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Original Article

Alteration in pancreatic protein expression in dexamethasone-treated mice

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Abstract

The aim of this study was to gain insight into pancreatic proteome changes induced by dexamethasone treatment. Six-week-old male ICR mice were subcutaneously injected with or without dexamethasone (10 mg/kg body weight; n= 5-6 mice per group) for 4 weeks. Body weight, food intake, and fasting blood glucose were measured at baseline and at the end of 4 weeks. The mice were sacrificed and pancreases were removed, frozen, and stored at -75˚C until analysis. Proteins were extracted, two-dimensional gel electrophoresis was performed and proteome profiles were examined with colloidal coomassie brilliant blue staining. Statistically significant protein spots (ANOVA, $p<0.05$) with 1.2-fold difference were excised and analyzed by LC-MS/MS. Proteomic data were evaluated using Western blot analysis. High-dose dexamethasone induced hyperglycemia, reduced body weight, reduced food intake, and altered pancreatic proteins in mice. The affected proteins mainly belonged to cell redox homeostasis, metabolism, endoplasmic reticulum stress, translation, and signaling pathways.

Keywords: mice, dexamethasone, pancreatic proteome, 2D electrophoresis, LC-MS/MS

1. Introduction

Pancreatic islet compensation is central to the development of type 2 diabetes mellitus (T2DM), and is the same mechanism observed in glucocorticoids-induced insulin resistance (Rafacho *et al.*, 2011; Rafacho, Giozzet, Boschero, & Bosqueiro, 2008). Rodent models of T2DM induced by glucocorticoids (e.g., dexamethasone) have been widely used to study the underlying mechanisms of this phenomenon. Research indicates that pancreatic β-cells are important targets for the diabetogenic effects of glucocorticoids (Delaunay *et al.*, 1997). Glucocorticoids exert a strong negative effect on β-cell development and insulin secretion (Gesina *et al.*, 2004; Lambillotte, Gilon, & Henquin, 1997). Furthermore, glucocorticoids are known to induce oxidative stress and the release of mitochondrial cytochromes (Renner, Kofler, & Gnaiger, 2002; Tome, Lutz, & Briehl, 2004).

Investigations in isolated islets and pancreatic β-cell lines have shown that glucocorticoid induces apoptosis (Ranta *et al.*, 2006; Reich, Tamary, Sionov, & Melloul, 2012). It has been mentioned that endoplasmic reticulum (ER) stress and modulation of the unfolded protein response (UPR) (Linssen *et al.*, 2011), as well as p38 MAPK (mitogen-activated protein kinase) activation and the subsequent up-regulation of thioredoxin-interaction protein (Reich *et al.*, 2012), are possible contributors to the cytotoxic action of glucocorticoids on pancreatic β-cells. Although several theories have been proposed and discussed, the mechanism of cytotoxic action in the β-cells is not fully understood.

The pancreas is a mixed gland with both exocrine and endocrine functions. Exocrine pancreatic cells have been shown to be important for providing a compatible environ-

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ment for pancreatic islets, given their proximity to each other. A plethora of studies have shown that the exocrine part of the pancreas secretes proteins that are important to the function, regeneration, and replication of β-cells (Gross *et al.*, 1998; Rouquier, Verdier, Iovanna, Dagorn, & Giorgi, 1991; Watanabe *et al.*, 1994). Additional studies that focus on the effect of dexamethasone on the pancreatic protein profile would be beneficial. Based on our review of the literature, reports describing molecular changes in whole pancreas are lacking.

Previously, Sanchez *et al.* (2001) published a proteomic database of mouse pancreatic islet cells using two-dimensional gel electrophoresis (Sanchez *et al.*, 2001). Another study identified and reported how pancreatic proteins are differentially expressed in normal mice compared to those with diet-induced T2DM (L. Qiu, List, & Kopchick, 2005). Proteomic techniques facilitate the comprehensive investigation of a proteome, with results that may lead to the identification of protein changes or the presence of signaling pathways.

Here, we set forth to explore the full spectrum of pancreatic proteins using two-dimensional electrophoresis and mass spectrometry. The specific aim of this study was to identify differentially expressed proteins in dexamethasone-treated mice pancreas in order to better understand the mechanism of cytotoxicity in pancreatic cells and pancreatic β-cells.

2. Methods

This study was conducted at the Department of Physiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. Siriraj Hospital is Thailand's largest university-based tertiary referral center.

