaling in DIO-60D rats. These data strongly support the idea that HGF signaling has a protective role in insulin resistance.

Overall, our data indicate that HGF is a growth factor performing a key role in islet mass increase and hyperinsulinemia in DIO animals and suggest that the HGF-Met axis has an important role on insulin signaling in the liver.

Our study provides additional evidence for a role of HGF in insulin signaling in liver and may greatly contribute toward a better understanding of insulin resistance mechanisms associated with obesity and type 2 diabetes mellitus.

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