The p66^{Shc} Protein Mediates Insulin Resistance and Secretory Dysfunction in Pancreatic β-Cells Under Lipotoxic Conditions

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We evaluated the role of the p66^{Shc} redox adaptor protein in pancreatic β-cell insulin resistance that develops under lipotoxic conditions and with excess body fat. Prolonged exposure to palmitate in vitro or the presence of overweight/obesity augmented p66^{Shc} expression levels and caused an impaired ability of exogenous insulin to increase cellular insulin content and secreted C-peptide levels in INS-1E cells and human and murine islets. In INS-1E cells, p66^{Shc} knockdown resulted in enhanced insulin-induced augmentation of insulin content and C-peptide secretion and prevented the ability of palmitate to impair these effects of insulin. Conversely, p66^{Shc} overexpression impaired insulin-induced augmentation of insulin content and C-peptide secretion in both the absence and presence of palmitate. Under lipotoxic condition, the effects of p66^{Shc} are mediated by a p53-induced increase in p66^{Shc} protein levels and JNK-induced p66^{Shc} phosphorylation at Ser^{36} and appear to involve the phosphorylation of the ribosomal protein S6 kinase at Thr^{389} and of insulin receptor substrate 1 at Ser^{307}, resulting in the inhibition of insulin-stimulated protein kinase B phosphorylation at Ser^{473}. Thus, the p66^{Shc} protein mediates the impaired β-cell function and insulin resistance induced by saturated fatty acids and excess body fat.

The loss of pancreatic β-cell functional mass is a necessary and early event during the development of type 2 diabetes, as well as a potential target for the treatment and potential cure of type 2 diabetes (1). Insulin-secreting β-cells are targeted by insulin itself, which acts in an autocrine manner to promote β-cell viability and function (2). Constitutively secreted insulin is necessary to maintain β-cell glucose sensitivity (3), and alterations in insulin receptor and insulin receptor substrate 1 (IRS-1) result in secretory dysfunction and glucose intolerance (2). However, the physiological relevance of autocrine insulin activity remains somewhat controversial because of the various insulin doses and glucose levels used to assess β-cell secretory function (2,4).

Several metabolic stressors can result in impaired insulin activity or insulin resistance in pancreatic β-cells, as shown in other insulin-targeted tissues. The exposure of pancreatic β-cells to high glucose levels induces the c-Jun N-terminal kinase (JNK)– and extracellular signal-regulated kinase 1/2–mediated inhibitory serine phosphorylation of IRS-1, resulting in inhibition of insulin signaling and consequent impairment of insulin's ability to regulate its own biosynthesis (5). Similarly, chronic exposure of β-cells to elevated free fatty acid levels, particularly long-chain saturated fatty acids (SFAs), can induce the JNK-mediated phosphorylation of IRS-1/2, inhibiting insulin-induced insulin gene transcription (6).

The Src homology 2 domain-containing transforming protein 1, 66-kDa isoform (p66^{Shc}), protein is a redox enzyme capable of sensing and generating reactive oxygen species, which also plays a role in metabolic dysfunction. In previous work (7), we described for the first time the proapoptotic role of p66^{Shc} in INS-1E cells, which depends on its Ser^{36} phosphorylation. Moreover, p66^{Shc} expression levels were found to be increased in INS-1E cells exposed to SFAs, in pancreatic islets isolated from mice fed a high-fat diet, and in human pancreatic islets isolated from overweight/obese subjects (7). Previously,

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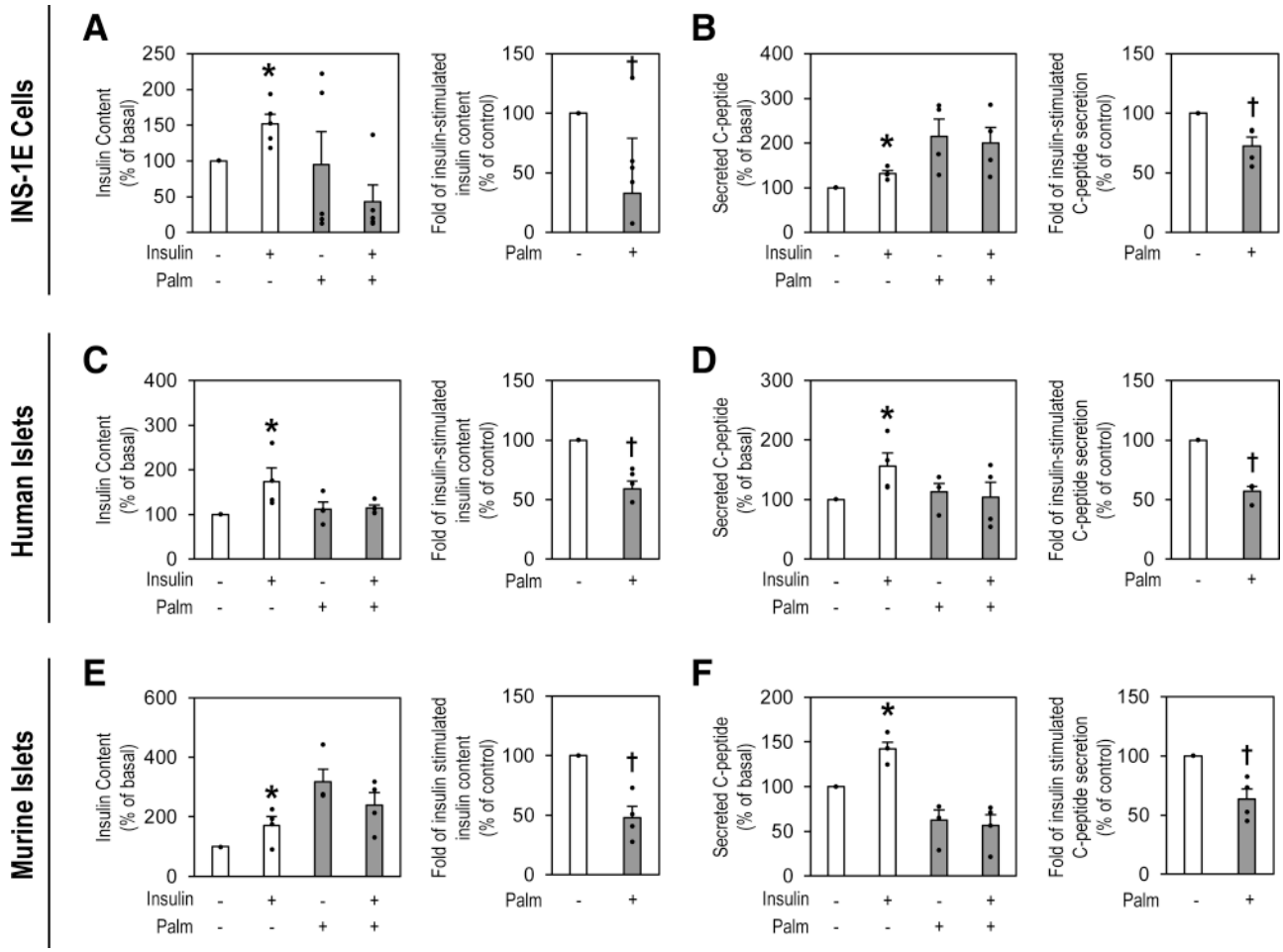

