Ghanaat-Pour and Sjöholm, Metabolomics 2011, 1:2
DOI: 10.4172/2153-0769.1000102

Metabolomics: Open Access

Research Article Open Access

Exenatide and Pioglitazone Regulate Fatty Acid-Induced Gene Expression in Normal and Diabetic Human Islets

Hamedeh Ghanaat-Pour and Åke Sjöholm*

Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital, SE-118 83 Stockholm, Sweden

Abstract

Chronically elevated free fatty acids (FFAs) are believed to have a detrimental effect on glucose-stimulated insulin secretion (GSIS), β-cell function, and β-cell survival in type 2 diabetes, a phenomenon often referred to as lipotoxicity. The objective of the present study was to investigate the global alterations in gene expression induced by long term exposure to palmitate with or without the PPAR-γ agonist pioglitazone (a thiazolidinedione) or the GLP-1 receptor agonist exenatide (synthetic exendin-4, an incretin mimetic) in human diabetic and non-diabetic pancreatic islets, using microarray technology and confirmed by qRT-PCR. Gene expression profiling revealed alterations in several functional categories, such as epigenetic regulation of gene expression, cell proliferation and differentiation, metabolism, response to stimulus, transport, and signal transduction. Moreover, *PPY*, *TCF4/TCF7L2*, *REG3A*, *GADD45B, TXNIP, ARNT, EGFR, CEL, ACACA,* and *GAPDH* are some of the interesting genes that were differently regulated in our study. The apoptotic pathway was not substantially influenced by either pioglitazone or exenatide in the presence of palmitate. The function and survival of the human β-cell seems to be controlled directly through the epigenetic control of gene expression rather than a direct effect on the apoptotic pathway. Considering that the nutritional state directly induces the epigenetic modifications, pioglitazone and exenatide appear to normalize these epigenetic misregulations and may protect the β-cell from lipotoxic insult. The epigenetic modifications of the genome provide new promising targets for clinical diagnostics and also therapeutic purposes in the treatment of human type 2 diabetes by conferring β-cell protection from lipotoxicity.

Keywords: Apoptosis; Exenatide; GK rat; Lipotoxicity; microarray, pancreatic islet, pioglitazone, Type 2 diabetes, ARNT, TCF7L2/TCF4, GLP-1, PPAR

Abbreviations: ACACA, Acetyl-Coenzyme A carboxylase alpha; ARNT, Aryl hydrocarbon receptor nuclear translocator; CEL, Carboxyl ester lipase; EGFR, Epidermal growth factor receptor; FFA, free fatty acids; GADD45B, Growth arrest and DNA-damage-inducible beta; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GLP-1, glucagon-like peptide 1; PPY, Pancreatic polypeptide; PPAR-γ, peroxisome proliferator-activated receptor γ; REG3A, Regenerating islet-derived 3 alpha; TZDs, thiazolidinediones; TXNIP, Thioredoxin interacting protein; TCF7L2/TCF4, Transcription factor 7-like 2

Introduction

Elevated plasma free fatty acids (FFAs), caused by the excessive release of fatty acids from an expanded adipose tissue mass, are often linked to β-cell dysfunction in diabetes [1,2]. β-cell mass appears to be reduced in many cases of type 2 diabetic patients [3-5] and considering that type 2 diabetic patients usually are obese, it is unclear whether this defect arises due to genetic and/or environmental factors. The respective role of genetic and environmental factors is controversial, since most of the previous studies have been performed in animal models with a genetic predisposition to diabetes/obesity. Considering the fact that non-diabetic obese subjects show an increased β-cell volume [6], a genetic predisposition may be essential to β-cell failure and thereby β-cell mass reduction.

β-cell failure in type 2 diabetes has been previously suggested to be induced by increased FFAs alone [7,8], or in synergy with glucotoxicity [9-12]. Whether FFAs alter β-cell function and apoptosis in synergy with glucose, or by its own right, thus remains controversial.

Pioglitazone, a PPAR-γ agonist (member of the TZD family), seems to attenuate FFA-induced apoptosis in β-cells [13]. In contrast, troglitazone (another member of the TZD family) showed no protective effect against FFA-induced toxicity in β-cells [14].

Exenatide (synthetic exendin-4), an incretin mimetic and GLP-1 receptor agonist, has been shown to increase β-cell mass by enhancing proliferation/neogenesis, and its function [15-19]. Moreover, it stimulates β-cell fat metabolism [20,21] and protects against β-cell apoptosis [22,23]. The mechanism and the protective effect of these drugs against lipotoxicity in the β-cell remain unclear.

The aim of this study was to determine the long-term *in vitro* effect of palmitate, with or without pioglitazone or exenatide, on global gene expression in human pancreatic islets from healthy non-obese non-diabetic and non-obese diabetic subjects by using microarray technology. We thus wanted to to identify genes influenced by palmitate, pioglitazone and/or exenatide, and also to compare the expression of genes in between the diabetic and non-diabetic groups of conditions.