2.1 Animal handling

Male 6-week-old ICR mice (37-39 g) were acclimatized for 1 week and then randomly assigned to either the treatment or control group, with a group size of 5-6 mice per group. Mice in both groups were maintained in a 12-h light/dark cycle environment at 25 ± 2 °C and 60% humidity. Mice were housed 5-6 per cage with wooden chip bedding, and were provided a standard rat chow pellet that they could consume ad libitum (Perfect Companion Group Co., Ltd., Bangkok, Thailand). Mice in the treatment and control groups were injected daily with dexamethasone sodium phosphate (10mg/kg, subcutaneously) and normal saline, respectively, for 4 weeks. Body weight, food intake, and fasting blood glucose (using glucometer) were measured at baseline and at the end of 4 weeks. After end-of-experiment data were collected, the mice were euthanized by intraperitoneal pentobarbital sodium injection (Nembutal; H. Lundbeck A/S, Copenhagen, Denmark). The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University. The animal experimentation protocol was approved by the Institutional Animal Care and Use Committee, Faculty of Medicine Siriraj Hospital, Mahidol University (Approval No: SI-ACUP 001/2559).

2.2 Preparation of pancreas proteins for two-dimensional gel electrophoresis

Pancreases were removed and washed with cold normal saline, flash-frozen in liquid nitrogen, and stored at -75 °C until analysis. Approximately 50 mg of pancreatic tissue was minced on ice and homogenized with a hand-held tissue homogenizer in 50 ml of lysis buffer (7M urea, 2M thiourea, 4% CHAPS) containing Protease Inhibitor Cocktail (Thermo Fisher Scientific, Inc., Waltham, MA, USA). After a 1 hour incubation period at room temperature with occasional shaking, the homogenate was centrifuged at 30,000xg for 30 min at 4 °C, after which the supernatant was collected. Concentrations of protein samples were assayed using Bradford method. Pancreatic tissue homogenate of the mice in each group were pooled

2.3 Two-dimensional gel electrophoresis

A fixed amount of pancreatic protein (350 mg) – one from each of the pooled samples from each group – was mixed in thiourea rehydration solution [7M urea, 2M thiourea, 2% CHAPS, 60 mM DTT, 0.5% (v/v), IPG buffer pH 3-11, trace of bromophenol blue] to a volume of 125 ml, which was then loaded onto a 7 cm IPG strip (pH 3-11NL). Rehydration was performed using the IPGphor Isoelectric Focusing System (GE Healthcare, Little Chalfont, United Kingdom) at 50 mA for 12 h at 20 °C. First-dimension isoelectric focusing (IEF) was performed at 20°C using the following parameters: 200 Vh, 300 Vh, 7,500 Vh, and 3,000 Vh for a total 11,000 Vh, according to the manufacturer's protocol. Strips were first equilibrated for 30 min in equilibration solution (pH 8.8) containing 75 mM Tris-HCl, 6M urea, 30% (w/w) glycerol, 2% (w/w) SDS, and 1% DTT. Strips were then subjected to an additional 30 min in equilibration solution (pH 8.8) containing 75 mM Tris-HCl, 6M urea, 30% (w/w) glycerol, 2% SDS, and 2.5% iodoacetamide. Second-dimension electrophoresis was conducted on the electrophoresis system using 10% vertical SDS-PAGE slab gels, which were run at a constant voltage of 70 V until bromophenol blue reached the gel bottom. Electrophoresis of each sample was repeated three times under the same condition. Individual gels of the two groups were run in pairs.

2.4 Visualization and image analysis of two-dimensional gel electrophoresis

A total of six gels were stained with coomassie brilliant blue R 250 for visualization of spots. Briefly, the gels were incubated in staining solution (0.2% coomassie brilliant blue R 250 in 1:1 ethanol and 20% trichloroacetic acid) with mild shaking for 2 h at room temperature, followed by destaining (8% acetic acid and 25% ethanol) for 2 h to clear background stain. The stained gels were scanned (300 dpi resolution) using Bio-Rad GS-710 Calibrated Imaging Densitometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Three pairs of gel images from the two groups were analyzed with ImageMaster 2D Platinum 7 software (GE Healthcare, Little Chalfont, United Kingdom). Spot detection and spot matching were performed automatically and

manually, respectively. Statistical analysis was performed using ANOVA and a *p*-value <0.05 was considered to be statistically significant. The cut-off value was set at 1.2-fold increase or decrease. Only proteins with significantly altered levels were excised for identification by mass spectrometry.

