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Original Research Article

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ABSTRACT

Aim. - Alteration of functional beta-cell mass in adults can be programmed by adverse events during fetal life. Previously, it was demonstrated that high glucocorticoid (GC) levels during fetal life participates in this programming by inhibition of beta-cell development. More specifically, it GC levels stimulate expression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), a transcriptional co-regulator of the GC receptor (GR), which *per se* impairs beta-cell mass and function when overexpressed. As PGC-1α is also a potent inducer of mitochondrial biogenesis, our study aimed to determine how PGC-1α modifies mitochondrial function in beta cells and how it might regulate insulin secretion.

Methods. - Beta-cell function was studied in mice overexpressing PGC-1α specifically in beta cells and in MIN6 cells overexpressing PGC-1α *in vitro*.

Results. – PGC-1α overexpression in beta cells *in vivo* leads to a reduced beta-cell mass early in fetal life, whereas PGC-1α overexpression *in vitro* stimulates mitochondrial biogenesis and respiratory activity without improving ATP production, while increasing oxidative stress and impairing insulin secretion in response to glucose. While oxidative stress with PGC-1α overexpression in beta cells activates AMPK, it has also been revealed that blocking such oxidative stress or AMPK activation may restore insulin secretion.

Conclusion. - PGC-1α induces oxidative stress, which disrupts insulin secretion by AMPK activation. Thus, control of oxidative or energetic stress in beta cells may help to restore insulin secretion.

Keywords: Beta cell; Energetic stress; Insulin secretion; Oxidative stress; PGC-1

INTRODUCTION

Type 2 diabetes (T2D) is characterized by two major defects: insulin resistance in insulin-responsive organs and impaired insulin secretion due to decreased functional beta-cell mass. The origins of these two T2D factors are multiple and far from being fully understood. Apart from genetic predisposition and the influence of lifestyle, the fetal environment now appears to be a key component in the risk of developing metabolic diseases in adulthood. The concept of fetal programming stipulates that changes in the fetal environment alter the development of organs, leading to dysfunction in adult life and contributing to the onset of adult diseases. Our laboratory, which focuses on clarification of fetal programming in T2D, has shown that high glucocorticoid (GC) levels in rodents during fetal life participate in this programming by inhibiting the development of beta cells [1]. This inhibition eventually leads to a reduced beta-cell mass, impaired insulin secretion and glucose intolerance in adult animals. Thus, excess GC levels during fetal life programme T2D by targeting beta cells.

GCs are pleiotropic hormones that regulate several key adaptive processes. Synthesized from cholesterol and secreted by the adrenal glands in response to stimuli such as stress and fasting, they act on target organs by binding to cytoplasmic receptors and subsequently migrating to the nucleus. It then acts as a transcription factor and regulates the expression of target genes through recruitment of other proteins, such as transcriptional co-regulators. PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α) is a crucial transcriptional regulator involved in the metabolic effects of GC receptors (GRs) in the liver [2], and also co-regulates other nuclear receptors, such as PPARs (peroxisome proliferator-activated receptors) in adipose tissue [2]. Previously, our team demonstrated that PGC-1α is involved in the fetal programming of beta-cell dysfunction: first, GCs stimulate expression of PGC-1α; second, PGC-1α forms a complex with GRs that bind to insulin promoter type 1 (Pdx1), a key beta-cell transcription factor, leading to a clear reduction of Pdx1 expression; and, finally, PGC-1α overexpression in beta cells leads to glucose intolerance, impaired glucose-stimulated insulin secretion (GSIS), decreased beta-cell mass and beta-cell hypotrophy in adult mice [3]. Surprisingly, overexpression of PGC-1α just during fetal life was sufficient to induce beta-cell dysfunction in adults. Thus, PGC-1α can be considered a major regulator of beta-cell mass and function.

Beta-cell function is highly dependent on energy production. In fact, insulin secretion is controlled by the adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio. Glucose, a source of energy production, is transported to beta cells by specific transporters (GLUT2 in rodents and GLUT1 in humans). Glucose is oxidized and its catabolism activates

the respiratory chain in mitochondria, which increases the ATP/ADP ratio. This latter event triggers closure of ATP-dependent potassium (K) channels, leading to membrane depolarization allowing the opening of calcium channels, which eventually induces insulin exocytosis. Beta cells in diabetic animals display deficiencies of glucose oxidation associated with reduced expression of GLUT2 and of several enzymes involved in glucose catabolism (glucokinase, glycerol-3-phosphate dehydrogenase). This results in a decreased ATP/ADP ratio and insulin release [4]. Interestingly, PGC-1α is a potent inducer of mitochondrial biogenesis [5], and can bring about the expression of mitochondrial genes that are mainly controlled by two nuclear transcription factors: TFAM (mitochondrial transcription factor A) and TFB (transcription factor B) [5, 6]. As this crucial role of PGC-1α is under the control of cellular energy production, our present study aimed to determine the consequences of PGC-1α overexpression on energy production and its potential link with altered insulin secretion induced by PGC-1α.

In fact, the present study has confirmed that PGC-1α overexpression, specifically in beta cells during fetal life, induces oxidative stress and decreases beta-cell mass in fetuses and newborns, and that PGC-1α expression in a beta-cell line and in isolated mouse islets leads to oxidative stress. Also, while 5'-AMP-activated protein kinase (AMPK) is activated in beta cells when PGC-1α is overexpressed, this activation seems not only linked to a deficit in ATP production, but also—and mostly—to high levels of reactive oxygen species (ROS), whereas preventing ROS production and AMPK activation counteracts the impaired GSIS induced by PGC-1α. These results therefore provide new insights into how PGC-1α regulates beta-cell function through induction of oxidative and energetic stress.

MATERIALS AND METHODS

Beta-cell-specific PGC-1α overexpression in mice

The present study's transgenic mice expressing PGC-1α in beta cells have been previously described elsewhere [3]. All animal experiments were done according to *Principles of Laboratory Animal Care* and French law under French Ministry Agreement N° 02886.03.

Mouse islet isolation, and cell-culture infection and treatment

Islets from wild-type or Ins-PGC-1α mice were isolated using a collagenase solution (1 mg/mL, Sigma-Aldrich, St. Louis, MO, USA), separated with Histopaque (Sigma-Aldrich) and handpicked using a binocular microscope (Leica Microsystems GmbH, Wetzlar, Germany). Isolated islets were cultured in Roswell Park Memorial Institute (RPMI) medium containing 11.1 mmol/L glucose (GE Healthcare France, Vélizy-Villacoublay), 0.2 mmol/L glutamine, 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL penicillin and 100 mg/mL streptomycin. Islets were then treated with Tempol 10 mmol/L or compound C (CC) 500 µmol/L (Sigma-Aldrich) for 48 h. MIN6 cells were similarly treated and cultured as described elsewhere [7], and infected with adenovirus overexpressing either green fluorescent protein (Ad-GFP) or PGC-1α (Ad-PGC-1α) for 24 h, as previously described [3]. These MIN6 cells were also treated with Tempol 10 mmol/L or CC 500 µmol/L for 48 h.