Materials and Methods

Materials

The chemicals were obtained from the following sources: Collagenase type CLS (EC 3.4.24.3) from Roche (Mannheim, Germany). Culture medium RPMI-1640, fetal calf serum, L-glutamine, benzylpenicillin and streptomycin from Flow Laboratories (Irvine,

***Corresponding author:** Åke Sjöholm, Karolinska Institutet, Department of Internal Medicine, Stockholm South Hospital, SE-118 83 Stockholm, Sweden, Tel: +4686163219; Fax: +4686163146; E-mail: ake.sjoholm@ki.se

Received August 25, 2011; **Accepted** October 27, 2011; **Published** October 29, 2011

Citation: Ghanaat-Pour H, Sjöholm Å (2011) Exenatide and Pioglitazone Regulate Fatty Acid-Induced Gene Expression in Normal and Diabetic Human Islets. Metabolomics 1:102. doi:10.4172/2153-0769.1000102

U.K.). Sodium palmitate from Sigma (St. Louis, MO). All other chemicals of analytical grade were obtained from E. Merck (Darmstadt, Germany). Pioglitazone (Actos®) was generously provided by Takeda Pharmaceuticals North America (Deerfield, IL) and exenatide (Byetta®) was graciously donated by Amylin Inc. (San Diego, CA).

Human islet preparation and culture

Diabetic or non-diabetic human pancreatic islets were kindly provided by the Uppsala University Hospital facility for isolation of human islets from Scandinavian brain-dead donors. The type 2 diabetic donor was a 63-year-old male with BMI 22.6 and the non-diabetic donor was a 57-year-old female with BMI 20.7. The islets were maintained in RPMI-1640 tissue culture medium in the presence of 6 mM glucose, supplemented with 1 % (v/v) FBS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 µg/ml streptomycin, and were used for experiments ~ a week after isolation. A total of 36 (18 non-diabetic and 18 diabetic) islet samples were used for gene chip analyses after 48 hrs of culture: 3 non-diabetic samples of islets cultured in 6 mM glucose, 3 non-diabetic samples of islets cultured in 6 mM glucose and 0.125 mM palmitate, 3 non-diabetic samples of islets cultured in 6 mM glucose and 10 µM pioglitazone, 3 non-diabetic samples of islets cultured in 6 mM glucose, 0.125 mM palmitate and 10 µM pioglitazone, 3 non-diabetic samples of islets cultured in 6 mM glucose and 1 nM exenatide, 3 non-diabetic samples of islets cultured in 6 mM glucose, 0.125 mM palmitate and 1 nM exenatide, 3 diabetic samples of islets cultured in 6 mM glucose, 3 diabetic samples of islets cultured in 6 mM glucose and 0.125 mM palmitate, 3 diabetic samples of islets cultured in 6 mM glucose and 10 µM pioglitazone, 3 diabetic samples of islets cultured in 6 mM glucose, 0.125 mM palmitate and 10 µM pioglitazone, 3 diabetic samples of islets cultured in 6 mM glucose and 1 nM exenatide, and 3 diabetic samples of islets cultured in 6 mM glucose, 0.125 mM palmitate and 1 nM exenatide. At the end of the culture period, batches of ~ 500 islets were washed in PBS and transferred to Eppendorf tubes with 0.5 ml Trizol (Invitrogen, Carlsbad, CA), in which the islets were homogenized and then snap-frozen in liquid nitrogen and maintained in –70°C pending analysis. All experiments were approved by the Karolinska Institute Ethics Committee.

RNA extraction and sample preparation for Affymetrix gene analysis

Total RNA was extracted using the Qiagen RNAesy kit (WVR, Stockholm, Sweden) according to the manufacturer's instructions. The integrity of the extracted RNA was confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Double-stranded cDNA was synthesized with 50 ng of total RNA using the SuperScript Choice system (Invitrogen, Carlsbad, CA). Synthesis of single-stranded complementary DNA (cDNA) and purification of double-stranded cDNA, synthesis and isolation of biotin-labeled complementary RNA (cRNA), quantification and fragmentation of cRNA, and target hybridization of fragmented cRNA, washing and staining of GeneChip expression probe arrays were all performed according to the manufacturer's instructions (Technical manual of Affymetrix GeneChip products). Samples were analyzed by the Affymetrix Human Genome U133 Plus 2.0 Array interrogating over 47,000 transcripts. The level of expression of each gene was evaluated by the intensity of hybridization of labeled mRNA.

The probe arrays were scanned using the Agilent Gene Array Scanner (Agilent, Santa Clara, CA) and the scanned images were inspected and analyzed using established quality control criteria according to manufacturer's instructions.

Data analysis

Data normalization and statistical analysis were performed using GeneSpring 7.3 software (Agilent, Santa Clara, CA). The data were normalized by utilizing the 50th percentile (per chip normalization) and the normalization to median (per gene normalization). To determine genes differentially expressed across conditions, the ANOVA test (considering False Discovery Rate, FDR) at P values <0.05, and a fold change of >1.5 were performed. Moreover, these genes were assigned to functional groups as described in GeneSpring (Agilent, Santa Clara, CA), NetAffx (Affymetrix) and other databases. Detailed descriptions of a selection of the differentially expressed transcripts, classified according to function and average fold change, are listed in Tables 1-4 and supplementary Tables 6-13.

cDNA synthesis

RNA was denatured for 10 min at 65°C and immediately chilled on ice. First strand cDNA synthesis was performed in a 20 µl reaction mixture containing 2 µg total RNA in a solution of 10 µl, 4 µl 5 × reverse transcriptase buffer (Invitrogen, Carlsbad, CA), 10 mM deoxynucleoside triphosphate, 1 µl random hexamer primers (100 pmol/µl), 1.5 µl dithiothreitol (Amersham Pharmacia Biotech, Piscataway, NJ) (100 mmol/l) and 1 µl RT1 reverse transcriptase (200 U/µl; Invitrogen, Carlsbad, CA). The reagents were mixed and incubated at 37°C for 45 min. cDNA solutions were incubated for 5 min at 95°C to inactivate reverse transcriptase and then stored at –20°C.