2.5 In-gel digestion and LC-MS/MS

Protein spots of interest were excised manually and destained 3 times with 50 mM AmBic and 50% methanol (1:1) for 10 min each. Gel pieces were dehydrated with 100% acetonitrile (CAN). Gels were then treated with 10 mM DDT in 10 mM AmBic for 1 h at 56°C, followed by 100 mM iodoacetamide in 10 mM AmBic for 1 h in the dark at room temperature. The following dehydration step involved treatment with 100% ACN for 5 min. For in-gel digestion, trypsin (Promega Corporation, Fitchburg, WI, USA) solution (10 ng/ml in 10 mM AmBic) was added to the gel pieces followed by incubation at 4°C for 30 min. Excess enzyme solution was removed and gel pieces were incubated with 10 mM AmBic for overnight at 37 °C. Peptides were extracted with 50% ACN/1% formic acid and dried using an incubator set at 40 ° C overnight.

After tryptic digestion, dry samples were dissolved in 15 µl of 0.1% formic acid in LC-MS grade water. Analysis of tryptic peptides was performed using a Synapt™ HDMS™ system (Waters Corporation, Milford, MA, USA). The mass spectrometer was operated in the V-mode of analysis with a resolution of at least 10,000 full-width at half-maximum. All analyses were performed using the positive nanoelectrospray ion mode. The time-of-flight analyzer of the mass spectrometer was externally calibrated with (Glu1) fibrinopeptide B from m/z 50 to 1,600 with acquisition lock mass corrected using the monoisotopic mass of the doubly charged precursor of (Glu1) fibrinopeptide B. The reference sprayer was sampled with a frequency of 20 seconds. Accurate mass LC-MS data were acquired using data direct acquisition mode. The energy of trap was set at collision energy of 6 V. In transfer collision energy control, low energy was set at 4 V. The quadrupole mass analyzer was adjusted so that ions from m/z 300 to 1,800 were efficiently transmitted. The MS\MS survey was over range 50 to 1,990 Da and scan time was 0.5 sec.

2.6 Protein quantitation and identification

Protein data was compared against data in the NCBI database for protein identification. The database interrogation was, as follows; taxonomy (*Mus musculus*); enzyme (trypsin); variable modifications (carbamidomethyl, oxidation of methionine residues); mass values (monoisotopic); protein mass (unrestricted); peptide mass tolerance (1.2 Da); fragment mass tolerance (±0.6 Da); peptide charge state (1+, 2+, and 3+); and, max missed cleavages (3). The maximum value of each group was used to determine the presence or absence of each identified protein.

2.7 Western blot analysis

Mouse pancreases were homogenized in RIPA buffer. Total protein concentration was quantified by Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Ten micrograms of total proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membrane was then blocked with 5% skimmed milk. The membrane was incubated overnight at 4°C with one of the following primary antibodies: mouse monoclonal anti-GSTP1(Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), sheep polyclonal anti-kallikrein-1 (R&D Systems, Inc., Minneapolis, MN, USA), or goat polyclonal anti-α tubulin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The membranes were then washed and incubated with HRP-conjugated secondary antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Protein bands were detected by enhanced chemiluminescence (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Band intensities of proteins were analyzed using ImageJ densitometry software version 1.43 (https://imagej.nih.gov/ij/download.html).

2.8 Statistical analysis

Values are expressed as mean ± standard deviation. Student's t-test was used to compare differences between groups. All comparisons were two-tailed and *p*-values<0.05 were regarded as being statistically significant. SPSS Statistics version 23 (SPSS, Inc., Chicago, IL, USA) was used for all data analysis.

3. Results

3.1 Body weight, food intake, and fasting blood glucose

Treatment and control group mice had similar body weight and food intake at the start of the study. However, mean body weight (37.8 ± 1.74 g *vs.* 41.54 ± 1.77 g) and mean daily food intake (3.7 ± 0.2 g *vs.* 4.2 ± 0.22 g) were both significantly decreased in dexamethasone-treated mice at the end of study, as compared to controls (Figure 1a and 1b). Conversely and in contrast to the start of the study, dexamethasone-treated mice had significantly higher fasting blood sugar at the conclusion of the study compared to controls (130 ± 8.5 mg/dl *vs.*107 ± 5.1 mg/dl, respectively) (Figure 1c).