Measurement of glucose-induced insulin secretion

MIN6 cells or batches of 50 islets were sequentially incubated for 1 h at 37° C with 2.8 mmol/L glucose, 16.7 mmol/L glucose and 50 mmol/L KCl in Krebs–Ringer bicarbonate HEPES buffer. Total islet insulin content was extracted by acid ethanol (1.5% HCl in 75% ethanol), and insulin contents and insulin secretion assayed by enzyme-linked immunosorbent assay (ELISA) kits (Mercodia AB, Uppsala, Sweden).

Pancreatic processing and quantitative morphometry

Pancreata from mouse fetuses [embryonic (E) days E13.5, E15.5 and E18.5] were fixed in a 3.7% formalin solution, then dehydrated and embedded in paraffin, and cut into 5-µm sagittal sections. These sections were collected on poly-L-lysine-coated slides, left at 37° C overnight and stored at 4° C until needed for immunohistochemistry. Morphometric beta-cell mass analysis was performed on eight random transverse sections from wild-type and Ins-PGC-1α pancreata, as previously described [1], and the beta-cell fraction calculated as the ratio of pancreatic insulin-positive cell area to total tissue area of the entire section, as determined by

computer-assisted measurements, using a Leica DMRB microscope equipped with a colour video camera coupled to a Leica Q500IW PC computer, as previously described [1].

RNA preparation and real-time PCR

Total RNA was extracted using RNeasy Plus extraction kits (Qiagen, Hilden, Germany) and reverse-transcribed into cDNA using SuperScript II reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, USA). Gene expression was quantified by real-time polymerase chain reaction (RT-PCR) using SYBR Green supermix in a MyiQ thermocycler (Bio-Rad Laboratories, Hercules, CA, USA). The value obtained for each specific gene product was normalized by 18S ribosomal RNA and expressed as the fold change of the value under control conditions. Primers were designed to span two exons where possible (primer sequences are available upon request).

Western blot tests

Total proteins were separated on SDS-PAGE gels and transferred to nitrocellulose membranes, which were then incubated with rabbit antibodies raised against PGC-1α (provided by Daniel P. Kelly), AMPK, anti-phospho-AMPK α (Thr172; Merck KGaA, Darmstadt, Germany) and beta-actin (Sigma-Aldrich).

Measurement of ROS content

Intracellular ROS generation was detected using a MitoSOX molecular probe (Invitrogen Corp.). After a 5-μmol/L MitoSOX probe reagent solution was prepared, the MIN6 cells were covered by 1 mL of this solution, then incubated for 10 min at 37˚ C (away from light), then washed three times with warm buffer. The fluorescence rate for each cell group was analyzed by flow cytometry (FACS) with an excitation wavelength of 510 nm and emission wavelength of 580 nm. For isolated islets, intracellular ROS levels were detected using a 2′,7′-dichlorofluorescein diacetate (H2DCF-DA) probe (Invitrogen Corp.). Islets were then resuspended in prewarmed HBSS (Hank's Buffered Salt Solution) buffer containing the H2DCF-DA probe (1 mmol/L) for 5 min at 37˚ C (away from light), then washed three times with culture media. The fluorescence rate in islets was measured with an excitation wavelength of 485 nm and emission wavelength of 530 nm on a fluorescence plate reader.

Determination of ATP content

Cells were lysed and their total ATP content assessed with a luciferin–luciferase kit (CellTiter-Glo, Promega Corporation, Madison, WI, USA). Luminescence was then measured with a Berthold LB96V MicroLumat Plus luminometer (Berthold Technologies, Bad Wildbad, Germany). ATP was expressed relative to protein quantity and compared with ATP quantified in control cells.

Measurement of oxygen consumption

Oxygen consumption was assessed with an XF24 Analyzer (Agilent Technologies, Santa Clara, CA, USA). MIN6 cells were seeded onto XF24 plates loaded with 25 mmol/L of glucose-stimulating solution and incubated to 37º C in a non-CO_2 incubator 30 min before the run, which was launched according to a programme designed to achieve the optimal mix, wait and measure cycle times. Variations of oxygen concentration in cell media were recorded every 5 min. To measure proton leaks, cells were first exposed to oligomycin A (1 μM) followed by rotenone (1 μM); the proton leak was then calculated as the difference between oxygen consumption after oligomycin A and after rotenone.

Assay of activity of respiratory complexes

Freshly prepared mitochondria were used for spectrophotometric determination of the activity of respiratory chain complexes using biochemical assays with a total volume of 200 μL. Protocols were adapted from previous publications for complexes I and II [8, 9], complex III [10], complex IV [9] and all complexes [11]. Activity of respiratory chain complexes was calculated as units per 1 μg of isolated mitochondrial fraction and normalized by citrate synthase activity.

Statistical analysis

All results are expressed as means ± SD. Statistical significance of all variations was tested by Mann–Whitney non-parametric tests. P values ≤ 0.05 were considered significant.

RESULTS

PGC-1α overexpression in fetal beta cells leads to oxidative stress in adults

Previously, it was found that when PGC-1α is overexpressed in beta cells in Ins-PGC-1α mice, they present with glucose intolerance and impaired insulin secretion in response to glucose along with a decreased beta-cell mass [3]. The present study found that PGC-1α expression is increased in Ins-PGC-1α fetuses at embryonic stages E15.5 and E17.5 (Fig. 1A) and that fetal beta-cell mass is altered at stages E15.5 and E17.5 in Ins-PGC-1α fetuses concomitantly with overexpression of PGC-1α (Fig. 1B). As PGC-1α is involved in mitochondrial function, a search was made for indirect evidence of increased respiration and increased oxidative stress. It was observed that the mRNA coding for antioxidant proteins such as SOD1, SOD2, catalase and Hmox was increased in fetal Ins-PGC-1α pancreata at both E15.5 and E17.5 compared with control fetuses (Fig. 1C). Next, measuring the expression of antioxidant genes in islets from adult Ins-PGC-1α mice revealed that specific beta-cell PGC-1α overexpression significantly increased mRNA levels of genes coding for antioxidant proteins compared with islets from control mice (Fig. 1D). In addition, increased expression of mRNA coding for transcription factors involved in mitochondrial biogenesis, such as NRF1, NRF2 and TFAM, was also observed in Ins-PGC-1α islets (Fig. 1D), while using a fluorescent probe to detect ROS levels (MitoSOX) revealed increased levels in adult Ins-PGC-1α pancreatic islets compared with control islets (Fig. 1E).

PGC-1α overexpression in beta cells *in vitro* leads to oxidative stress

To evaluate how PGC-1α overexpression might modulate mitochondrial function and respiration, our study used an adenovirus to prompt PGC-1α expression in a beta-cell line, the MIN6 cells. The MIN6 cells that overexpressed PGC-1α presented with increased mRNA levels of transcription factors controlling mitochondrial biogenesis compared with cells infected with the control adenovirus (Fig. 2A). ROS production was also increased in Ad-PGC-1α cells compared with control cells (Fig. 2B), and there was increased expression of eight genes encoding for proteins involved in ROS scavenging—namely, *SOD1*, *SOD2*, *CAT*, *TXN2*, *GCLC* and *UCP2* (Fig. 2C)—which is similar to what was observed in islets from Ins-PGC-1α mice (Fig. 1D). Thus, PGC-1α overexpression *in vitro* was also associated with an increase of oxidative stress and expression of genes coding for antioxidant proteins.

