



Glucagon-Like Peptide-1 Increases Mitochondrial Biogenesis and Function in INS-1 Rat Insulinoma Cells

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Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone that increases glucose-stimulated insulin secretion in pancreatic β -cells. Since mitochondrial function is crucial to insulin secretion, we hypothesized that GLP-1 may increase mitochondrial biogenesis in pancreatic β -cells. We treated INS-1 rat insulinoma cells with GLP-1 or exendin-4 for 48 hours and measured mitochondrial mass and function. Both GLP-1 and exendin-4 increased mitochondrial mass by approximately 20%. The mitochondria/cytosol ratio was increased from $7.60 \pm 3.12\%$ to $10.53 \pm 2.70\%$ by exendin-4. In addition, GLP-1 increased the mitochondrial membrane potential and oxygen consumption. Proliferator-activated receptor-gamma coactivator 1 α expression was increased approximately 2-fold by GLP-1 treatment. In conclusion, the present study presents evidence for a new mechanism of action by which GLP-1 improves pancreatic β -cell function via enhanced mitochondrial mass and performance.

Keywords: Glucagon-like peptide-1; Mitochondria; Diabetes mellitus; Beta cell; Insulin

INTRODUCTION

Mitochondrial dysfunction causes both insulin resistance and β -cell dysfunction, leading to glucose intolerance and diabetes [1,2]. In skeletal muscle, decreased mitochondrial fatty acid oxidation results in increased amounts of cytosolic long-chain acyl CoA and diacylglycerol, which leads to increased serine phosphorylation of insulin receptor substrate-1, thereby eliciting insulin resistance [3]. In pancreatic β -cells, decreased ATP generation due to impaired mitochondrial oxidative phosphorylation is linked to impaired insulin secretion, via a mechanism that reduces closure of the ATP-sensitive potassium channel [4]. In this regard, measures to improve mitochondrial

function, either in the skeletal muscle or in the pancreatic β -cell, should be effective for treating diabetes.

Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone that stimulates insulin secretion and suppresses glucagon secretion, inhibits gastric emptying, and reduces appetite and food intake [5,6]. Because of its efficacy in lowering blood glucose by stimulating β -cell insulin secretion, GLP-1 analogues and incretin enhancers (i.e., dipeptidyl peptidase-4 inhibitors) are widely used in the clinic to treat diabetes [7]. Interestingly, it was reported that GLP-1 stimulates ATP synthesis in pancreatic MIN6 β -cells [8], suggesting a new mechanism for improving β -cell function using GLP-1 therapy. In this study, we examined the effect of GLP-1 and its analogue

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on mitochondrial biogenesis in pancreatic β -cells.

METHODS

INS-1 cell culture and glucose-stimulated insulin secretion

INS-1 rat insulinoma cells (passages 28 to 34) were grown in monolayer culture in RPMI-1640 supplemented with 10% fetal bovine serum, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 μ M β -mercaptoethanol, 100 U/mL penicillin, and 100 μ g/mL streptomycin, at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. For glucose-stimulated insulin secretion, INS-1 cells were seeded in 24-well plates and treated with 0, 100, or 200 nM GLP-1 (Sigma-Aldrich, St. Louis, MO, USA) for 48 hours. Cells were then washed twice with Krebs' ringer bicarbonate buffer (KRBB), incubated in KRBB at 37°C for 1 hour, and exposed to 5 or 10 mM glucose for 1 hour. The culture supernatant was collected and stored at -20°C until assayed for insulin concentration using enzyme-linked immunosorbent assay (ELISA, Linco Research, St. Charles, MO, USA).

Measurement of mitochondrial mass and membrane potential

INS-1 cells (1×10^5) were seeded in six-well plates and incubated with GLP-1 (100 to 400 nM) or exendin-4 (100 to 200 nM, Sigma-Aldrich) for 48 hours. Mitochondrial mass was measured by 10-n-nonyl-acridine orange staining (NAO, Invitrogen, Carlsbad, CA, USA), and the mitochondrial membrane potential was measured using tetramethylrhodamine ethyl ester perchlorate (TMRE, Invitrogen) with a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA), following the manufacturers' protocols. The strength of NAO or TMRE staining was expressed as the mean fluorescence intensity (MFI).