Figure 1—Effects of insulin and palmitate on insulin content and C-peptide release in INS-1E cells and human and murine islets. INS-1E cells (*A* and *B*), human islets (*C* and *D*), and murine islets (*E* and *F*) were cultured in the presence of 0.5 mmol/L palmitate (gray bars) or BSA (white bars), as a control, for 24 h, then incubated in KRBH for 100 min and finally stimulated with 10 nmol/L (*A* and *B*) or 100 nmol/L (*C*–*F*) insulin in fresh KRBH for 40 min. *A*, *C*, and *E*: Insulin content was measured by ELISA, normalized to protein concentration, and expressed as a percentage of untreated control (at least $n = 4$ independent experiments); the fold increase of insulin-stimulated insulin content over control (not treated with palmitate) is also shown. *B*, *D*, and *F*: Secreted C-peptide levels were measured by ELISA, normalized against total protein concentration, and expressed as a percentage of the untreated control (at least $n = 3$ independent experiments); the fold increase of insulin-stimulated secreted C-peptide levels over control (not treated with palmitate) is also shown. Data are mean ± SEM. *$P < 0.05$ vs. control without insulin; †$P < 0.05$ vs. control without palmitate. Palm, palmitate.

p66^{Shc} has been implicated in the development of obesity-induced insulin resistance through the ribosomal protein S6 kinase (S6K)/IRS-1/protein kinase B (AKT) pathway in endothelial cells and adipose tissue (8,9).

To date, the effects of SFAs and excess body fat on insulin action and signaling in pancreatic β-cells have not been explored, and the role of p66^{Shc} in this process has not been investigated. In this study, we provide evidence that p66^{Shc} is crucially involved in lipotoxicity-induced β-cell insulin resistance.

RESEARCH DESIGN AND METHODS

Pancreatic Islet Isolation and Culture

Human islets were isolated from pancreata obtained from multiorgan donors at the Islet Cell Laboratory of the University of Pisa or from pancreatic fragments obtained from patients undergoing pancreatectomy to treat tumors in the ampulla of Vater at the Division of General Surgery of the University Hospital Polyclinic in Bari. Human pancreatic tissues were processed with the approval of the local ethics committee in Pisa and Bari, respectively, after informed consent was obtained. Anonymized patient information is provided in the Human Islets Checklist (Supplementary Material). Twenty male 4–8-week-old C57BL/6 mice were purchased from Charles River Laboratories (Calco, Lecco, Italy). Animal experiments were conducted after obtaining approval from the Ethics Committee of the Genetic Research Centre "Gaetano Salvatore" BioGeM, in accordance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition, and the regulations established in Italy and the European Union for animal experiments. Mouse and human pancreatic