PGC-1α overexpression in beta cells *in vitro* leads to improved respiration despite AMPK activation

Next, our study addressed the effect of PGC-1α overexpression on beta-cell mitochondrial and respiratory capacity. Based on quantification of the ratio between mitochondrial and nuclear DNA, the number of mitochondria was increased in PGC1α-overexpressing cells compared with control cells (Fig. 3A). Also, to study how Ad-PGC-1α in MIN6 cells regulates oxidative phosphorylation in response to glucose, the oxygen consumption rate was measured and was significantly increased in Ad-PGC-1α *vs* control cells (Fig. 3B). Also, measuring proton leaks using oligomycin A and rotenone (Fig. 3B) revealed that proton leaks were increased when PGC-1α was overexpressed in MIN6 cells (Fig. 3C). Assessing enzyme activity of the individual respiratory chain complexes of MIN6 cells showed that the overall activity of each complex was significantly increased in cells overexpressing PGC-1α, except for complexes I (NADH reductase) and III (ubiquinol cytochome C reductase; Fig. 3D), while the activity of ATP synthase (complex V) was increased when PGC-1α was overexpressed, but with no changes at the transcription level (data not shown). These increases in the mitochondrial pool and overall respiratory chain activity suggested that Ad-PGC-1α cells could produce more ATP, and measurements revealed that ATP content under basal conditions (2.5 mM of glucose) was similar in cells expressing PGC-1α and control cells (Fig. 3E). With stimulation by 25 mM of glucose, Ad-PGC-1α cells had decreased ATP contents compared with control cells (Fig. 3E). When levels of expression of the ATP/ADP nucleotide carrier (SLC25 family) were measured, they were increased in cells overexpressing PGC-1α cells (Fig. 3F). The blunted ATP production suggests that Ad-PGC-1α cells might be under energetic stress. In fact, Western blots of Ad-GFP and Ad-PGC-1α cells showed that a fivefold increased PGC-1α protein level was associated with an increased ratio of AMPK phosphorylated on Thr172 over total AMPK, which is classically associated with AMPK activation (Fig. 3G). Thus, in MIN6 beta cells, PGC-1α overexpression led to AMPK activation.

PGC-1α-induced oxidative stress and AMPK are both involved in decreased GSIS

Mice overexpressing PGC-1α in beta cells displayed impaired GSIS [3]. Similarly, an altered GSIS was observed in Ad-PGC-1α cells in response to 16.7 mM of glucose (Fig. 4A) compared with control cells. As both oxidative and energetic stress can control insulin secretion, our study aimed to determine whether PGC-1α-induced oxidative stress and AMPK activation were involved in impaired insulin secretion. To address this question, ROS

production and AMPK activation in Ad-GFP and Ad-PGC-1α cells were blocked by 10 mM of Tempol (a superoxide dismutase mimetic that inhibits ROS accumulation) and 500 μM of CC (AMPK inhibitor), respectively. After 48-h treatment with Tempol, ROS production, measured by a MitoSOX fluorescent probe, was reduced (Fig. 4B). On analyzing the expression of mRNA coding for antioxidant proteins, PGC-1α overexpression in the presence of Tempol led to increased levels of *SOD1*, *HMOX*, *Txn2* and *Prdx2*, but not *SOD2*, *CAT*, *GCLC* and *UCP2* (Fig. 4C). Concerning insulin secretion, Tempol significantly restored GSIS in Ad-PGC-1α cells (Fig. 4D) to similar levels as in control MIN6 cells. Thus, oxidative stress induced by PGC-1α overexpression in beta cells was involved in impaired insulin secretion. Interestingly, on investigating Thr172-AMPK phosphorylation in Ad-GFP and Ad-PGC-1α cells treated with 10 mM of Tempol for 48 h, the antioxidant strongly decreased AMPK activation (Fig. 4E), suggesting that ROS are responsible for AMPK activation.

In addition, when PGC-1α-overexpressing cells were treated with CC for 48 h, AMPK phosphorylation was decreased (Fig. 4F), while GSIS in Ad-PGC-1α cells was partially restored in response to glucose (Fig. 4G). Thus, PGC-1α-induced AMPK activation in beta cells participates in GSIS inhibition.

ROS-activated AMPK is also involved in decreased GSIS in Ins-PGC-1α mice

The influence of oxidative and energetic stress was also explored in isolated pancreatic islets from Ins-PGC-1α mice, which detected an increase of Thr172-AMPK in Ins-PGC-1α isolated islets (Fig. 5A). As previously shown in MIN6 cells, treatment of Ins-PGC-1α islets with 10 mM of Tempol for 24 h reversed AMPK phosphorylation and partially restored GSIS in isolated islets (Fig. 5B) to levels similar to those of control islets. Thus, Tempol treatment of adult islets can reverse AMPK activation and partially restore insulin secretion.

DISCUSSION

Understanding the underlying mechanisms of beta-cell dysfunction helps to define strategies to restore normal insulin secretion and improve glycaemic control in diabetes patients. It had previously been shown that PGC-1α overexpression in beta cells *in vivo* led to decreases in beta-cell mass, insulin secretion and glucose intolerance [3]. In the present study, the aim was to clarify how PGC-1α overexpression inhibits insulin secretion, with a particular focus on mitochondrial function. Indeed, it was demonstrated that PGC-1α overexpression induces mitochondrial biogenesis and oxidative and energetic stress, and impairs insulin secretion. It also revealed that energetic stress is induced by oxidative stress and that blockade of either oxidative or energetic stress can restore insulin secretion.

Previous studies suggested that PGC-1α is a major actor in the aetiology of diabetes. PGC-1α expression in pancreatic beta cells is increased in several animal models of obesity and diabetes as well as in diabetic patients [12]. It was also found that glucolipotoxicity has deleterious effects on beta-cell function [13] through induced expression of PGC-1α, whereas inhibiting PGC-1α expression can restore insulin secretion even in the presence of fatty acids [14–16]. This suggested that the increased expression of PGC-1α in islets might contribute to beta-cell dysfunction induced by chronic hyperlipidaemia. In addition, previous research by our team showed that PGC-1α is induced by GCs in fetal beta cells and that PGC-1α adds to the deleterious effect of GCs on beta-cell development and function [3]. However, other authors proposed the opposite situation wherein reduced PGC-1α expression correlated with reduced insulin secretion in islets from patients with T2D, and was ascribed to both genetic and epigenetic factors [17]. Yet, despite these contradictory observations, elevated PGC-1α expression seems clearly deleterious in the pancreas and is linked to the metabolic syndrome, although few data on beta-cell type are available regarding the specific effects of PGC-1α on beta-cell dysfunction.