Quantitative real-time PCR

Expression of selected genes (Table 5) was performed from total RNA using an ABI Prism 7700 polymerase chain reaction machine (PE Applied Biosystems, Foster City, CA). These genes were selected based on differential expression grounded on statistical analysis and fold change threshold, a select range of intensities, biological interest and potential roles in diabetes, and primer availability. Results are expressed relative to with and the ratio of different groups (Table 5).

cDNA samples were used as templates whereas an internal control (18S rRNA), was quantified as a positive control and used for normalization of the different template values. 18S rRNA was chosen among 34 candidate control genes previously checked using the TaqMan Human Endogenous Control Plate. Data were normalized using 18S rRNA because of the consistency of its level from sample to sample. To amplify the cDNA, 100 ng of the reversed transcribed cDNA from each sample were subjected to 40 cycles of real-time quantitative PCR in 20 µl of total reaction volume. Moreover, *GADD45B*, *GAPDH*, *PPY*, *REG3A*, *TXNIP*, *CEL*, *ARNT*, *ISL1*, *TCF7L2*/*TCF4*, and *DAD1* probes and primers were used to flank the target DNA sequence. Each real-time TaqMan PCR reaction (20 µl) consisted of the cDNA template (except for the negative controls) and H_2O to final volume of 9 µl, 1µl of TaqMan® Gene Expression Assay in 20 X format (containing the target primers and TaqMan probe) and 10 µl of TaqMan Fast Universal PCR Master Mix (2X), No AmpErase UNG (According to manufacturer's instruction "TaqMan Gene Expression Assays", Applied Biosystems). Aliquots were then amplified by an initial period of 2 min at 50°C and 10 min at 95°C followed by 40 concurrent cycles involving denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min The samples were placed in 96-well optical PCR plate (N 801-0560, Perkin Elmer) and amplified in an automated fluorometer (ABI PRISM 7700 Sequence Detection System, Applied Biosystems). For relative quantification of different mRNAs, the following arithmetic formula was used according to the Perkin-Elmer Instruction Manual

Function	Gene Title	Public ID	Fold	change	
			Palmitate	Palmitate + pioglitazone	Palmitate + exenatide
Regulation of gene expression, epigenetic	Thyroid hormone receptor associated protein 1 (THRAP1)	BC024236	11.2		
	Phosphoinositide-3-kinase, class 3 (PIK3C3)	BC010388	9.1		
	Cardiac-MyBP-C associated Ca/CaM kinase (MLCK)	BC039103	7.1		
	Acetyl-Coenzyme A carboxylase alpha (ACACA)	BC007115	5.2		
	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome) (GLI3)	BC032660	5.6		
	Deoxynucleotidyltransferase, terminal (DNTT)	AA585152	5.4		
	RAD51 homolog (RAD51)	AL833420	4.7		
	Pleckstrin homology, Sec7 and coiled-coil domains 1(cytohesin 1)(PSCD1)	CA442689	3.4		
	Sterol regulatory element binding transcription factor 1 (SREBF1)	S66168	3.3		
	Rho-guanine nucleotide exchange factor (RGNEF)	AB082529	3.2		
	Leukemia inhibitory factor receptor (LIFR)	BC038371	3.0		
	Insulin-like growth factor binding protein 2, 36kDa (IGFBP2)	NM_000597	2.9		
	Glutamate receptor, metabotropic 3 (GRM3)	AI733361	2.8		
	Glutathione peroxidase 3 (GPX3)	AW149846	2.5		
	Pre-B-cell leukemia transcription factor 1 (PBX1)	AL832146	2.4		
	Transforming growth factor, beta receptor II (TGFBR2)	AI809493	2.4		
	CAMP responsive element binding protein 1 (CREB1)	AW945589	2.2		
	Nuclear receptor subfamily 1, group H, member 2 (NR1H2)	NM_007121	1.8		
	Arginase (ARG1)	AV649309	1.7		
	Fibroblast growth factor 10 (FGF10)	NM_004465	1.7		
	Nodal homolog (NODAL)	AI670948	1.7		
	X (inactive)-specific transcript (XIST)	BE644917	1.6		
	Aryl hydrocarbon receptor nuclear translocator (ARNT)	AL137290	1.6		
	Sp3 transcription factor (SP3)	AW470841	1.5		
	Ribosomal protein L28 (RPL28)	NM_000991		21.2	5.6
	Chromatin modifying protein 5 (CHMP5)	NM_015961		18.4	15.6
	Hypothetical protein LOC285463 (CTBP1)	BF984434		14.0	14.9
	Glutathione S-transferase A1(GSTA1)	NM_000846		11.7	4.9
	Protease, serine, 3 (PRSS3)	NM_002771		9.4	3.2
	Aldolase B, fructose-bisphosphate (ALDOB)	BC005314		8.0	8.0
	ISL1 transcription factor, LIM/homeodomain, (islet-1)(ISL1)	NM_002202		7.3	8.6
	Trypsinogen C (TRY6)	U66061		7.3	
	Calreticulin (CALR)	AA910371		7.2	9.8
	Ectonucleotide pyrophosphatase/ phosphodiesterase 2 (autotaxin)(ENPP2)	L35594		7.2	3.2
	Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5)	X90579		7.1	1.9
	Cysteine-rich, angiogenic inducer, 61 (CYR61)	NM_001554		7.0	6.6
	Protease, serine, 2 (trypsin 2)(PRSS2)	NM_002770		6.9	3.3
	Fibrinogen beta chain (FGB)	NM_005141		6.9	4.3
	Human T-cell leukemia virus enhancer factor (HTLF)	BF590117		5.7	6.4
	Regulator of G-protein signalling 4 (RGS4)	AL514445		5.3	4.4
	Pancreatic polypeptide (PPY)	M15788		5.2	
	CD53 antigen (CD53)	NM_000560		4.8	3.6
	Leukocyte-derived arginine aminopeptidase (LRAP)	BE889628		4.7	4.3
	Colipase, pancreatic (CLPS)	NM_001832		4.6	1.9
	Potassium large conductance calcium-activated channel, subfamily M, beta member 2 (KCNMB2)	AF209747		4.6	4.7