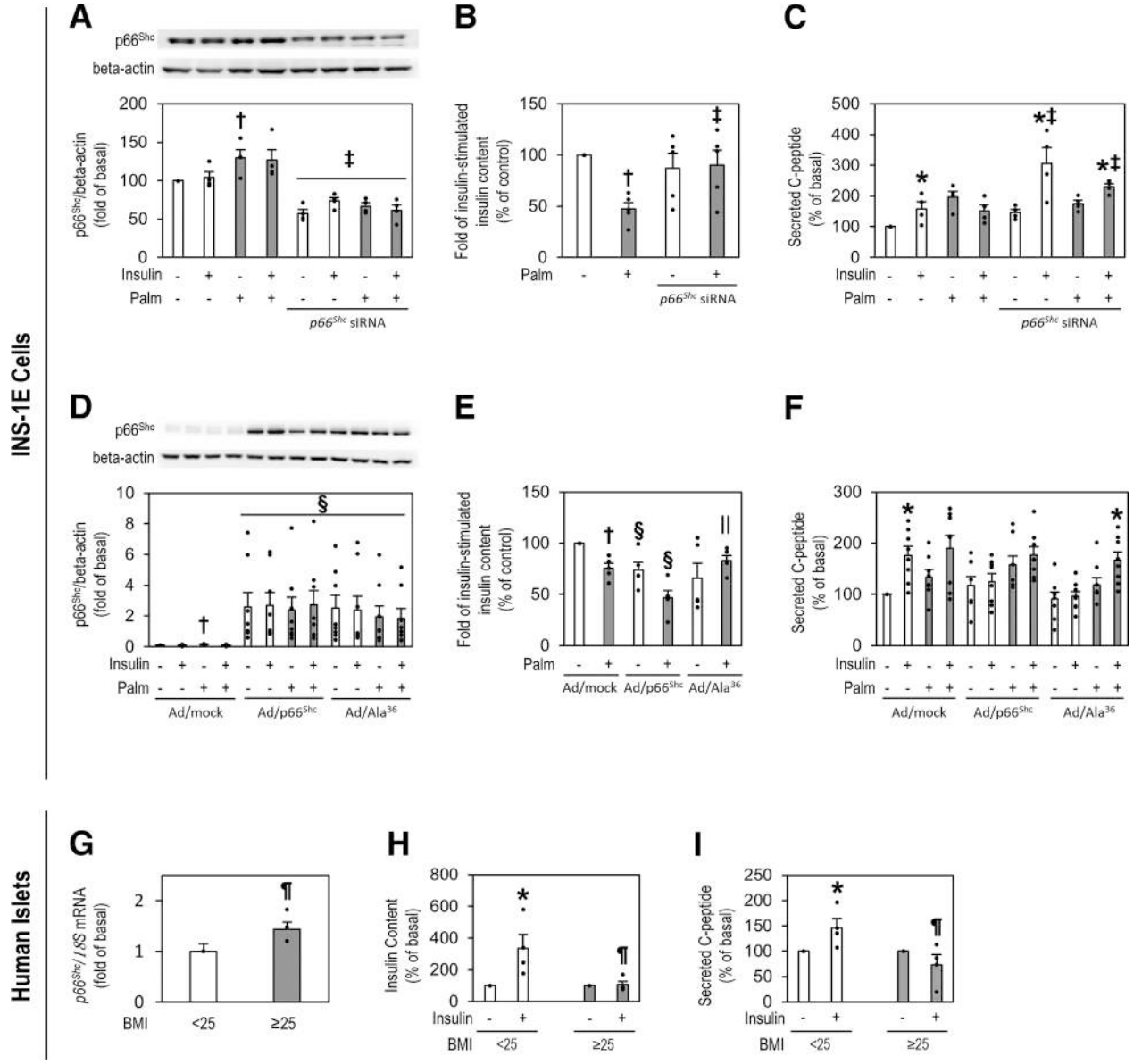

Figure 2—Role of p66^{Shc} protein in lipotoxicity-induced β-cell insulin resistance. *A–C*: INS-1E cells were transfected with *p66^{Shc}* siRNA for 24 h, then cultured in the presence of 0.5 mmol/L palmitate (gray bars) or BSA (white bars), as a control, for an additional 24 h. Successively, cells were incubated in KRBH for 100 min and finally stimulated with 10 nmol/L insulin in fresh KRBH for 40 min. The transfection reagent only was used as control. *A*: Representative immunoblot of p66^{Shc} and β-actin protein levels with the densitometric analysis of the bands, expressed as relative optical density. Values were corrected using total β-actin levels as a loading control and normalized against untreated control ($n = 4$ independent experiments). *B*: Insulin content was measured with a specific ELISA, normalized to protein concentration, and shown as fold increase of insulin-stimulated insulin content over control (not treated with palmitate) ($n = 5$ independent experiments). *C*: Secreted C-peptide levels were measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the untreated control ($n = 4$ independent experiments). *D–F*: INS-1E cells were infected with a recombinant adenoviral vector encoding p66^{Shc} (Ad/*p66^{Shc}*), a phosphorylation-defective p66^{Shc} protein containing a Ser^{36} to Ala mutation (Ad/Ala^{36}), or empty adenovirus (Ad/mock) for 24 h. Cells were then cultured in the presence of 0.5 mmol/L palmitate (gray bars) or BSA (white bars), as a control, for 24 h and then incubated in KRBH for 100 min and finally stimulated with 10 nmol/L insulin in fresh KRBH for 40 min. *D*: Representative immunoblot of p66^{Shc} and β-actin protein levels with the densitometric analysis of bands (expressed as the relative optical density), corrected using total β-actin levels as a loading control, and normalized against control ($n = 8$ independent experiments). *E*: Insulin content was measured by ELISA, normalized to total protein concentration, and shown as fold increase over control (not treated with palmitate) ($n = 5$ independent experiments). *F*: Secreted C-peptide levels were measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the control ($n = 8$ independent experiments). *G*: *p66^{Shc}* mRNA levels in pancreatic islets from lean (BMI <25 kg/m^2, white bars) and overweight/obese (BMI ≥25 kg/m^2, gray bars) subjects ($n = 4$ independent experiments). *H* and *I*: Pancreatic islets were isolated from lean or overweight/obese subjects, incubated in KRBH for 100 min, and finally stimulated with 100 nmol/L insulin in fresh KRBH for 40 min. *H*: Insulin content was measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the untreated control ($n = 4$ independent experiments). *I*: Secreted C-peptide levels were measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the untreated control ($n = 4$ independent experiments). Data are mean ± SEM. *$P < 0.05$ vs. control without insulin; †$P < 0.05$ vs. control without palmitate; ‡$P < 0.05$ vs. transfection reagent; §$P < 0.05$ vs. Ad/mock; ||$P < 0.05$ vs. Ad/*p66^{Shc}*; ¶$P < 0.05$ vs. BMI <25 kg/m^2. Palm, palmitate.