In our present study, PGC-1α overexpression led to increases in ROS production and AMPK activation, two signs already associated with beta-cell dysfunction [18–20]. Pancreatic beta cells are vulnerable to oxidative stress due to their low levels of antioxidant enzyme systems, whereas oxidative stress can inhibit insulin gene expression, suppress insulin gene transcription and alter GSIS by damaging mitochondrial DNA. Our study has also shown that insulin (INS)-1 and INS-2 genes are decreased when PGC-1α is overexpressed in MIN6 cells and in Ins-PGC-1α islets [3]. On the other hand, antioxidant treatment or overexpression of antioxidant enzymes preserved insulin promoter activity, Pdx1-binding and levels of insulin mRNA despite high glucose concentrations in studies *in vitro* [21] while, in animal studies,

antioxidant treatment decreased markers of oxidative stress and had beneficial effects on levels of insulin mRNA, Pdx1-binding and glycaemia [22]. In our experiments, antioxidant treatment reduced AMPK phosphorylation induced by PGC-1α overexpression, suggesting that ROS are responsible for AMPK activation. Interestingly, one study has shown that ROS production is an absolute requirement for proper GSIS [23]. In our present study, GSIS was not changed in either Tempol-treated or untreated Ad-GFP cells, suggesting that lowering ROS levels did not hamper GSIS. Although ROS are not major AMPK activators, several authors have shown that such activation is possible under specific conditions [24, 25], although the mechanism remains unclear. AMPK is mainly activated by increased concentrations of AMP, which is in equilibrium between ADP and ATP contents. In our study, no significant increase was observed in ATP production after PGC-1α overexpression. Yet, increased mitochondrial activity was detected after PGC-1α overexpression. A decoupling process of the respiratory chain might explain this discrepancy. In fact, there was an increase in proton leaks after PGC-1α overexpression, although their origin remains unclear because, in our two models (MIN6 cells + Ad-PGC-1α or Ins-PGC-1α), expression of the uncoupling protein UCP1 was undetectable (data not shown). Paradoxically, however, a twofold increase was measured in mitochondrial membrane potential (data not shown), as well as a significant increase in ATP synthase activity, while the expression of two kinases known to activate AMPK—namely, LKB1 and CamKK—were unchanged after PGC-1α overexpression (data not shown). Given that AMPK can directly phosphorylate PGC-1α [26], it would be of interest to investigate whether the activation of AMPK after PGC-1α overexpression reinforces PGC-1α action in beta cells. Also, as phosphorylation by AMPK increases PGC-1α-dependent activation of its own promoter [26], these modifications of PGC-1α, taken together, could induce a positive loop of PGC-1α action, thus supporting the phenotype observed in beta cells.

To understand the defect in ATP production after PGC-1α overexpression, several key points were studied. First, whether cytosolic ADP/matrix ATP transport is impaired was determined by measuring the expression of the transporter ATP/ADP nucleotide carrier (SLC25 family), which revealed that expression of this ADP/ATP transporter was also increased when PGC-1α was overexpressed, suggesting that the deficit in ATP production was not due to a defect of nucleotide transport. Also, as PGC-1α controls mitochondria biogenesis, the quality of the mitochondrial network was analyzed to determine the number, size and distribution of mitochondria in Ad-PGC-1α *vs* Ins-PGC-1α beta cells. Electron microscopy revealed no significant changes or alterations of mitochondrial structures (data

not shown). The energetic status of beta cells overexpressing PGC-1α might also be explained by the fact that mitochondrial biogenesis mobilizes a huge amount of energy to generate new mitochondria at the expense of secretory function. Apart from AMPK activation, another hallmark of altered energy production has been reported: a smaller cellular size when PGC-1α is overexpressed in beta cells [3]. As S6 kinase, a target of AMPK that controls protein synthesis, controls beta-cell size [27] and insulin secretion, measurements of S6 kinase activation revealed a reduction of the phosphorylated S6 ribosomal protein, the target of S6 kinase, when PGC-1α is overexpressed (data not shown). Thus, activation of this pathway may explain the observed phenotype of secretory defect, as small beta cells secrete less insulin than large beta cells, and the membrane surface is directly related to cell exocytosis capacity [28].

Previously, adenovirus-induced overexpression of PGC-1α in rat islets reduced expression of the glucose sensors involved in GSIS—namely, GLUT2 and glucokinase—and increased expression of G6Pase, leading to a decrease in ATP/ADP by reducing glucose oxidation and thereby contributing to impaired GSIS [12]. Our present study has found that, in Ad-PGC-1α MIN6 cells, G6Pase was significantly increased compared with Ad-GFP cells, whereas GLUT2 and glucokinase levels remained unchanged (data not shown). Thus, elucidating why ATP production is blunted remains a challenge. An increased expression of *UCP2* was found with PGC-1α overexpression in beta cells. Recently, the role of *UCP2* as a mitochondrial uncoupler has been questioned, and studies have suggested it might actually act as an antioxidant enzyme [29, 30]. Yet other studies have proposed that uncoupling protein might modify mitochondrial function. For example, *UCP2* is activated by high glucose and ROS levels, which are associated with reduced mitochondrial membrane potential and heat production [31]. In our present study, *UCP2* expression was increased after PGC-1α overexpression and normalized after Tempol treatment. Moreover, two other studies *in vitro* have shown that PGC-1α can induce expression of *UCP2* in INS-1 cells and in rat pancreatic islets, resulting in reduced insulin secretion [15, 32]. Thus, *UCP2* increases could contribute to the onset of beta-cell dysfunction.

The present results for GSIS were obtained from both MIN6 cells and isolated islets from Ins-PGC-1α mice. Blockade of AMPK activation by either CC or Tempol was clearly associated in MIN6 cells with complete restoration of GSIS. In contrast, in isolated islets from Ins-PGC-1α mice, GSIS was only weakly, yet significantly, restored by Tempol treatment despite a clear reduction of AMPK activation. To explain this discrepancy, two explanations may be proposed: first, PGC-1α overexpression starts early in fetal life, as shown by the

present study, and strongly impairs beta-cell function and hampers its restoration by Tempol treatment whereas, in MIN6 cells, PGC-1α was only overexpressed during the 48 h prior to GSIS measurement; and second, the fact that, in beta cells from Ins-PGC-1α mice, factors other than oxidative stress may be responsible for AMPK activation, or factors other than AMPK may be responsible for the decrease in GSIS, cannot be excluded. Thus, further studies are needed to elucidate these points.

In conclusion, our present study data point to the involvement of PGC-1α in beta-cell dysfunction while also exposing the mechanisms by which PGC-1α impairs beta-cell development and the ability of cells to secrete insulin. Our study also identifies a new network of interactions among beta cells linking oxidative and energetic stress, thereby reinforcing the idea that both are major regulators of insulin secretion.

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REFERENCES

[1] Valtat B, Dupuis C, Zenaty D, Singh-Estivalet A, Tronche F, Breant B, et al. Genetic evidence of the programming of beta cell mass and function by glucocorticoids in mice. Diabetologia 2011;54:350-9.

[2] Besseiche A, Riveline JP, Gautier JF, Breant B, Blondeau B. Metabolic roles of PGC-1alpha and its implications for type 2 diabetes. Diabetes Metab 2015; 41:347-57.

[3] Valtat B, Riveline JP, Zhang P, Singh-Estivalet A, Armanet M, Venteclef N, et al. Fetal PGC-1alpha overexpression programs adult pancreatic beta-cell dysfunction. Diabetes 2013;62:1206-16.

[4] Fu Z, Gilbert ER, Liu D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. Curr Diabetes Rev 2013;9:25-53.

[5] Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell 1999;98:115-24.

[6] Gleyzer N, Vercauteren K, Scarpulla RC. Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. Mol Cell Biol 2005;25:1354-66.

[7] Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, et al. Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology 1990;127:126-32.

[8] Janssen AJ, Trijbels FJ, Sengers RC, Smeitink JA, van den Heuvel LP, Wintjes LT, et al. Spectrophotometric assay for complex I of the respiratory chain in tissue samples and cultured fibroblasts. Clin Chem 2007;53:729-34.