3.2 Two-dimensional gel electrophoresis and mass spectrometry

The two-dimensional gel electrophoresis imaging revealed a total of 276 spots matched between dexamethasone-treated mice and control group mice. Differential image analysis showed 228 changed spots, of which 60 were higher and 168 were lower in the dexamethasone-treated group, when compared with those in the control group. Fifty-two statistically significant spots with either a 1.2 fold increase or decrease were processed for identification by mass spectrometry (Figure 2). Of the 52 statistically significant spots, 14 were found to be up-regulated and 38 were down-regulated (Table 1).

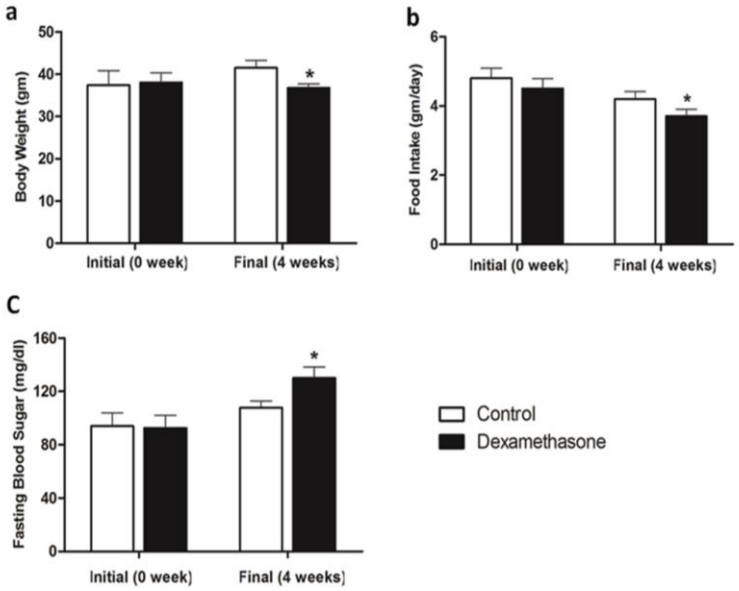

Figure 1. Mean body weight (a), food intake (b), and fasting blood sugar (c) in the dexamethasone-treated group and control group.

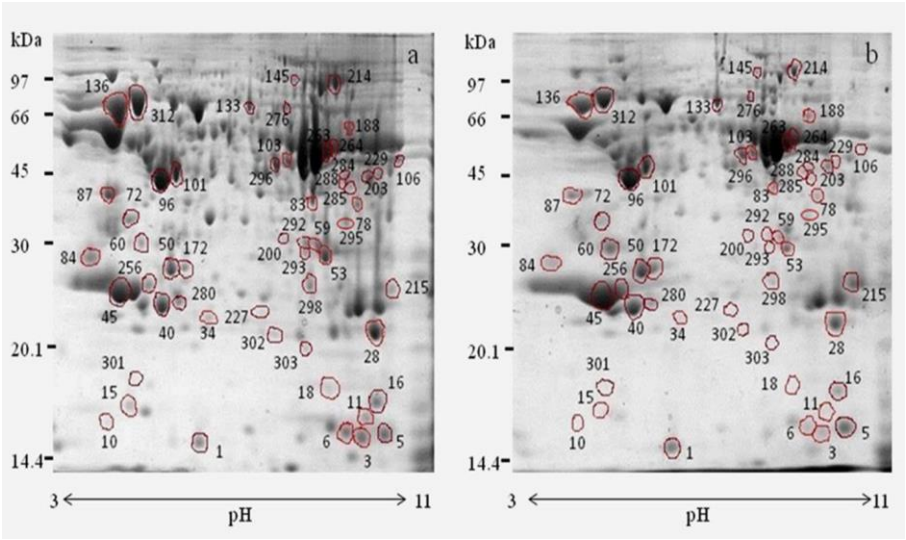

Figure 2. Two-dimensional gel electrophoresis of mice pancreatic lysate: coomassie blue-stained gels from control (a); and, dexamethasone-treated mice (b). Proteins were resolved on 7 cm pH 3-11 IEF strips (NL), followed by SDS-PAGE (10%). Differentially expressed spots detected by ImageMaster 2D Platinum v7.0 software are circled. The gels shown are representative of three independent experiments.

3.3 Western blot analysis

Among the proteins identified by mass spectrometry, GSTpi and kallikrien-1 were selected for validation by immunoblotting (Figure 3a and 3b). Western blot analysis corroborated the proteomic data, showing lower expression of GSTpi and higher expression of kallikrien-1 in dexamethasone-treated pancreases, when compared to controls.