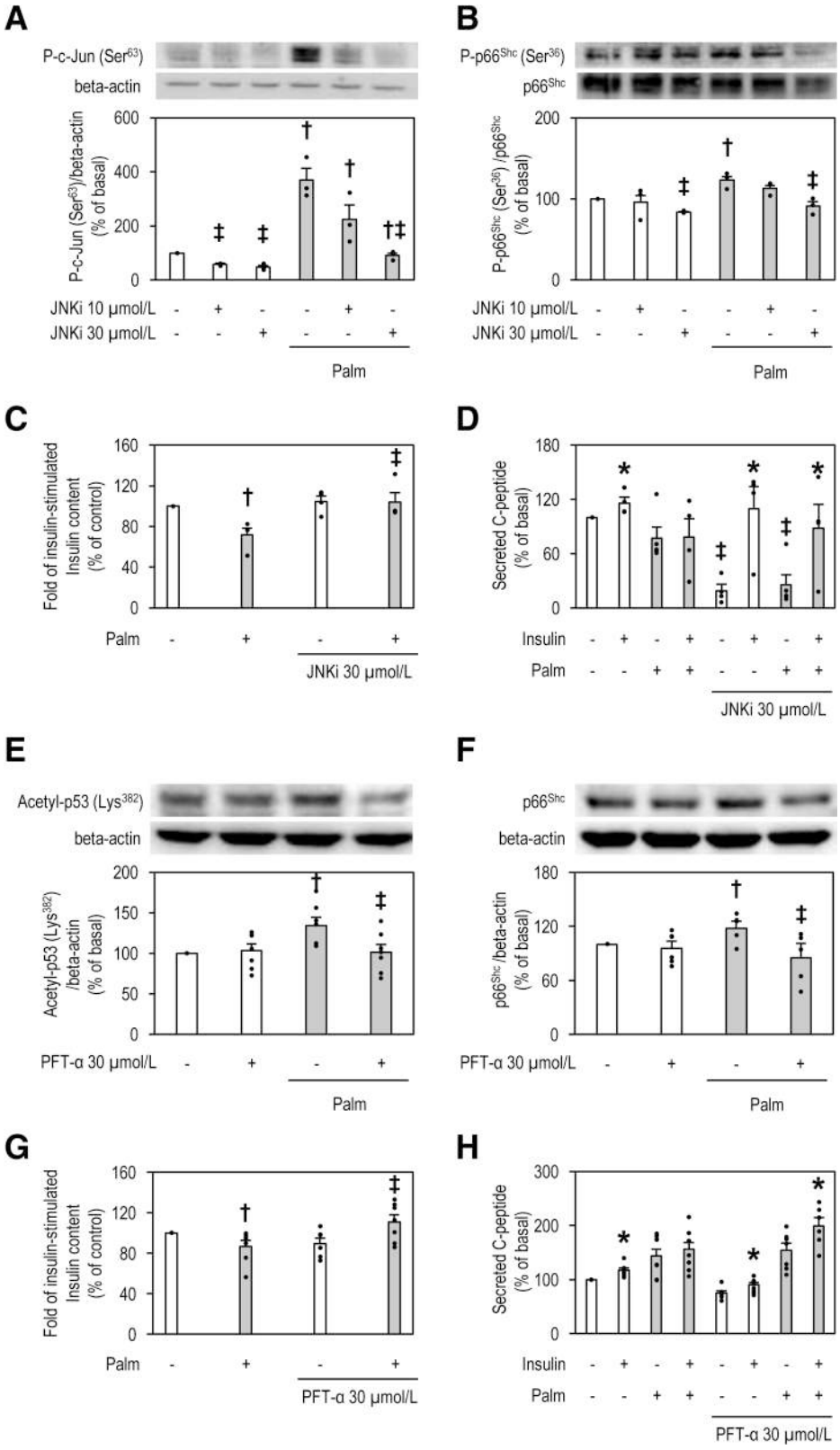

Figure 3—Role of JNK and p53 in palmitate effects on insulin-induced insulin content and C-peptide release in INS-1E cells. *A* and *B*: Cells were stimulated with 10 or 30 μmol/L JNK inhibitor (JNKi) (SP600125) or dimethyl sulfoxide (DMSO) as control for 2 h, then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for 24 h. *A*: Representative immunoblot of c-Jun (Ser^{63}) phosphorylation and β-actin protein levels, quantified by densitometric analysis of the bands and expressed as relative optical density. C-Jun (Ser^{63}) phosphorylation values were corrected using total β-actin levels as a loading control and normalized against untreated control ($n = 3$ independent experiments). *B*: Representative immunoblot of $p66^{Shc}$ (Ser^{36}) phosphorylation and protein levels, quantified by densitometric analysis of the bands and expressed as relative optical density. The $p66^{Shc}$ (Ser^{36}) phosphorylation values were normalized against $p66^{Shc}$ protein,

islets were isolated and cultured as described in Li et al. (10), Lupi et al. (11), and the Supplementary Methods.