[9] Medja F, Allouche S, Frachon P, Jardel C, Malgat M, Mousson de Camaret B, et al. Development and implementation of standardized respiratory chain spectrophotometric assays for clinical diagnosis. Mitochondrion 2009;9:331-9.

[10] Luo C, Long J, Liu J. An improved spectrophotometric method for a more specific and accurate assay of mitochondrial complex III activity. Clin Chim Acta 2008;395:38-41.

[11] Spinazzi M, Casarin A, Pertegato V, Salviati L, Angelini C. Assessment of mitochondrial respiratory chain enzymatic activities on tissues and cultured cells. Nat Protoc 2012;7:1235-46.

[12] Yoon JC, Xu G, Deeney JT, Yang SN, Rhee J, Puigserver P, et al. Suppression of beta cell energy metabolism and insulin release by PGC-1alpha. DevCell 2003;5(1):73-83.

[13] Nolan CJ, Prentki M. The islet beta-cell: fuel responsive and vulnerable. Trends EndocrinolMetab 2008;19:285-91.

[14] De Souza CT, Araujo EP, Prada PO, Saad MJ, Boschero AC, Velloso LA. Short-term inhibition of peroxisome proliferator-activated receptor-gamma coactivator-1alpha expression reverses diet-induced diabetes mellitus and hepatic steatosis in mice. Diabetologia 2005;48:1860-71.

[15] De Souza CT, Gasparetti AL, Pereira-da-Silva M, Araujo EP, Carvalheira JB, Saad MJ, et al. Peroxisome proliferator-activated receptor gamma coactivator-1-dependent uncoupling protein-2 expression in pancreatic islets of rats: a novel pathway for neural control of insulin secretion. Diabetologia 2003;46:1522-31.

[16] Zhang P, Liu C, Zhang C, Zhang Y, Shen P, Zhang J, et al. Free fatty acids increase PGC-1alpha expression in isolated rat islets. FEBS Lett 2005;579:1446-52.

[17] Ling C, Del Guerra S, Lupi R, Ronn T, Granhall C, Luthman H, et al. Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia 2008;51:615-22.

[18] da Silva Xavier G, Leclerc I, Varadi A, Tsuboi T, Moule SK, Rutter GA. Role for AMP-activated protein kinase in glucose-stimulated insulin secretion and preproinsulin gene expression. Biochem J 2003;371(Pt 3):761-74.
[19] Leclerc I, Rutter GA. AMP-activated protein kinase: a new beta-cell glucose sensor?: Regulation by amino acids and calcium ions. Diabetes 2004;53 Suppl 3:S67-74.
[20] Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes 2015;6:456-80.
[21] Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. Endocr Rev 2008;29:351-66.
[22] Robertson RP, Harmon JS. Diabetes, glucose toxicity, and oxidative stress: A case of double jeopardy for the pancreatic islet beta cell. Free Radic Biol Med 2006;41:177-84.
[23] Leloup C, Tourrel-Cuzin C, Magnan C, Karaca M, Castel J, Carneiro L, et al. Mitochondrial reactive oxygen species are obligatory signals for glucose-induced insulin secretion. Diabetes 2009;58:673-81.
[24] Li L, Chen Y, Gibson SB. Starvation-induced autophagy is regulated by mitochondrial reactive oxygen species leading to AMPK activation. Cell Signal 2013;25:50-65.
[25] Sarre A, Gabrielli J, Vial G, Leverve XM, Assimacopoulos-Jeannet F. Reactive oxygen species are produced at low glucose and contribute to the activation of AMPK in insulin-secreting cells. Free Radic Biol Med 2012;52:142-50.
[26] Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 2007;104:12017-22.
[27] Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, et al. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. Nature 2000;408:994-7.
[28] Giordano E, Cirulli V, Bosco D, Rouiller D, Halban P, Meda P. B-cell size influences glucose-stimulated insulin secretion. Am J Physiol 1993;265(2 Pt 1):C358-64.
[29] Produit-Zengaffinen N, Davis-Lameloise N, Perreten H, Becard D, Gjinovci A, Keller PA, et al. Increasing uncoupling protein-2 in pancreatic beta cells does not alter glucose-induced insulin secretion but decreases production of reactive oxygen species. Diabetologia 2007;50:84-93.
[30] Robson-Doucette CA, Sultan S, Allister EM, Wikstrom JD, Koshkin V, Bhattacharjee A, et al. Beta-cell uncoupling protein 2 regulates reactive oxygen species production, which influences both insulin and glucagon secretion. Diabetes 2011;60:2710-9.
[31] Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802-12.
[32] Oberkofler H, Klein K, Felder TK, Krempler F, Patsch W. Role of peroxisome proliferator-activated receptor-gamma coactivator-1alpha in the transcriptional regulation of the human uncoupling protein 2 gene in INS-1E cells. Endocrinology 2006;147:966-76.