4. Discussion

A plethora of studies have found and reported that dexamethasone administration causes metabolic dysfunction in mice. Glucocorticoid is known to induce muscle wasting and a decline in bone density (Schacke, Docke, & Asadullah, 2002). Accordingly, reduced body weight is a commonly observed feature in mice and rats that are made insulin-resistant by dexamethasone treatment (Protzek *et al.*, 2014;

Table 1. List of identified pancreatic proteins with significantly altered expression after dexamethasone treatment.

Spot no.	Protein	Accession no.	Theoretical Mw (kDa)/ PI	Dex./Con. ratio
1	Lithostathine-1 precursor	gi 6677703	18906/6.08	1.8 ↑
3	Peptidyl-prolyl cis-trans isomerase A	gi 6679439	18131/7.74	1.8 ↓
5	Ribonuclease pancreatic	gi 133221	17265/8.62	1.3 ↑
10	Cytochrome b5	gi 13385268	15232/4.96	2.0 ↓
11	Destrin	gi 9790219	18852/8.14	3.7 ↓
15	Eukaryotic translation initiation factor 5A	gi 31712036	17049/5.08	2.5 ↓
16	Armet protein	gi 28913725	19399/8.38	1.3 ↓
18	Ribosomal protein L23a	gi 46430508	17684/10.44	5.6 ↓
23	Cellular nucleic acid binding protein	gi 50471	20053/8.13	6.8 ↓
28	Glutathione-S-transferase pi	gi 576133	23634/8.13	1.6 ↓
34	Cystidine monophosphate kinase 1	gi 17389257	26125/8.13	1.6 ↓
40	Trypsin 4 precursor	gi 6755893	26941/5.48	1.5 ↑
45	Chymotripsinogen B1	gi 148679565	24653/5.00	3.4 ↑
50	Unnamed protein product	gi 12843302	29642/5.39	1.8 ↑
53	Guanine nucleotide-binding protein subunit beta-2	gi 6680047	35511/7.60	2.8 ↓
59	Electron transferring flavoprotein, alpha polypeptide	gi 13097375	35360/8.62	3.0 ↓
60	Kallikrein	gi 9989702	29354/4.96	5.2 ↑
72	Elongation factor 1 delta	gi 13124192	31388/4.91	1.3 ↓
78	Branched chain amino acid aminotransferase, Mitochondrial isoform 1 precursor	gi 33859514	44669/8.61	1.9 ↓
83	Poly (rC) binding protein 1	gi 6754994	37987/6.66	1.9 ↓
84	14-3-3 Protein epsilon	gi 226874906	29326/4.63	1.9 ↓
87	Protein 40 KD	gi 226005	32846/4.80	2.5 ↓
96	Carboxypeptidase B precursor	gi 56550071	47943/5.19	1.3 ↑
101	Carboxypeptidase A1 precursor	gi 54312076	47469/5.41	2.3 ↑
103	Pancreatic triacylglycerol lipase precursor	gi 37674236	52137/6.37	1.7 ↓
106	Elongation factor Tu	gi 556301	50474/9.10	1.7 ↓
133	Bile salt activated lipase precursor	gi 6753406	66170/5.88	2.2 ↑
136	Pdia 2 protein	gi 109731005	58209/4.90	1.5 ↓
145	Elongation factor 2	gi 18202285	96222/6.41	1.3 ↓
164	Insulin-like growth factor II	gi 393423	5716/4.31	4.9 ↓
172	Unnamed protein product	gi 12843302	29642/5.39	2.1 ↑
188	Pyruvat kinase PKM isoform M2	gi 31981562	58378/7.18	1.8 ↓
200	60S Ribosomal protein L5	gi 23956082	34607/9.87	5.1 ↓
203	Phosphoglycerate kinase 1	gi 70778976	44921/8.02	2.4 ↓
214	P100 Co-activator	gi 6009521	99934/6.64	1.8 ↓
215	Rpl 13 protein	gi 55716028	24639/9.99	3.1 ↑
227	Acyl-protein thioesterase 1	gi 6678760	25013/6.14	3.1 ↓
229	NADP specific isocitrate dehydrogenase	gi 120003362	51358/8.88	2.7 ↓
256	Rho GDP-dissociation inhibitor 1	gi 31982030	23450/5.12	5.8 ↑
263	Pancreatic alpha-amylase precursor	gi 111607467	57966/6.88	2.4 ↑
264	Unnamed protein product	gi 49929	58065/7.18	2.1 ↑
276	Phosphoenolpyruvate carboxykinase, mitochondrial	gi 52783203	71338/6.92	1.5 ↓
280	Precursor apoprotein A-I	gi 50015	30569/5.64	1.5 ↑
284	S-adenosyl homocysteine hydrolase	gi 56541076	48156/5.98	3.5 ↓
285	Unnamed protein product	gi 12844116	47661/4.76	2.3 ↓
288	Cystathione gamma-lyase	gi 22122387	44166/7.58	2.3 ↓
293	G protein beta subunit like	gi 475012	35453/8.08	4.4 ↓
295	L- lactate dehydrogenase a chain isoform 1	gi 6754524	36817/7.62	1.8 ↓
296	P38-2G4	gi 790470	38233/8.11	2.0 ↓
298	Triosephosphate isomerase	gi 54855	27021/6.90	1.7 ↓
302	Cell division control protein 42 homolog isoform 1 precursor	gi 6753364	21587/6.15	1.3 ↓
303	Ubiquitin-fold modifer-conjugating enzyme 1	gi 13384768	19640/6.90	1.8 ↓