INS-1E Cell Culture

Rat insulin secreting INS-1E cells (passage 15–30) were a gift from C.B. Wollheim (University of Geneva, Geneva, Switzerland). INS-1E cells were cultured as described in the Supplementary Methods.

Pancreatic Islets and INS-1E Cell Treatment

Human (preparation 13–17 in the Human Islets Checklist [Supplementary Material]) and mouse islets and INS-1E cells were treated with 0.5 mmol/L palmitate or oleate solution (Sigma-Aldrich Inc., St. Louis, MO) or with the same volume of a 10% w/v fatty acid–free BSA solution as a control. After 24 h, islets and cells were stimulated with 10–100 nmol/L human recombinant insulin (Roche Diagnostics, Mannheim, Germany) for different times. To achieve $p66^{Shc}$ knockdown, INS-1E cells were transfected with 100 nmol/L $p66^{Shc}$-targeted siRNA (QIAGEN, Hilden, Germany) using Lipofectamine RNAiMAX reagent and Opti-MEM medium (Thermo Fisher Scientific, Waltham, MA) for 48 h (7). The overexpression of $p66^{Shc}$ was achieved by transducing INS-1E cells with recombinant adenoviruses carrying a construct encoding the $p66^{Shc}$ protein (7). Where indicated, INS-1E cells were transfected with an adenoviral $p66^{Shc}$ construct harboring a Ser^{36} to Ala^{36} mutation. An empty adenovirus vector was used as control (mock). See the Supplementary Methods for further details.

Measurement of Insulin Content and Secreted C-Peptide Levels

After a wash with PBS 1×, murine and human pancreatic islets and INS-1E cells were incubated in Krebs-Ringer bicarbonate HEPES buffer (KRBH) (0.1% w/v BSA, 3 mmol/L glucose, 114 mmol/L NaCl, 4.4 mmol/L KCl, 1 mmol/L $MgSO_4$, 29.5 mmol/L $NaHCO_3$, 1.28 mmol/L $CaCl_2$, and 10 mmol/L HEPES, pH 7.4, all from Sigma-Aldrich) (12) for 100 min, followed by stimulation with insulin (10–100 mol/L) diluted in fresh KRBH solution for 40 min. To evaluate insulin content, cells were washed twice with PBS 1×, then mechanically lysed in a nondenaturing lysis buffer (13). The level of C-peptide released in the medium, as a measure of endogenously produced insulin, and insulin content were measured using ELISAs specific for mouse (Shibayagi Co., Ltd., Ishihara, Japan), humans, and rats (Mercodia AB, Uppsala, Sweden).

Immunoblotting

Cell lysates were obtained and analyzed by immunoblotting assays (13) (Supplementary Methods). Supplementary Table 1 lists the antibodies used.

Quantitative Real-Time PCR

RNA isolation, cDNA synthesis, and quantitative real-time PCR were conducted as previously reported (13) (Supplementary Methods). Supplementary Table 2 lists the primer sequences.

Statistical Analysis

Data are presented as the mean ± SEM. Statistical analysis was performed using the two-tailed unpaired Student *t* test. Significance was set at $P < 0.05$.

Data and Resource Availability

The data sets generated and/or analyzed during the study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during this study.

RESULTS

Insulin Increases Its Own Content and C-Peptide Release in INS-1E Cells and Human and Murine Islets

Insulin stimulation increased insulin content and C-peptide secreted levels in INS-1E cells (Fig. 1*A* and *B*) and human (Fig. 1*C* and *D*) and murine (Fig. 1*E* and *F*) pancreatic islets. This was associated with increased gene expression levels of insulin 1, but not insulin 2, in INS-1E cells (Supplementary Fig. 1).

Palmitate Impairs Insulin Effects in INS-1E Cells and Human and Murine Islets

Prior exposure of cells and islets to the SFA palmitate impaired the stimulatory effects of insulin on its own cellular content (Fig. 1*A*, *C*, and *E*) and C-peptide secretion

then against untreated control ($n = 3$ independent experiments). *C* and *D*: Cells were stimulated with 30 μmol/L SP600125 or DMSO as control for 2 h, then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for 24 h before being incubated in KRBH for 100 min and finally stimulated with 10 nmol/L insulin in fresh KRBH for 40 min. *E* and *F*: Cells were stimulated with 30 μmol/L pifithrin-α (PFT-α) or DMSO as control for 1 h, then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for 24 h. *E*: Representative immunoblot of p53 (Lys^{382}) acetylation and β-actin protein levels, quantified by densitometric analysis of the bands and expressed as relative optical density. The p53 (Lys^{382}) acetylation values were corrected using total β-actin levels as a loading control and normalized against untreated control ($n = 6$ independent experiments). *F*: Representative immunoblot of $p66^{Shc}$ protein levels, quantified by densitometric analysis of the bands and expressed as relative optical density. The $p66^{Shc}$ values were normalized against β-actin levels, then against untreated control (at least $n = 5$ independent experiments). *G* and *H*: Cells were stimulated with 30 μmol/L PFT-α or DMSO as control for 1 h, then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for 24 h before being incubated in KRBH for 100 min and finally stimulated with 10 nmol/L insulin in fresh KRBH for 40 min. *C* and *G*: Insulin content was measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the untreated control (at least $n = 3$ independent experiments). *D* and *H*: Secreted C-peptide levels were measured by ELISA, normalized to total protein concentration, and expressed as a percentage of the untreated control (at least $n = 3$ independent experiments). Data are mean ± SEM. *$P < 0.05$ vs. control without insulin; †$P < 0.05$ vs. control without palmitate; ‡$P < 0.05$ vs. control without inhibitor. P, phosphorylated; Palm, palmitate.