Figures

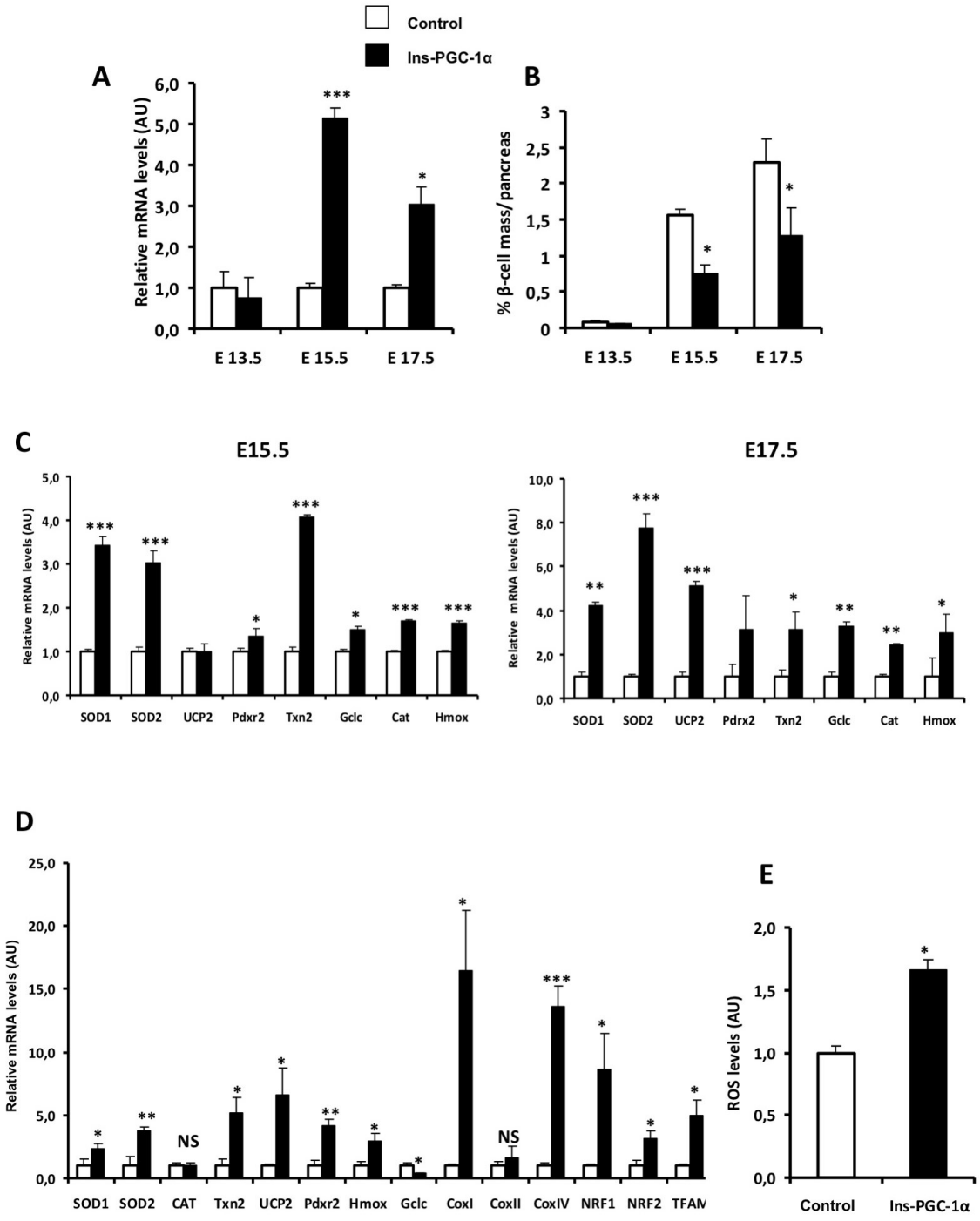


Fig. 1. PGC-1α overexpression in control (white bars) and transgenic (black bars) mice leads to oxidative stress in beta cells: (A) PGC-1α expression in fetal pancreata at embryonic (E) stages E13.5, E15.5 and E17.5; (B) beta-cell mass in stage-5 fetal pancreata; (C) antioxidant gene expression in fetal pancreata at stages E15.5 and E17.5; (D) gene expression in isolated

adult pancreatic islets; and (E) levels of reactive oxygen species (ROS) measured in isolated adult pancreatic islets. Results are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ *vs* controls by Mann–Whitney non-parametric tests (n = 4 per group).

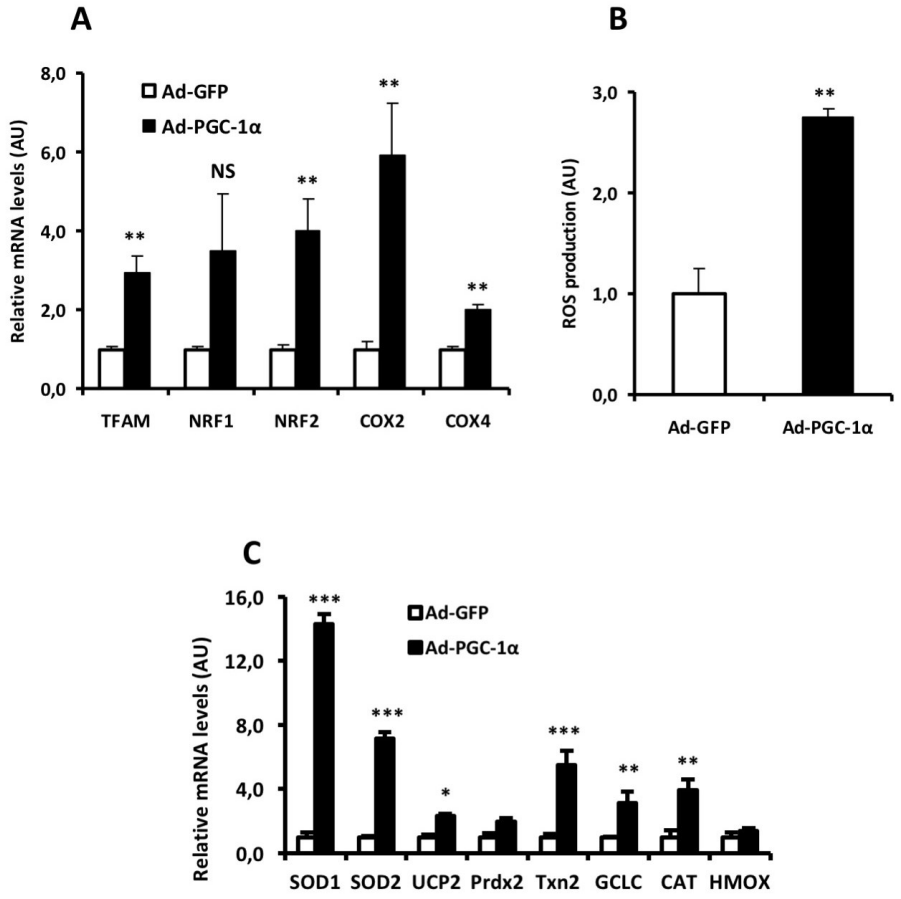


Fig. 2. PGC-1α overexpression in beta cells *in vitro* [control MIN6 cells (Ad-GFP, white bars) and cells overexpressing PGC-1α (Ad-PGC-1α, black bars)] leads to oxidative stress: (A) mRNA levels assayed by quantitative polymerase chain reaction (qPCR); (B) measured levels of reactive oxygen species (ROS); and (C) mRNA levels assayed by qPCR. Results are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Ad-PGC-1α *vs* Ad-GFP cells by Mann–Whitney non-parametric tests (n = 4 per group).