Dex. = Dexamethasone, Con. = Control

Rafacho *et al.*, 2008). Furthermore, the observation that dexamethasone decreases food intake could be due to the anorexic effect of glucocorticoids (Minet-Quinard *et al.*, 2000). Our finding that dexamethasone injection causes significant increase in blood sugar compared to controls is consistent with the findings of previous studies (Rafacho, Cestari, Taboga, Boschero, & Bosqueiro, 2009; Roma, Souza, Carneiro, Boschero, & Bosqueiro, 2012). This increase in blood sugar could be due to reduced glucose disposal and/or increased hepatic glucose production (Rose, Vegiopoulos, &

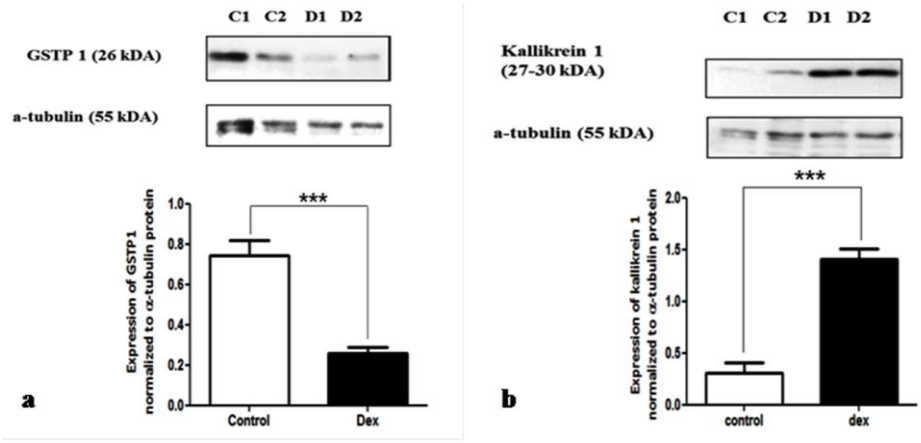

Figure 3. Dexamethasone reduced pancreatic GSTpi, but induced pancreatic kallikrien-1. A representative Western-blot analysis of GSTpi and α-tubulin is shown. The bar graphs above show fold change of GSTpi protein levels normalized to α-tubulin (a). A representative Western-blot analysis of kallikrien-1and α-tubulin is shown. Bar graphs above show fold change of kallikrien-1 protein levels normalized to α-tubulin (b). Data is presented as mean ± S.E.M. of 5-6 mice;***$P< 0.01$ compared to control group.

Herzig, 2010; Vegiopoulos & Herzig, 2007).

In the present study, two-dimensional gel electrophoresis, a gel-based proteomic approach, was employed to examine changes in the pancreatic proteome. Proteomic studies have been conducted in both whole pancreatic tissues, as well as in isolated islets of Langerhans from mice (Sanchez *et al.*, 2001; Xie *et al.*, 2008). Based on our review of the literature, this is the first study to report dexamethasone-induced proteomic alterations in mouse pancreas. Findings from this study may enhance our understanding of the mechanism of steroid-induced β-cell damage. The proteins that were identified are responsible for various molecular functions, as shown in Figure 4.