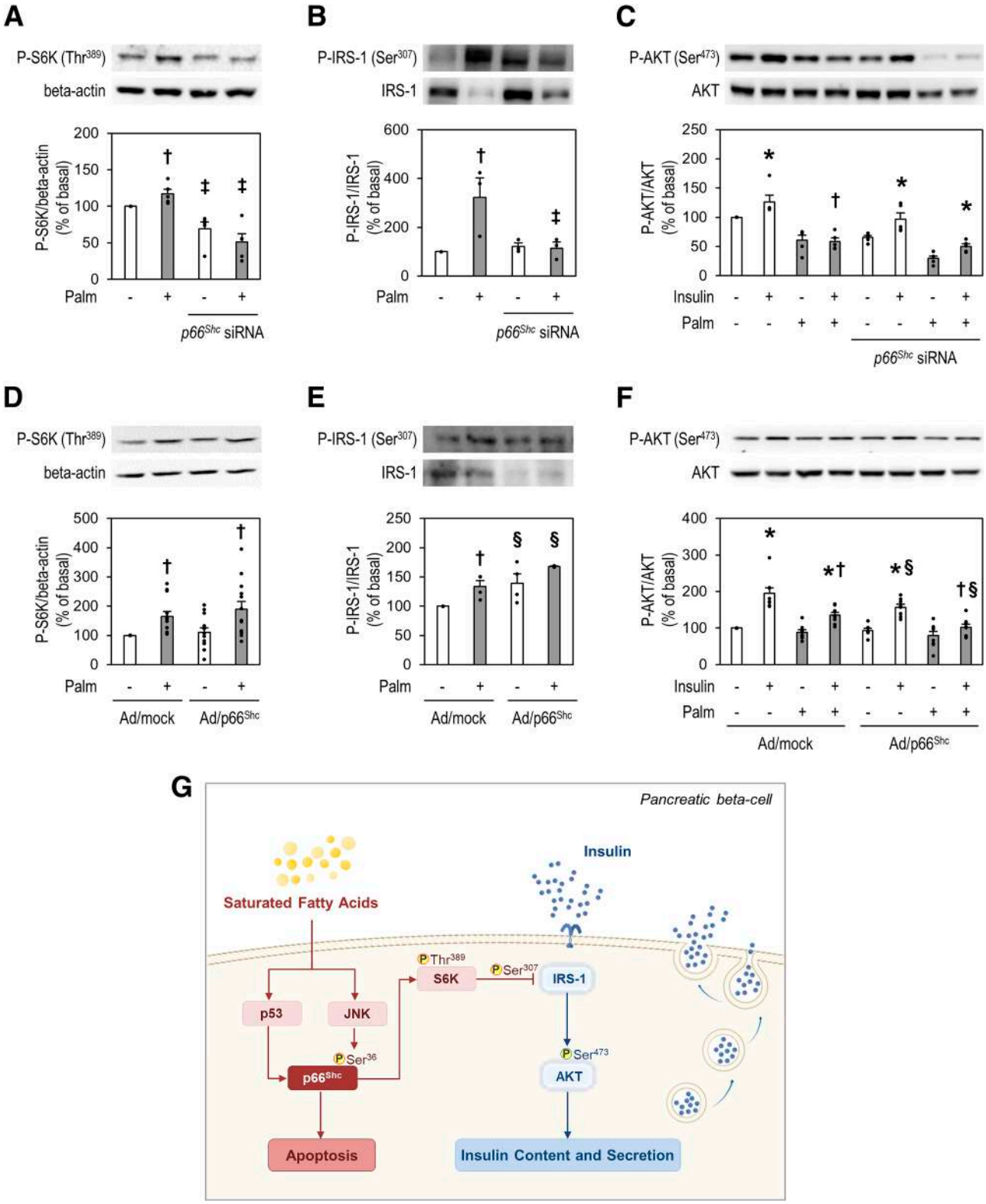

Figure 4— Molecular mechanisms by which p66^{Shc} mediates palmitate-induced insulin resistance in β-cells. *A–C*: INS-1E cells were transiently transfected with *p66^{Shc}* siRNA for 24 h, then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for an additional 24 h and stimulated with 100 nmol/L insulin for 10 min. Only the transfection reagent was used as a control. S6K (Thr^{389}) (*A*), IRS-1 (Ser^{307}) (*B*), and AKT (Ser^{473}) (*C*) phosphorylation was measured by immunoblotting and quantified by densitometric analysis of the related bands, normalized against β-actin, total IRS-1, and total AKT, respectively. The resulting relative optical density values are expressed as percentages of the untreated control. *D–F*: INS-1E cells were infected with recombinant adenoviral vector encoding p66^{Shc} (Ad/*p66^{Shc}*) or with empty adenovirus (Ad/mock) for 24 h. Cells were then cultured in the presence of 0.5 mmol/L palmitate (or BSA as control) for 24 h and stimulated with 100 nmol/L insulin for 10 min. S6K (Thr^{389}) (*D*), IRS-1 (Ser^{307}) (*E*), and AKT (Ser^{473}) (*F*) phosphorylation was measured by

(Fig. 1*B*, *D*, and *F*). This occurred also in INS-1E cells exposed to the unsaturated fatty acid oleate (Supplementary Fig. 2*A* and *B*).

