

**Title:**

**Mitochondrial proteome analysis reveals altered expression of voltage dependent anion channels in pancreatic  $\beta$ -cells exposed to high glucose**

**Authors:** Meftun Ahmed<sup>1,2,3</sup>, Sarheed J Muhammed<sup>3</sup> Benedikt Kessler<sup>4</sup>, Albert Salehi<sup>3</sup>

<sup>1</sup>Department of Physiology, Ibrahim Medical College, Dhaka, Bangladesh

<sup>2</sup>The Oxford Centre for Diabetes, Endocrinology & Metabolism, Oxford University, UK

<sup>3</sup>Department of Clinical Science, Clinical Research Center, University of Lund, Sweden

<sup>4</sup>Central Proteomics Facility Headington, Henry Wellcome Building for Cellular and Molecular Physiology, University of Oxford, UK

**Corresponding author:**

Meftun Ahmed

Associate Professor

Department of Physiology

Ibrahim Medical College

122 Kazi Nazrul Islam Avenue

Shahbag, Dhaka-1000, Bangladesh

Email: meftun@hotmail.com

## **Abstract**

*Background and aims:* Chronic hyperglycemia leads to deterioration of insulin release as well as insulin action on peripheral tissues. However, the mechanism underlying  $\beta$  cell dysfunction resulting from glucose toxicity has not been fully elucidated. Several studies have examined the toxic effect of high glucose on activities of individual mitochondrial proteins in  $\beta$  cells. Nevertheless there have been no studies of the effects of high glucose on the entire mitochondrial proteome. The aim of the present study was to define a set of alterations in mitochondrial protein profiles of pancreatic  $\beta$  cell line using proteomic approaches.

*Methods:* INS1E cells were incubated in the presence of 5.5 and 20 mM glucose for 72hrs and mitochondria were isolated. Mitochondrial protein profiles were obtained by two-dimensional gel (2-DE) electrophoresis and ESI-LC-MS/MS.

*Results:* Separation of mitochondrial proteins by 2 DE showed higher resolution and a high level of reproducibility was obtained among the gels (correlation coefficient,  $r = 0.78$ ). More than 400 spots were detected on the colloidal Coomassie stained 2D gels; of these protein spots, 75 displayed two fold or more significant changes ( $p < 0.05$ ) in relative abundance in the presence of 20 mM glucose compared to the controls. Thirty-three protein spots appear only on the control mitochondrial map. Mitochondrial proteins down regulated under glucotoxic conditions includes ATP synthase  $\alpha$  chain and  $\delta$  chain, malate dehydrogenase, aconitase, trifunctional enzyme  $\beta$  subunit, NADH cytochrome b5 reductase and voltage-dependent anion-selective channel protein (VDAC) 2. VDAC1, 75 kDa glucose-regulated protein, heat shock protein (HSP) 60 and HSP10 were found to be upregulated. Protein identification revealed contamination of the mitochondrial fraction with proteins from other organelles. These differentially expressed proteins includes proinsulin, calreticulin, Pdia6, PKC substrate 60.1 kDa protein, Orp150, endoplasmin, Hsc70,

heterogeneous nuclear ribonucleoproteins D0 and A2/B1, lamin B1, histones H2B, H3.3 and H4 and elongation factor 1- $\alpha$ -1.

*Conclusions:* The orchestrated changes in expression of VDACs and multiple other proteins involved in nutrient metabolism, ATP synthesis, cellular defense, glycoprotein folding and mitochondrial DNA stability may explain cellular dysfunction in glucotoxicity resulting in altered insulin secretion.