	Regenerating islet-derived 3 alpha (REG3A)	NM_002580		4.2	2.0
	Phospholipase C, epsilon 1 (PLCE1)	NM_016341		4.0	1.8
	Transcription factor 4 (TCF4)	BF592782		3.9	5.3
	cAMP responsive element binding protein 1 (CREB1)	M34356		3.7	2.5
	Transforming growth factor, beta 2 (TGFB2)	BF061658		3.4	3.7
	lymphoid-restricted membrane protein (LRMP)	NM_006152		3.3	2.3
	Cytochrome P450, family 2, subfamily A, polypeptide 6 (CYP2A6)	M33318		3.3	3.2
	Bombesin-like receptor 3 (BRS3)	Z97632		3.2	2.3
	Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	AF182273		3.2	1.8
	Eukaryotic translation initiation factor 2-alpha kinase 1 (EIF2AK1)	NM_014413		3.1	2.0
	Glutathione peroxidase 2 (gastrointestinal)(GPX2)	NM_002083		3.3	3.3
	Ferritin, heavy polypeptide 1(FTH1)	AA083483		3.1	3.7
	Meningioma expressed antigen 5 (hyaluronidase) (MGEA5)	AK002091		3.0	1.7
	ADAM metallopeptidase domain 28 (ADAM28)	NM_021778		3.0	
	Nuclear receptor subfamily 4, group A, member 1 (NR4A1)	D85245		2.9	3.5
	High-mobility group box 1 (HMGB1)	N92507		2.8	2.5
	Peripherin (PRPH)	NM_006262		2.8	4.1
	Activating transcription factor 6 (ATF6)	NM_007348		2.8	1.9
	GLIS family zinc finger 3 (GLIS3)	AW025602		2.7	2.3
	DNA-damage-inducible transcript 3 (DDIT3)	BC003637		2.7	
	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (CACNA1A)	AA769818		2.6	2.5
	Paired box gene 6 (PAX6)	NM_000280		2.6	
	Interleukin 7 receptor (IL7R)	NM_002185		2.5	2.2
	Villin 2 (ezrin)(VIL2)	BF663141		2.5	2.0
	Xanthine dehydrogenase (XDH)	U06117		2.4	3.2
	Glutamate receptor, metabotropic 2 (GRM2)	NM_000839		2.4	2.4
	Heparin-binding EGF-like growth factor (HBEGF)	NM_001945		2.3	2.3
	Tumor necrosis factor (ligand) superfamily, member 13b (TNFSF13B)	AW151360		2.3	2.5
	Major histocompatibility complex, class II, DR alpha (HLA-DRA)	M60333		2.3	
	S100 calcium binding protein A8 (calgranulin A) (S100A8)	NM_002964		2.2	2.0
	Major histocompatibility complex, class I, A (HLA-A)	AI923492		2.2	2.1
	Major histocompatibility complex, class II, DR beta 1(HLA-DRB1)	NM_002125		2.1	
	Apolipoprotein C-I (APOC1)	W79394		2.1	1.9
	Pantothenate kinase 2 (PANK2)	AV703394		2.0	2.0
	Neuropeptide Y (NPY)	NM_000905		2.0	2.1
	Rho guanine nucleotide exchange factor (GEF) 12 (ARHGEF12)	AI807672		2.0	1.8
	keratin 20 (KRT20)	AI732381		2.0	1.9
	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 (NFATC3)	U85430		2.0	
	Fms-related tyrosine kinase 1 (FLT1)	AA058828		1.9	2.3
	Sp4 transcription factor (SP4)	BF438799		1.9	2.1
	Synaptotagmin I (SYT1)	AV731490		1.8	
	Major histocompatibility complex, class II, DQ beta 1(HLA-DQB1)	AI583173		1.8	
	Interleukin 10 receptor, beta (IL10RB)	BC001903		1.8	
	Phosphofructokinase, muscle (PFKM)	U24183		1.7	
	Rap guanine nucleotide exchange factor (GEF) 3 (RAPGEF3)	U78168		1.7	2.3
	Epidermal growth factor receptor (EGFR)	BE878463		1.7	
	Chemokine (C-C motif) receptor 1 (CCR1)	NM_001295		1.6	2.1
	Carboxypeptidase M (CPM)	AW663908		1.6	