Palmitate-Induced Impairment of Insulin Effects in INS-1E Cells Is Mediated by p66^{Shc}

The *p66^{Shc}* silencing approach resulted in a 40–50% reduction of p66^{Shc} protein levels in all experimental conditions compared with control cells treated with vehicle only (Fig. 2*A*). Under these conditions, the palmitate-induced impairment of the insulin stimulatory effect on its own cellular content was largely prevented (Fig. 2*B*). In addition, *p66^{Shc}* knockdown resulted in increased insulin-induced C-peptide secretion in both the absence and presence of palmitate compared with control cells (Fig. 2*C*).

Conversely, p66^{Shc} overexpression in INS-1E cells resulted in a marked increase of p66^{Shc} protein levels (Fig. 2*D*) and in the inhibition of the ability of insulin to augment insulin content (Fig. 2*E*) and secreted C-peptide levels (Fig. 2*F*) in cells not exposed to palmitate. In the presence of palmitate, these effects of insulin were impaired further (Fig. 2*E* and *F*).

Following overexpression of the p66^{Shc}Ala^{36} mutant protein (Fig. 2*D*), which is unable to undergo phosphorylation at the key Ser^{36} site (7), the palmitate-induced impairment of the effects of insulin to enhance its own content (Fig. 2*E*) and C-peptide secretion (Fig. 2*F*), respectively, were no longer observed.

Islets From Overweight/Obese Subjects Show Elevated *p66^{Shc}* mRNA Expression and Reduced Response to Insulin

In islets (preparation 1–12 in the Human Islets Checklist [Supplementary Material]) obtained from overweight/obese subjects, *p66^{Shc}* mRNA levels were higher than in islets from lean subjects (Fig. 2*G*). In addition, the effects of insulin to increase insulin content (Fig. 2*H*) and secreted C-peptide (Fig. 2*I*) were blunted.

Role of JNK, Tumor Suppressor Protein 53, and Oxidative Stress in SFA-Induced Insulin Resistance in INS-1E cells

The inhibition of JNK (Fig. 3*A*) and tumor suppressor protein 53 (p53) protein activity (Fig. 3*E*) reduced palmitate-induced p66^{Shc} phosphorylation at Ser^{36} (Fig. 3*B*) and p66^{Shc} protein expression (Fig. 3*F*), respectively, and prevented the palmitate effects on insulin-induced insulin content (Fig. 3*C* and *G*) and C-peptide secretion (Fig. 3*D* and *H*). Of note, the reduction in reactive oxygen species levels impaired insulin effects under both basal and palmitate-stimulated conditions (Supplementary Fig. 3).

p66^{Shc} Mediates the Palmitate-Induced Alterations of Insulin Signaling Through S6K/IRS-1/AKT in INS-1E Cells

Palmitate treatment increased S6K Thr^{389} phosphorylation (Fig. 4*A*) and IRS-1 Ser^{307} phosphorylation, resulting in both reduced IRS-1 protein levels (Fig. 4*B*) and impaired ability of insulin to stimulate AKT Ser^{473} phosphorylation (Fig. 4*C*). However, in β-cells with *p66^{Shc}* knockdown, the effects of palmitate on phosphorylation of S6K (Fig. 4*A*) and IRS-1 (Fig. 4*B*) proteins were abrogated, and this enhanced insulin-stimulated AKT Ser^{473} phosphorylation (Fig. 4*C*). Conversely, p66^{Shc} overexpression did not alter S6K Thr^{389} phosphorylation (Fig. 4*D*) and exacerbated the palmitate-mediated IRS-1 Ser^{307} phosphorylation and reduction of IRS-1 protein levels (Fig. 4*E*), leading to complete abrogation of insulin-stimulated AKT Ser^{473} phosphorylation (Fig. 4*F*). Of note, p66^{Shc} overexpression also increased IRS-1 Ser^{307} phosphorylation, reduced IRS-1 protein levels (Fig. 4*E*), and impaired insulin-stimulated AKT Ser^{473} phosphorylation (Fig. 4*F*) in cells not exposed to palmitate.