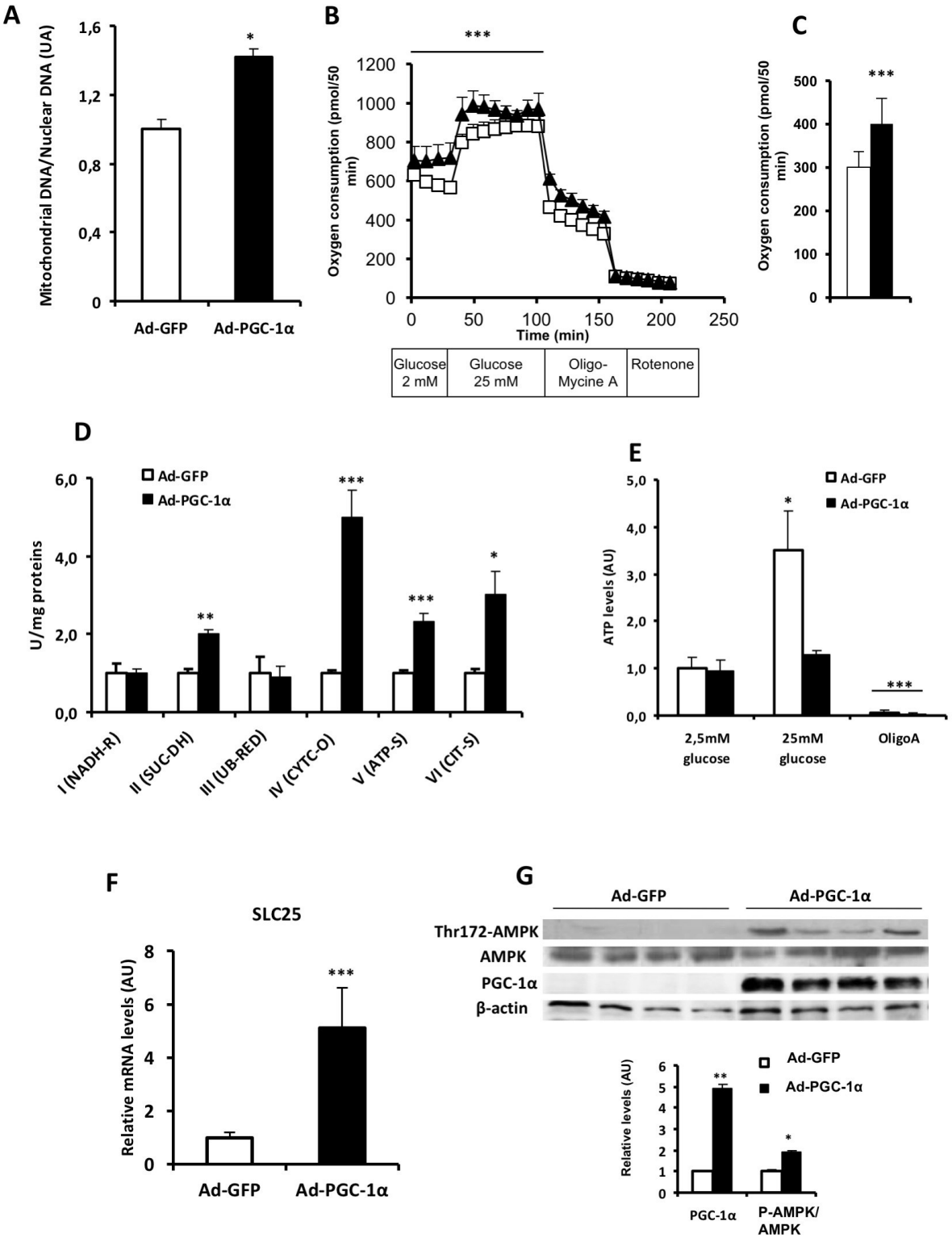
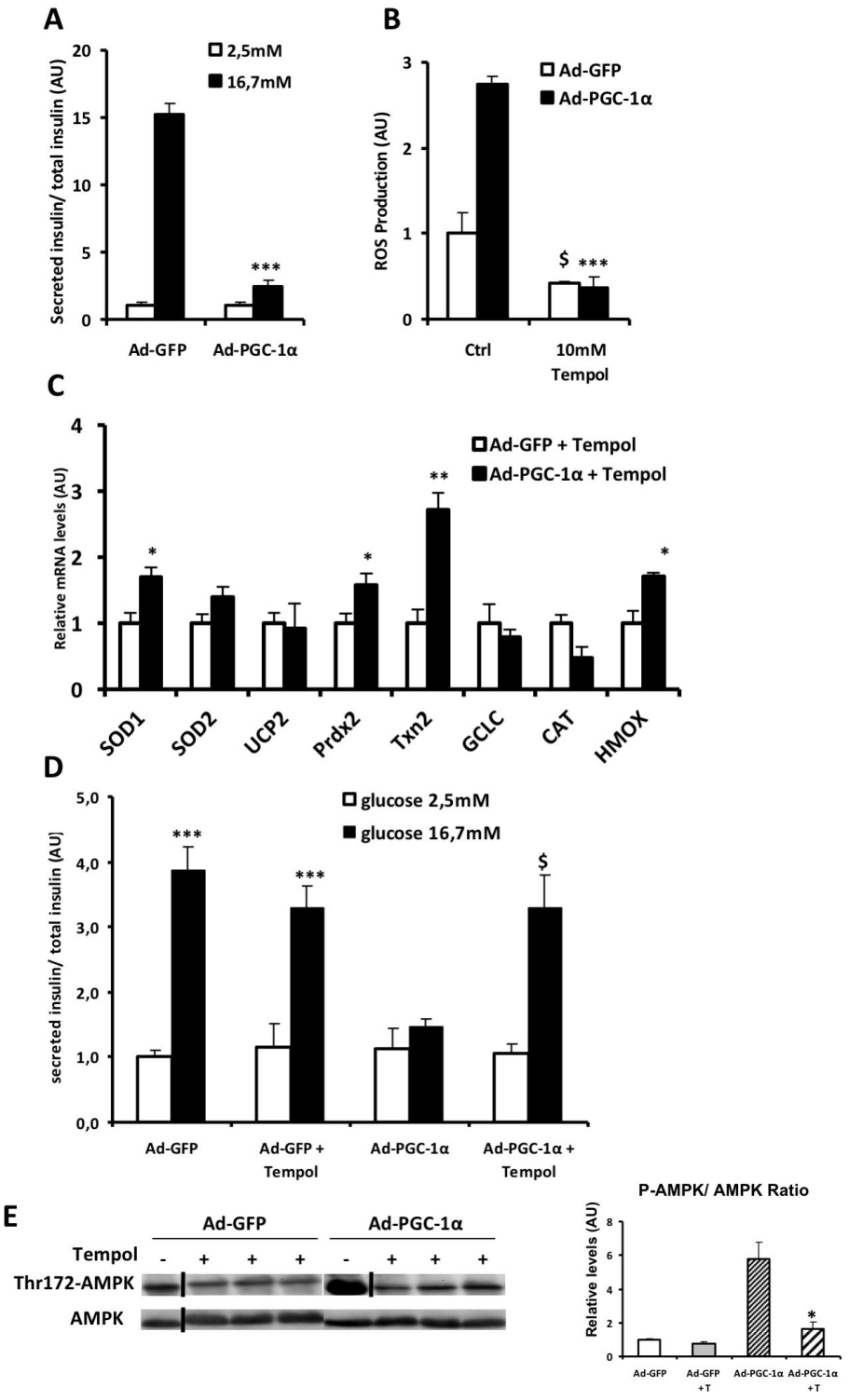


Fig. 3. PGC-1α overexpression in beta-cells *in vitro* [control MIN6 cells (Ad-GFP, white bars) and cells overexpressing PGC-1α (Ad-PGC-1α, black bars)] leads to improved respiration and AMPK activation: (A) mitochondrial to nuclear DNA ratio; (B) oxygen consumption; (C) proton leak; (D) enzyme activity of respiratory chain complexes; (E)

quantified ATP contents; (F) mRNA levels for SLC25 carrier assayed by qPCR; and (G) Western blot tests, with graph showing quantification by group. Results are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Ad-PGC-1α *vs* Ad-GFP cells by Mann–Whitney non-parametric test (n = 4 per group).