	FXYD domain containing ion transport regulator 3 (FXYD3)	NM_005971		3.1	3.6
	PR domain containing 1, with ZNF domain (PRDM1)	AI692659		1.9	3.1
	Myosin binding protein C (MYBPC3)	NM_000256		2.7	2.4
	Bombesin-like receptor 3 (BRS3)	Z97632		3.2	2.3
	Arachidonate 5-lipoxygenase (ALOX5)	NM_000698			2.1
	Regenerating islet-derived 3 alpha (REG3A)	NM_002580		4.2	2.0
	Bone morphogenetic protein 2 (BMP2)	NM_001200			1.9
	Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5)	X90579		7.1	1.9
	Major histocompatibility complex, class II, DR beta 4 (HLA-DRB4)	BC005312		1.6	1.9
	Colipase, pancreatic (CLPS)	NM_001832		4.6	1.9
	Alpha-2-HS-glycoprotein (AHSG)	AF130057			1.6
	WNT1 inducible signaling pathway protein 3 (WISP3)	AF143679			1.5
Metabolism	Potassium channel, subfamily K, member 1 (KCNK1)	AL833343	4.3		
	Choline dehydrogenase (CHDH)	AA609488	3.7		
	Nephronophthisis 3 (adolescent)(NPHP3)	NM_152530	2.9		
	Hydroxysteroid (17-beta) dehydrogenase 12 (HSD17B12)	BC012536	2.9		
	NADPH oxidase 1 (NOX1)	AI826277	2.9		
	Tyrosinase (TYR)	BC027179	2.9		
	Eukaryotic translation initiation factor 3, subunit 9 eta (EIF3S9)	AA628539	2.6		
	Proteasome 26S subunit, non-ATPase, 7 (Mov34 homolog)(PSMD7)	AW361702	2.3		
	Solute carrier family 18, member 2 (SLC18A2)	NM_003054	2.3		
	Mevalonate kinase (MVK)	AU150926	1.9		
	Mitochondrial ribosomal protein S12 (MRPS12)	NM_021107	1.9		
	Transferrin receptor (TFRC)	N76327	1.9		
	Nitric oxide synthase 1 (NOS1)	BE299830	1.8		
	Transient receptor potential cation channel, subfamily M, member 6 (TRPM6)	AK026281	1.8		
	Sorbin and SH3 domain containing 1 (SORBS1)	AI377221	1.7		
	Solute carrier family 22, member 5 (SLC22A5)	NM_003060	2.0	1.8	2.0
	Cystathionine-beta-synthase (CBS)	BE613178	1.7		
	Sucrase-isomaltase (alpha-glucosidase)(SI)	NM_001041	1.6		
	Secretin (SCT)	NM_021920	1.5		
	Glutamate decarboxylase 2 (GAD2)	NM_000818		10.7	8.2
	Glutaredoxin (thioltransferase) (GLRX)	AF162769		5.2	2.3
	Voltage-dependent anion channel 3 (VDAC3)	U90943		4.9	2.1
	Lectin, mannose-binding, 1 (LMAN1)	U09716		4.7	4.7
	Collagen, type I, alpha 2 (COL1A2)	NM_000089		4.0	3.8
	NADH dehydrogenase 1 beta subcomplex, 8 (NDUFB8)	NM_005004		3.9	
	Ferritin, light polypeptide (FTL)	BG538564		3.8	
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	AFFX-HUMGAPDH/ M33197_5		3.6	
	Potassium inwardly-rectifying channel, subfamily J, member 15 (KCNJ15)	D87291		3.6	2.0
	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 2 (NDUFA2)	BC003674		3.3	2.4
	Thioredoxin interacting protein (TXNIP)	AI439556		3.2	1.8
	Potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8)	NM_004982		3.2	1.8
	Glycogenin (GYG)	NM_004130		3.1	
	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase) (KMO)	AI074145		2.7	2.2
	Ceruloplasmin (ferroxidase) (CP)	NM_000096		2.7	