Transmission electron microscopy

INS-1 cells (5×10^5) were seeded in 60-mm plates and incubated with exendin-4 for 48 hours. Cells were collected and processed for electron microscopy using standard methods. Ten random pictures were taken at a magnification of $\times 5,000$, using an H-7100 transmission electron microscope (Hitachi, Tokyo, Japan). The volume density of mitochondria was estimated using a point-counting method in a blinded fashion by two separate examiners. For each set of 10 pictures, the average volume density was calculated, and the mean of 10 values was used to estimate the vol-

ume density for each individual cell.

Measurement of oxygen consumption

Oxygen consumption was measured using a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) according to the manufacturer's instructions. A suspension of INS-1 cells at 1×10^6 cells/mL in a 2-mL volume of culture medium (RPMI-1640) was measured in the respirometer at 37°C.

Reverse transcriptase polymerase chain reaction

Total RNA from cells was prepared using an RNeasy Mini kit (Qiagen, Valencia, CA, USA). cDNA was obtained from 1 μ g RNA using random hexamers and avian myeloblastosis virus reverse transcriptase (Invitrogen). Samples were amplified in a Gene Amp polymerase chain reaction (PCR) system 9600 (Perkin-Elmer/Cetus, Norwalk, CT, USA). Digital images of the PCR products separated on 1% agarose gels were analyzed using a Gel Doc 2000 (BioRad Inc., Richmond, CA, USA).

Statistical analysis

Data are presented as means \pm SD. Nonparametric methods, including Kruskal-Wallis tests, Wilcoxon signed-rank tests, and Mann-Whitney tests, were used to evaluate statistical significance. Values for $P < 0.05$ were considered significant.

RESULTS

Effects of GLP-1 and its analogue on insulin secretion and mitochondrial biogenesis in INS-1 cells

GLP-1 treatment of INS-1 cells for 48 hours enhanced glucose-stimulated insulin secretion (Fig. 1A) and mitochondrial mass (Fig. 1B, C). GLP-1 at 100, 200, and 400 nM increased NAO staining intensity to 121.64 ± 11.54 , 127.48 ± 15.47 , and 126.94 ± 12.89 MFI, respectively ($n = 10$) (Fig. 1C). A similar result was obtained using another mitochondrial dye (MitoTracker Green, data not shown). When mitochondrial density was measured using a point-counting method on transmission electron microscopy images, the mitochondria/cytosol area ratio was significantly increased by 100 nM exendin-4, from $7.60 \pm 3.12\%$ to $10.53 \pm 2.70\%$ (Fig. 1D, E). The expression of proliferator-activated receptor-gamma coactivator 1 α (PGC1 α), a key regulator of mitochondrial biogenesis, was increased dramatically after 1 hour of GLP-1 treatment; after 4 hours, expression decreased gradually but remained above control levels for up to 48 hours of treatment