Pancreatic proteome analysis of high fat diet-induced diabetic mice revealed decreased oxidative stress protections (L. Qiu *et al.*, 2005). A decreased level of glutathione-S-transferase pi (GSTpi) was observed in our study, indicating dexamethasone-induced oxidative stress in mice pancreases. GTSpi is an important enzyme that acts as a cellular antioxidant. GTSpi plays a role in the conjugation of reduced glutathione to a highly diverse group of compounds, thereby protecting tissues from oxidation (Hayes & Strange, 1995). A decreased level of GSTpi has also been observed in other studies where increased oxidative stress was a major finding (Schroer *et al.*, 2011; Sharma *et al.*, 2014). It is important to note that oxidative stress is central to the pathogenesis of T2DM.

Pancreatic β-cells are heavily involved in the synthesis and secretion of insulin. They are, therefore, particularly sensitive to ER stress and the subsequent unfolded protein response. Severe or prolonged episodes of ER stress can lead to death of β-cells (Eizirik, Cardozo, & Cnop, 2008). Proteins related to ER stress, including ubiquitin-fold modifier-conjugating enzyme 1 (UFM 1), Armet proteins, and Pdia 2, were found to be differentially expressed in the pancreases of dexamethasone-treated mice. It is known that UFM 1 protects pancreatic β-cells from ER stress in mouse isolated islet and INS 1 (Lemaire *et al.*, 2011). The decreased level of UFM 1 in dexamethasone-treated pancreas indicates increased ER stress and possible damage to pancreatic β-cells.

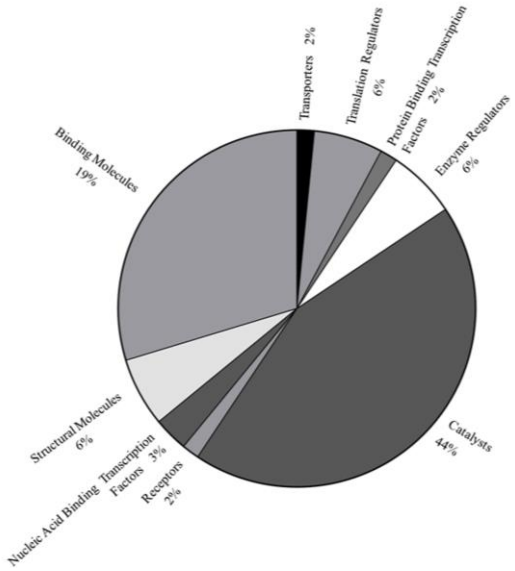

Figure 4. Mass spectrometry analysis of pancreatic proteins in response to dexamethasone treatment. Proteins were classified according to their molecular function using PANTHER software.

Armet, an ER stress response protein, gets induced by the unfold protein response (Mizobuchi *et al.*, 2007), whereas Pida 2 catalyzes protein folding (Desilva *et al.*, 1996). Armet inhibits ER-induced cell death (Apostolou, Shen, Liang, Luo, & Fang, 2008) and its level was also found to be diminished in response to dexamethasone treatment. Peptidyl-prolyl cis-trans isomerase A (PPIA), another molecule that accelerates the folding of proteins, was decreased in dexamethasone-treated mouse pancreas. PPIA was reported to prevent apoptosis in response to oxidative stress via Akt and NF-kB regulating Bcl-2, an anti-apoptotic protein (Wei *et al.*, 2013). In summary, these findings suggest that dexamethasone induces ER stress by affecting protein folding in pancreatic β-cells, a phenomenon that could result in cell death.

One of the most markedly changed protein groups in our study was metabolism-related enzymes. The precursor of pancreatic alpha-amylase, a protein related to carbohydrate metabolism, was increased in dexamethasone-treated mice. However, decreased expression of pancreatic alpha-amylase was previously reported in islets of Langerhans cultured with high glucose concentration (Ahmed & Bergsten, 2005), as well as in hyperlipidemic rat pancreas (Zhang *et al.*, 2010). Precursor of pancreatic alpha-amylase is required to be cleavage and glycosylated (Miyata & Akazawa, 1982). It is possible that dexamethasone might impair conversion process from precursor to mature pancreatic alpha-amylase. This impairment produces increased level of precursor of pancreatic alpha-amylase but decreased pancreatic alpha-amylase expression. However, this notion requires further investigation. Phosphoglycerate kinase 1, triosephosphate isomerase, phosphoenolpyruvate carboxykinase, and mitohondrial and pyruvate kinase PKM isoform M2 were also differentially expressed in mouse pancreas. Decreased levels of these enzymes suggest that carbohydrate metabolism is severely disturbed in dexamethasone-treated pancreatic cells. The metabolism of proteins and lipids were also affected, as indicated by differential expression of corresponding metabolic enzymes.