	Biotinidase (BTD)	AI767414		2.5	1.6
	Sterol O-acyltransferase 2 (SOAT2)	AF059203		2.4	2.6
	Carbohydrate (chondroitin 4) sulfotransferase 11 (CHST11)	AI806905		2.3	2.7
	SH3-domain GRB2-like 2 (SH3GL2)	NM_003026		2.3	1.8
	Syntaxin 7 (STX7)	NM_003569		2.2	1.9
	Matrix metallopeptidase 3 (stromelysin 1, progelatinase) (MMP3)	NM_002422		2.2	2.1
	Carbonic anhydrase I (CA1)	NM_001738		2.1	2.2
	Pyruvate dehydrogenase kinase, isoenzyme 3 (PDK3)	NM_005391		2.1	1.6
	Catalase (CAT)	AY028632		2.0	
	Synaptotagmin V(SYT5)	NM_003180		2.0	1.8
	Phosphogluconate dehydrogenase (PGD)	NM_002631		1.9	
	Steroid sulfatase, arylsulfatase C, isozyme S (STS)	NM_000351		1.8	1.7
	ATP-binding cassette, sub-family C (CFTR/MRP), member 1 (ABCC1)	NM_004996		1.8	2.3
	Glutathione S-transferase A3 (GSTA3)	NM_000847		1.8	
	Sorbitol dehydrogenase (SORDa)	L29008		1.7	2.1
	Carnitine palmitoyltransferase 1A (CPT1A)	BC000185		1.7	
	Holocarboxylase synthetase (biotin-(proprionyl-Coenzyme A-carboxylase (ATP-hydrolysing)) ligase) (HLCS)	D87328		1.6	
	Argininosuccinate synthetase (ASS)	NM_000050		1.6	
	Monoglyceride lipase (MGLL)	BC006230		1.6	
	NADH dehydrogenase 1 alpha subcomplex, 9 (NDUFA9)	AF050641		1.5	
	NADH dehydrogenase 1 alpha subcomplex, 10 (NDUFA10)	NM_004544		1.5	
	DEAD (Asp-Glu-Ala-Asp) box polypeptide 18 (DDX18)	NM_006773		9.2	9.8
	Proprotein convertase subtilisin/kexin type 2 (PCSK2)	AL031664		7.2	6.5
	Cyclic nucleotide gated channel alpha 1 (CNGA1)	NM_000087		3.7	2.8
	Cytochrome b-245, alpha polypeptide (CYBA)	NM_000101		2.6	2.1
	Cyclic nucleotide gated channel alpha 3 (CNGA3)	NM_001298		1.8	2.1
	Ceruloplasmin (ferroxidase)(HPS3, CP)	AI922198		2.4	2.0
	Phospholipase A2, group IIA (PLA2G2A)	NM_000300			1.6
	Interleukin 27 receptor, alpha (IL27RA)	NM_004843			1.5
Apoptosis	Mitogen-activated protein kinase 1 (MAPK1)	AL833111	4.7		
	BCL2/adenovirus E1B interacting protein like (BNIPL)	NM_138279	3.2		
	Serine/threonine kinase 17b (STK17B)	AI221707	2.3		
	Ras homolog gene family, member B (RHOB)	AI263909		9.5	13.3
	Defender against cell death 1 (DAD1)	NM_001344		8.4	3.4
	Lectin, galactoside-binding, soluble, 1 (galectin 1) (LGALS1)	NM_002305		6.6	3.0
	Engulfment and cell motility 1 (ELMO1)	NM_014800		5.5	4.8
	Synuclein, alpha (SNCA)	L36675		3.6	2.7
	Ring finger and FYVE-like domain containing 1(RFFL)	AI760772		3.3	3.5
	Growth arrest and DNA-damage-inducible, beta (GADD45B)	AF078077		2.9	1.7
	Serine/threonine kinase 4 (STK4)	BE222274		2.8	3.0
	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)(CASP1)	U13699		2.7	
	Mitogen-activated protein kinase kinase kinase 10 (MAP3K10)	NM_002446		2.7	2.7
	Protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), beta isoform (PPP2R1B)	AF163473		2.0	1.9
	Peroxiredoxin 2 (PRDX2)	L19185		1.8	
	NADH dehydrogenase 1 alpha subcomplex, 13 (NDUFA13)	NM_015965		1.7	

	Programmed cell death 8 (PDCD8)	NM_004208		1.7	
	TNF receptor-associated factor 2 (TRAF2)	NM_021138		1.6	
	BCL2-associated athanogene (BAG1)	AA394039		1.5	
	Apolipoprotein E (APOE)	N33009		1.5	1.8
	Caspase 10, apoptosis-related cysteine peptidase (CASP10)	NM_001230		1.5	
	Synuclein, alpha (non A4 component of amyloid precursor)(SNCA)	L36675		3.6	2.7
	Secreted phosphoprotein 1 (osteopontin)(SPP1)	AB019562			1.8
	S100 calcium binding protein, beta (S100B)	BC001766			1.5
Response to stimulus	Lymphotoxin beta (TNF superfamily, member 3) (LTB)	BC018898	2.8		
	Calcium-sensing receptor (CASR)	BF061333	2.1		
	Decay accelerating factor for complement (CD55) (DAF)	CA448665	2.1		
	DnaJ (Hsp40) homolog, subfamily A, member 1(DNAJA1)	NM_001539		8.0	5.6
	Chemokine (C-X-C motif) ligand 2 (CXCL2)	M57731		5.0	5.1
	CD59 antigen p18-20 (CD59)	BF983379		4.5	1.6
	CD58 antigen, (lymphocyte function-associated antigen 3) (CD58)	D28586		3.6	2.3
	Decay accelerating factor for complement (DAF)	BC001288		2.1	
	Coagulation factor XII (Hageman factor)(F12)	BF530348		1.7	1.8
	Chemokine (C-C motif) ligand 5 (CCL5)	NM_002985			1.8
	Interleukin 1 receptor antagonist (IL1RN)	U65590			1.8
Signal transduction	Leucine-rich repeat-containing G protein-coupled receptor 7 (LGR7)	NM_021634	3.0		
	SH3 multiple domains 2 (SH3MD2)	AB062480	3.0		
	GTPase, IMAP family member 1 (GIMAP1)	NM_130759	2.1		
	Glutamate receptor, metabotropic 5 (GRM5)	S64316	1.9		
	Growth differentiation factor 15 (GDF15)	BC000529	1.7		
	Thromboxane A2 receptor (TBXA2R)	U27325		1.7	2.1
	Protein tyrosine phosphatase, non-receptor type 1 (PTPN1)	NM_002827		1.5	
	SH3-domain binding protein 2 (SH3BP2)	AB000462		1.5	1.9
	TBC1 domain family, member 4 (TBC1D4)	NM_014832		2.7	2.0

Table 1: Transcripts down-regulated by palmitate, with or without pioglitazone or exenatide, in diabetic human islets.

of 1997: Relative alteration = $2^{-\Delta\Delta CT}$, where Ct is the point (cycle) at which the amplification plot crosses the threshold, which is defined as ΔCt = (Ct_sample2 – Ct_18S rRNA) - (Ct_sample1 Ct_18S rRNA). Therefore, the final value indicates an increase or decrease in mRNA for selected genes after normalization against 18S rRNA (Table 5).