DISCUSSION

We show that acute insulin stimulation increased cellular insulin content and C-peptide release in rat INS-1E cells and human and murine pancreatic islets. This occurred independently of glucose interference, as all experiments were conducted under low-glucose conditions. Moreover, both exposure to palmitate in vitro and presence of excess body fat in vivo resulted in the impaired ability of insulin to stimulate its biosynthesis and secretion in β-cells and murine and human pancreatic islets in vitro and in human pancreatic islets ex vivo, respectively. These results mirror and corroborate the previous observation of blunted insulin-induced insulin gene transcription following exposure of mouse pancreatic islets to palmitate (6). Our results in INS-1E cells indicate that palmitate alters the insulin signaling pathway by activating the phosphorylation of Thr^{389} in the S6K protein and the inhibitory phosphorylation of Ser^{307} in IRS-1, which are known to result in reduced IRS-1 protein levels and impairment of insulin-induced AKT activation (9,14). These results support the emerging concept that lipotoxicity is capable of inducing an insulin resistant

immunoblotting and quantified by densitometric analysis of the related bands, normalized against β-actin, total IRS-1, and total AKT, respectively. The resulting relative optical density values are expressed as percentages of the untreated control. Number of independent experiments was 5 (*A* and *C*), 3 (*B* and *E*), 13 (*D*), and 8 (*F*). Data are mean ± SEM. *$P < 0.05$ vs. control without insulin; †$P < 0.05$ vs. control without palmitate; ‡$P < 0.05$ vs. transfection reagent; §$P < 0.05$ vs. Ad/mock. *G*: SFAs increase p66^{Shc} levels and its phosphorylation in Ser^{36} through the p53 protein and JNK kinase, respectively, thus inducing apoptosis and insulin resistance in pancreatic β-cells (7). In addition, p66^{Shc} activation induces the phosphorylation of Thr^{389} in the S6K protein and the inhibitory phosphorylation of Ser^{307} in IRS-1, which in turn reduce IRS-1 protein levels and impair insulin-induced AKT activation, thus impairing the ability of insulin to increase its own cellular content and C-peptide secretion in pancreatic β-cells. P, phosphorylated; Palm, palmitate.

state in pancreatic β-cells similar to other classical insulin target tissues.

Importantly, we show for the first time that p66^{Shc} plays a key role in this lipotoxicity-mediated insulin resistance in pancreatic β-cells, since *p66^{Shc}* knockdown and overexpression respectively prevented and worsened palmitate-induced insulin resistance by reducing and boosting palmitate-induced activation of the S6K/IRS-1/AKT pathway (Fig. 4*G*). These results resemble the effects of lipotoxicity in endothelial and adipose cells (8,9). Experiments with overexpression of the wild-type and mutant (Ala^{36}) forms of p66^{Shc} suggested that p66^{Shc} phosphorylation at Ser^{36} is required to mediate the effects of palmitate on insulin action. Furthermore, we found that palmitate-induced insulin resistance in INS-1E cells is mediated by p53 and JNK proteins. These results resemble the mechanisms already demonstrated for palmitate-induced β-cell apoptosis (7).

The ex vivo experiments performed in human pancreatic islets isolated from donors without diabetes support the involvement of p66^{Shc} in lipotoxicity-induced β-cell insulin resistance in human obesity, as both elevated *p66^{Shc}* mRNA expression and impaired insulin effects were observed in islets obtained from overweight/obese subjects compared with islets obtained from lean subjects. Overall, p66^{Shc} can be identified as a crucial mediator of lipotoxicity-promoted β-cell failure in the context of human obesity. Interestingly, elevated p66^{Shc} levels have also been found in the liver of patients with alcoholic and nonalcoholic steatohepatitis (15,16), in the heart in response to cardiac dysfunction (17) and hyperglycemia (18), and in endothelial cells exposed to dyslipidemia (19), hyperglycemia (20), and proinflammatory cytokines (21). Taken together, this evidence suggests that p66^{Shc} is ubiquitously involved in multiorgan damage in response to metabolic stressors.

The physiological significance of the effects of autocrine basal insulin in vivo are still debated (2,4,22–24), even though under low-glucose conditions insulin could regulate its own content and release, representing a physiological mechanism to replenish insulin stores and make β-cells prone to rapidly satisfy insulin demand in response to secretagogues (3,22,25–27). Of note, insulin signaling in β-cells can be triggered by insulin binding at high concentrations to insulin receptor, IGF-I receptor, or insulin receptor/IGF-I receptor hybrids (28). It has been suggested that insulin promotes its own biosynthesis and secretion mainly through insulin receptor/IRS-1 signaling (29–31). On the other hand, insulin binding to IGF-I receptor mainly leads to the activation of IRS-2, which could play a major role in the regulation of β-cell mass (32,33).

In conclusion, p66^{Shc} mediates lipotoxicity-induced β-cell insulin resistance, an emerging mechanism for β-cell failure in obesity and type 2 diabetes. Of interest, glucagon-like peptide 1 receptor agonists, which reportedly inhibit palmitate-induced apoptosis by preventing JNK phosphorylation (34), also inhibited p66^{Shc} phosphorylation on Ser^{36} (7) and may thus use this mechanism as well to enhance insulin secretion. Since the loss of pancreatic β-cell functional mass is a necessary and early event in type 2 diabetes (1), the combined effects of p66^{Shc} on β-cell secretory function and survival suggest that this protein may represent a potential target for the prevention or treatment type 2 diabetes onset or progression.

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Prior Presentation. Parts of this study were presented in abstract form at the 79th Scientific Sessions of the American Diabetes Association, San Francisco, CA, 7–11 June 2019; 40th National Meeting of the Italian Society of Endocrinology, Rome, Italy, 29 May–1 June 2019; 27th National Meeting of the Italian Society of Diabetology, Rimini, Italy, 16–19 May 2018; 53rd Annual Meeting of the European Association for the Study of Diabetes, Lisbon, Portugal, 11–15 September 2017; International Symposium on Insulin Receptor and Insulin Action, Nice, France, 20–22 April 2017; and 39th National Meeting of the Italian Society of Endocrinology, Rome, Italy, 21–24 June 2017.

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