Proteolytic pathway-related proteins, such as carboxypeptidase A1 (CPA1) precursor, carboxypeptidase B (CPB) precursor, kallikrein, and chymotripsinogen B1, were significantly increased in dexamethasone-treated mouse pancreas. CPB has been linked to activation of insulin (Hu *et al.*, 2005). The increase in CPA1 and CPB precursors in our study may represent a compensatory effort against the suppression of insulin release. Similar to our findings, a high level of kallikrein was reported in the pancreases of diet-induced diabetic mice (Perez-Vazquez *et al.*, 2014). Kallikreins are a subgroup of serine proteases, which are enzymes that are capable of cleaving peptide bonds in proteins. A high level of kallikrein is known to be present in T2DM patients (Campbell *et al.*, 2010), and its presence attenuates insulin resistance (Yuan *et al.*, 2007). Based on these findings, it appears that pancreatic β-cells make an effort to attenuate or compensate for insulin resistance caused by dexamethasone by increasing these proteolytic molecules.

The precursor of lithostathine-1 (or REG 1) was increased in response to dexamethasone treatment in mice. High expression of REG 1 and REG 2 has also been reported in diet-induced diabetic mouse pancreases using two-dimensional gel electrophoresis. This high expression likely reflects an effort to stimulate the proliferation of pancreatic β-cells in order to enhance insulin secretion (L. Qiu *et al.*, 2005). It has also been mentioned that REG 1 and REG 2 can induce neutrophil activation (Keel *et al.*, 2009), which may contribute to the diabetic inflammatory process. Higher expression of REG 1 has been observed in db/db mouse model [36]. Similarly, the precursor of trypsin IV was increased in response to dexamethasone treatment, and it is known that trypsin IV can induce inflammation (Knecht *et al.*, 2007). It can, therefore, be predicted that dexamethasone induces inflammation in the pancreas while exerting its diabetogenic effect.

Several cell signaling molecules, translation factors, and ribosomal proteins were also differentially expressed in dexamethasone-treated mice. Molecules, such as guanine nucleotide-binding protein subunit beta-2, elongation factor 1 delta, elongation factor Tu, elongation factor 2, 60S ribosomal protein L5, G protein beta subunit-like, and cell division control protein 42 (CDC42) homolog isoform 1 precursor, were decreased after commencing dexamethasone treatment, whereas the level of Rho GDP-dissociation inhibitor 1 (GDI) was increased. Studies suggest the involvement of small G-proteins, such as CDC42, in glucose-stimulated insulin secretion (GSIS) from normal rat islets and human islets (Kowluru, 2003; Nevins & Thurmond, 2003). GSIS involves well-regulated trafficking of insulin-laden secretory granules for their docking and fusion with the plasma membrane (Newgard & McGarry, 1995). Emerging evidence suggests that G-proteins have a pivotal role in cytoskeletal remodeling to facilitate granule movement (Kowluru, 2003). Moreover, it was reported that rho guanosine diphosphate dissociation inhibitor plays a negative modulatory role in glucose-stimulated insulin secretion (Kowluru & Veluthakal, 2005). Guanine nucleotide-binding protein subunit beta-2 or RACK1, another important molecule, was decreased in pancreas in response to dexamethasone treatment. RACK 1 function as a key component in regulating the inositol-requiring enzyme 1 α signaling pathway in pancreatic β-cells. It has been reported that RACK 1 level decreases in diabetic mouse model (Y. Qiu *et al.*, 2010).

Insulin-like growth factor 2 (IGF-2) promotes the development of fetal pancreatic β-cells. In our study, the level of IGF-2 was decreased in response to dexamethasone treatment. Dysregulation of IGF-2 has been reported in numerous diseases, most notably diabetes, obesity, and cancers (Livingstone & Borai, 2014). Cytochrome b5, another differentially expressed protein, is an electron transfer component in a number of oxidative reactions. It has previously been associated with obesity in mice (Takahashi, Oh-Ishi, Shimizu, & Mori, 2001), but not with diabetes.

In conclusion, high-dose dexamethasone induced hyperglycemia, reduced body weight, reduced food intake, and altered pancreatic proteins in mice. The affected proteins mainly belonged to cell redox homeostasis, metabolism, endoplasmic reticulum stress, translation, and signaling pathways. These observed changes in the pancreatic proteome could be beneficial in helping us to better understand the phenomenon of glucocorticoid-induced pancreatic cells/β-cells damage.

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