Statistical analysis

Means ± S.E.M. were calculated and groups of data compared using Student's *t*-test for paired or unpaired data. Differences were deemed statistically significant when $P<0.05$.

Results

Microarray analysis of global gene expression changes regulated by palmitate, pioglitazone and exenatide in normal and diabetic human islets

To gain detailed insight into the molecular mechanisms of palmitate, pioglitazone and exenatide action in isolated islets, we performed global gene expression profiling studies using Affymetrix GeneChip technology. Among the ~ 47,000 transcripts interrogated on the array, there were over 2,700 genes with known or inferred function that were affected by both pioglitazone and exenatide treatment, during palmitate exposure (Figure 1). A large number of differentially expressed genes belonged to several functional categories, such as epigenetic regulation of gene expression, cell proliferation and differentiation, metabolism, response to stimulus, transport, and signal transduction (supplementary Figure 2). Interestingly, epigenetic regulation of gene expression turned out to be the largest and most influenced transcript group in this study. The expressional profiles for these genes or ESTs are listed in Table 1 to 4 and supplementary Tables 6-13. In none of the analyses, the apoptotic pathway was noticeably influenced either by palmitate, pioglitazone or exenatide.

Differential expression of a subset of genes confirmed with qrt-PCR

To confirm the gene regulation observed with microarray analysis, we performed qRT-PCR on samples for selected genes that were differently regulated on the microarrays. The results generated by qRT-PCR showed a highly significant correlation between the levels of gene expression measured by both methods (Table 5).

Discussion

The interaction between the genetic predisposition to β-cell failure with environmental or lifestyle factors appears to lead to diabetes

Function	Gene Title	Public ID	Fold	change	
			Palmitate	Palmitate + pioglitazone	Palmitate + exenatide
Regulation of gene expression, epigenetic	Chromatin modifying protein 5 (CHMP5)	NM_015961	17.5		
	Aldolase B, fructose-bisphosphate (ALDOB)	BC005314	17.4		
	PHD finger protein 20 (PHF20)	AL109965	12.7		
	ISL1 transcription factor, LIM/homeodomain, (ISL1)	NM_002202	12.4		
	Potassium large conductance calcium-activated channel, subfamily M, beta member 2 (KCNMB2)	AF209747	12.1		
	Nuclear receptor subfamily 2, group F, member 2 (NR2F2)	AL037401	11.9		
	Ectonucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2)(autotaxin)	L35594	10.5		
	Forkhead box N2 (FOXN2)	BF590117	9.7		
	Transcription factor 4 (TCF4)	BF592782	8.6		
	Regulator of G-protein signalling 4 (RGS4)	AL514445	8.6		
	Calreticulin (CALR)	AA910371	8.5		
	Fibrinogen beta chain (FGB)	NM_005141	8.4		
	Glutathione S-transferase A1 (GSTA1)	NM_000846	7.4		
	KH domain containing, RNA binding, signal transduction associated 1 (KHDRBS1)	NM_006559	6.1		
	Leukocyte-derived arginine aminopeptidase (LRAP)	BE889628	5.5		
	Ribosomal protein S19 (RPS19)	BC000023	5.5		
	Cysteine-rich, angiogenic inducer, 61 (CYR61)	NM_001554	5.5		
	GLIS family zinc finger 3 (GLIS3)	AW025602	5.0		
	High-mobility group box 1(HMGB1)	N92507	4.2		
	Regenerating islet-derived 3 alpha (REG3A)	NM_002580	4.1		
	Adducin 1 (alpha) (ADD1)	NM_001119	3.9		
	Protease, serine, 2 (trypsin 2)(PRSS2)	NM_002770	3.8		
	MAX-like protein X (MLX)	N40555	3.7		
	Eukaryotic translation initiation factor 2-alpha kinase 1 (EIF2AK1)	NM_014413	3.6		
	Sp4 transcription factor (SP4)	BF438799	3.5		
	Aldolase B, fructose-bisphosphate (ALDOB)	AV652403	3.5		
	Meningioma expressed antigen 5 (MGEA5)	AK002091	3.4		
	Major histocompatibility complex, class I, A (HLA-A)	AI923492	3.4		
	Runt-related transcription factor 1 (RUNX1)	D43968	3.3		
	CD53 molecule (CD53)	NM_000560	3.3		
	Corticotropin releasing hormone (CRH)	NM_000756	3.2		
	Lymphoid-restricted membrane protein (LRMP)	NM_006152	3.2		
	Platelet/endothelial cell adhesion molecule (PECAM1)(CD31 antigen)	AW574504	3.2		
	Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5)	X90579	3.0		
	Major histocompatibility complex, class II, DQ beta 1 (HLA-DQB1)	AI583173	3.0		
	Retinoic acid receptor responder 2 (RARRES2)	BC000069	3.0		
	Major histocompatibility complex, class II, DR beta 1 (HLA-DRB1)	NM_002125	2.9		
	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (CACNA1A)	AA769818	2.9		
	Major histocompatibility complex, class II, DR beta 4 (HLA-DRB4)	BC005312	2.8		
	Glutathione peroxidase 1(GPX1)	NM_000581	2.8		
	Bombesin-like receptor 3 (BRS3)	Z97632	2.8		
	Aldolase A, fructose-bisphosphate (ALDOA)	AK026577	2.7		
	Neuropeptide Y (NPY)	NM_000905	2.7		
	Major histocompatibility complex, class II, DR alpha (HLA-DRA)	M60333	2.7		
	Fibroblast growth factor receptor 2 (FGFR2)	NM_022975	2.7		
	ADAM metallopeptidase domain 28 (ADAM28)	NM_021778	2.7		
	PHD finger protein 13 (PHF13)	AL039384	2.7		
	Heparin-binding EGF-like growth factor (HBEGF)	NM_001945	2.7		