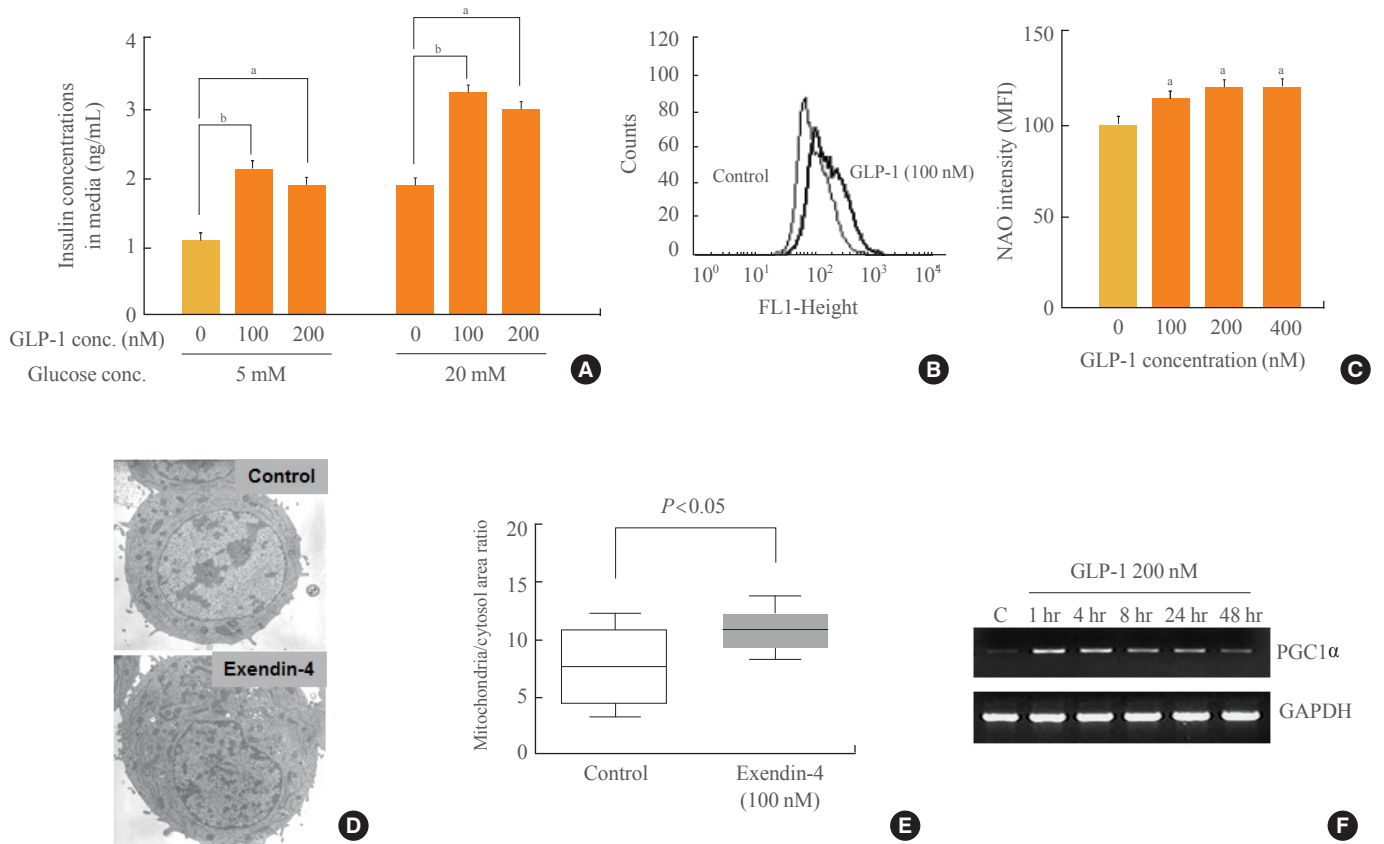


Fig. 1. Effects of glucagon-like peptide-1 (GLP-1) or exendin-4 on insulin secretion (A, $n=6$), mitochondrial mass (C, $n=10$), mitochondrial density (E, $n=10$), and proliferator-activated receptor-gamma coactivator 1 α (PGC1 α) expression (F) in INS-1 cells. (B) A representative fluorescence activated cell sorting analysis for 10-n-nonyl-acridine orange staining (NAO) intensity, as summarized in (C). (D) Representative transmission electron microscopy images used to estimate the mitochondria/cytosol area ratios shown in concentration (conc). MFI, mean fluorescence intensity; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. ^a $P < 0.05$; ^b $P < 0.01$ compared with the control.