A
2,5mM
16,7mM
Secreted insulin/ total insulin (AU)
20
15
10
5
0
***
Ad-GFP
Ad-PGC-1α
B
Ad-GFP
Ad-PGC-1α
ROS Production (AU)
3
2
1
0
$ ***
Ctrl
10mM Tempol
C
Ad-GFP + Tempol
Ad-PGC-1α + Tempol
Relative mRNA levels (AU)
4
3
2
1
0
*
*
**
*
SOD1
SOD2
UCP2
Prdx2
Txn2
GCLC
CAT
HMOX
D
glucose 2,5mM
glucose 16,7mM
secreted insulin/ total insulin (AU)
5,0
4,0
3,0
2,0
1,0
0,0
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Ad-GFP
Ad-GFP + Tempol
Ad-PGC-1α
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Thr172-AMPK
AMPK
P-AMPK/ AMPK Ratio
Relative levels (AU)
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Ad-GFP
Ad-GFP +T
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Ad-PGC-1α +T

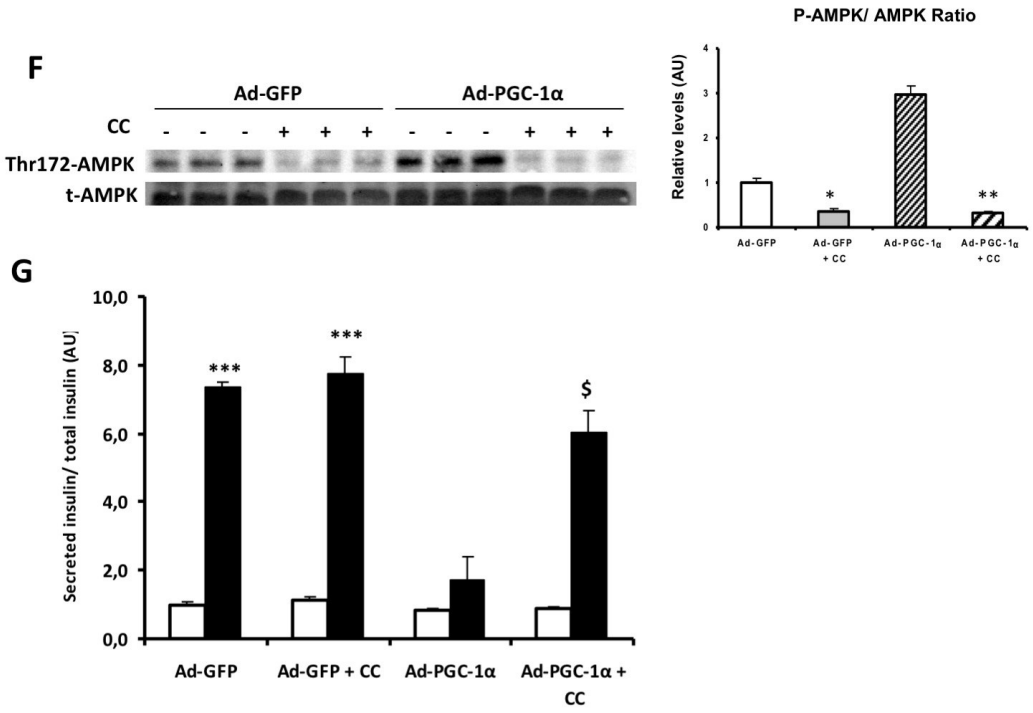

Fig. 4. Oxidative stress and AMPK are both involved in decreased glucose-stimulated insulin secretion (GSIS) following PGC-1α overexpression [control MIN6 cells (Ad-GFP, white bars) *vs* cells overexpressing PGC-1α (Ad-PGC-1α, black bars)]: (A) GSIS; (B) ROS production with/without Tempol treatment; (C) gene expression after Tempol treatment; (D) GSIS with/without Tempol; (E) Western blot protein test with/without Tempol or compound C (CC), with graph showing quantifications for controls (white bar), Ad-GFP + CC (grey bar), Ad-PGC-1α (grey hatched bar) and Ad-PGC-1α (white hatched bar); (F) Western blot with/without Tempol and CC, with graph showing quantifications by group; and (G) GSIS with CC. Results are means ± SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Ad-PGC-1α *vs* Ad-GFP cells by Mann–Whitney non-parametric test (n = 4 per group); $^{\$}$ $P < 0.05$, Ad-PGC-1α cells treated with *vs* without Tempol by Mann–Whitney non-parametric test (n = 4 per group).

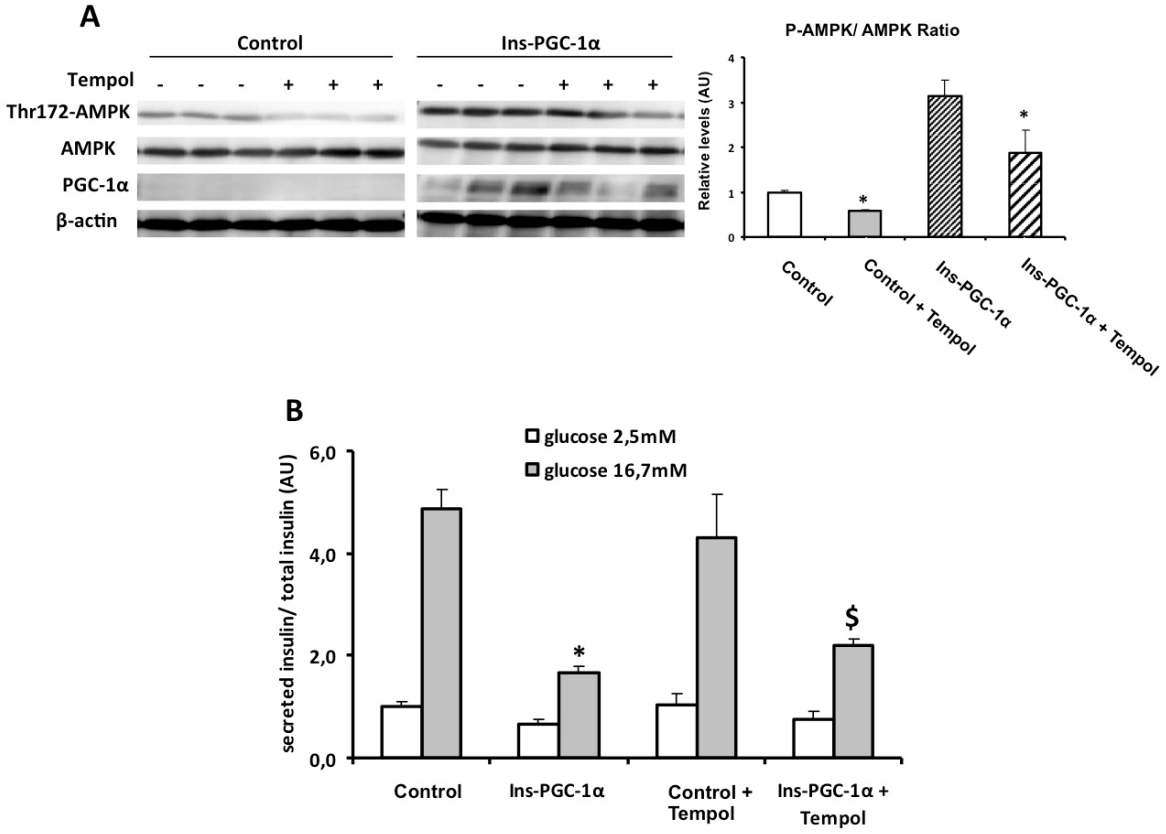

Fig. 5. Tempol treatment partially restores insulin secretion by isolated islets from Ins-PGC-1α mice (grey bars) *vs* controls (white bars): (A) Western blot protein tests of isolated islets with/without Tempol, with graph showing quantifications by group; and (B) GSIS in islets with/without Tempol. Results are means ± SD. * $P < 0.05$, Ins-PGC-1α *vs* control mice by Mann–Whitney non-parametric test (n = 4 per group); $^{\$}$ $P < 0.05$, Ad-PGC-1α islets treated with *vs* without Tempol by Mann–Whitney non-parametric test (n = 4 per group).