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Original article

# Oxidative and energetic stresses mediate beta-cell dysfunction induced by PGC-1α

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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 participate in this programming by inhibition of beta-cell development. More specifically, 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 restores 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.


## 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. peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-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 peroxisome proliferator-activated receptors (PPARs) in adipose tissue [2]. Previously, our team

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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: mitochondrial transcription factor A (TFAM) and transcription factor B (TFB) [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 performed according to *Principles of Laboratory Animal Care* and French law under French Ministry Agreement No. 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

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