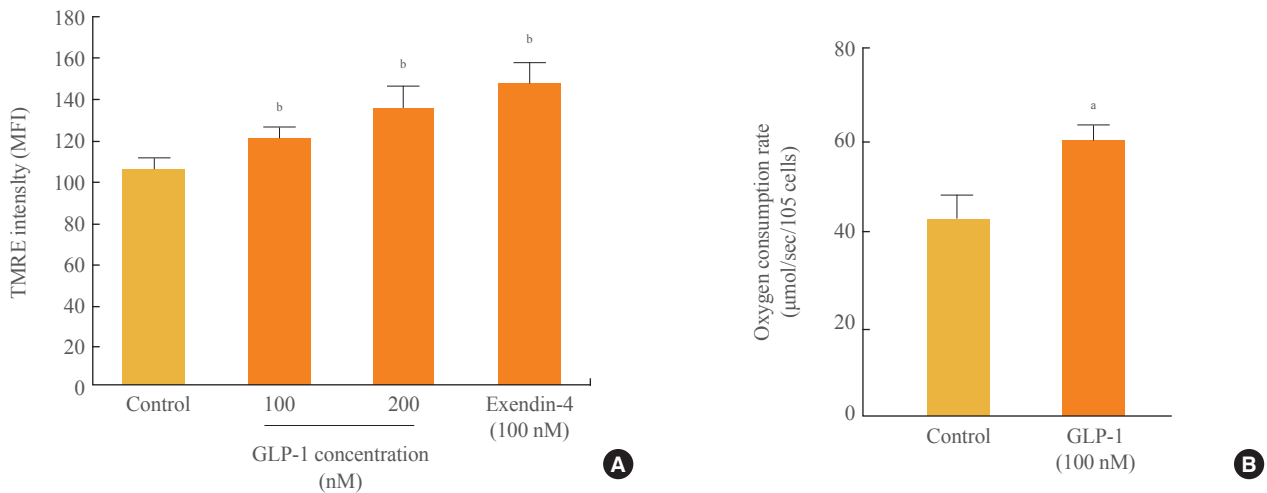


Fig. 2. Glucagon-like peptide-1 (GLP-1) increases mitochondria membrane potential (A, $n=6$) and cellular oxygen consumption rate (B, $n=4$). TMRE, tetramethylrhodamine ethyl ester perchlorate; MFI, mean fluorescence intensity. ^a $P < 0.05$; ^b $P < 0.01$ compared with control.

(Fig. 1F).

Effects of GLP-1 and exendin-4 on mitochondrial function in INS-1 cells

The intensity of TMRE staining, which is an indicator of the strength of the mitochondrial membrane potential, was increased in INS-1 cells treated with GLP-1 or exendin-4 for 48 hours (Fig. 2A). In line with this finding, the oxygen consumption rate of cells treated with GLP-1 for 48 hours exhibited a significant increase relative to controls ($56.3 \pm 2.8 \mu\text{mol}/\text{sec}/10^5$ cells vs. $42.0 \pm 1.6 \mu\text{mol}/\text{sec}/10^5$ cells, $P < 0.05$) (Fig. 2B).

DISCUSSION

Because mitochondrial oxidative phosphorylation is crucial to glucose-stimulated insulin secretion [4], the mechanisms mediating the effects of GLP-1 on mitochondria in pancreatic β -cells deserve further study. One possible mechanism is the mobilization of calcium into the mitochondrial matrix after GLP-1 treatment, which has been reported to enhance mitochondrial function in pancreatic MIN6 β -cells, possibly through the activation of several Krebs cycle dehydrogenases [8] and also in vascular smooth muscle cells [9]. Interestingly, in hepatocytes, nonapeptide fragments of GLP-1 (i.e., GLP-1 [28-36] amide) were shown to enter the cytoplasm rapidly and target mitochondria in a GLP-1 receptor-independent manner, and to be associated with decreased gluconeogenesis and oxidative stress [10]. However, in the present study, exendin-4 also exhibited a stimulatory effect on mitochondrial mass and function. Therefore, signaling through the GLP-1 receptor appears to be sufficient to increase both mitochondrial mass and function in pancreatic β -cells.

We report here a novel effect of GLP-1 on mitochondrial biogenesis and function. In INS-1 cells, GLP-1 and exendin-4 treatment triggered an increase in mitochondrial mass, mitochondrial density, mitochondrial membrane potential, and oxygen consumption. These increases were accompanied by up-regulation of PGC1 α , a key regulator of mitochondrial biogenesis [11]. However, further studies are needed to characterize the time-course of GLP-1-stimulated biogenesis, as it relates to that of PGC1 α expression. In conclusion, the present study provides evidence for a new mechanism of action of GLP-1 in pancreatic β -cells, linking mitochondrial biogenesis and function to glucose-stimulated insulin secretion.

CONFLICTS OF INTEREST

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