	Amylase, alpha 2A (AMY2A)	NM_004038	2.7		
	Signal transducer and activator of transcription 6, interleukin-4 induced (STAT6)	BC004973	2.6		
	Pantothenate kinase 2 (PANK2)	AV703394	2.6		
	Pancreatic polypeptide (PPY)	M15788	2.5		
	Phosphofructokinase, muscle (PFKM)	U24183	2.5		
	Major histocompatibility complex, class II, DR beta 5 (HLA-DRB5)	NM_021983	2.5		
	PHD finger protein 15 (PHF15)	AI735639	2.5		
	Guanine nucleotide binding protein (G protein), beta 5 (GNB5)	NM_016194	2.5		
	Chemokine (C-C motif) receptor 1 (CCR1)	NM_001295	2.4		
	Colipase, pancreatic (CLPS)	NM_001832	2.4		
	Nuclear receptor subfamily 4, group A, member 1 (NR4A1)	D85245	2.3		
	Acetyl-Coenzyme A carboxylase beta (ACACB)	AI970898	2.3		
	Fms-related tyrosine kinase 1 (FLT1)	AA058828	2.3		
	Rho guanine nucleotide exchange factor (GEF) 12 (ARHGEF12)	AI807672	2.3		
	Vav 3 oncogene (VAV3)	AF118887	2.2		
	Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	AF182273	2.2		
	Islet cell autoantigen 1 (ICA1)	L21181	2.2		
	Tumor necrosis factor receptor superfamily, member 13B (TNFRSF13B)	NM_012452	2.1		
	S100 calcium binding protein A8 (S100A8)	NM_002964	2.1		
	keratin 20 (KRT20)	AI732381	2.1		
	Mitogen-activated protein kinase kinase 3 (MAP2K3)	AA780381	2.1	2.4	2.0
	V-set domain containing T cell activation inhibitor 1 (VTCN1)	NM_024626	2.0		
	Apolipoprotein C-I (APOC1)	W79394	2.0		
	CDK5 regulatory subunit associated protein 1 (CDK5RAP1)	NM_016408	2.0		
	Paired box gene 6 (PAX6)	NM_000280	1.9		
	Phospholamban (PLN)	NM_002667	1.9		
	DNA-damage-inducible transcript 3 (DDIT3)	BC003637	1.9		
	Major histocompatibility complex, class II, DM alpha (HLA-DMA)	X76775	1.8		
	Interleukin 7 receptor (IL7R)	NM_002185	1.8		
	Solute carrier family 3, member 2 (SLC3A2)	NM_002394	1.8		
	Epidermal growth factor receptor (EGFR)	BE878463	1.8		
	Bone morphogenetic protein 2 (BMP2)	NM_001200	1.8		
	Transforming growth factor, beta 2 (TGFB2)	BF061658	1.7		
	Kruppel-like factor 5 (KLF5)	AB030824	1.7		
	Glutamate-ammonia ligase (GLUL)(glutamine synthetase)	U08626	1.7		
	Melanocortin 4 receptor (MC4R)	NM_005912	1.7		
	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 (NFATC3)	U85430	1.7		
	YY1 transcription factor (YY1)	AA748649	1.7		
	EPH receptor B2 (EPHB2)	AL530874	1.7		
	Mitogen-activated protein kinase kinase kinase 3 (MAP3K3)	BG231756	1.6	2.1	
	Activating transcription factor 6 (ATF6)	NM_007348	1.6		
	Xanthine dehydrogenase (XDH)	U06117	1.6		
	Acetyl-Coenzyme A carboxylase beta (ACACB)	AI057637	1.6		
	Forkhead box A3 (FOXA3)	R99562	1.5	2.6	2.6
	Potassium voltage-gated channel, Isk-related family, member 3 (KCNE3)	AI692703	1.5		
	Potassium voltage-gated channel, Shab-related subfamily, member 2 (KCNB2)	AF338730	1.5		
	Thyroid hormone receptor associated protein 1 (THRAP1)	BC024236		14.5	15.1
	LATS, large tumor suppressor, homolog 1 (LATS1)	BC015665		11.6	
	Phosphoinositide-3-kinase, class 3 (PIK3C3)	BC010388		11.4	11.0
	ATG10 autophagy related 10 homolog (ATG10)	AF318326		11.1	12.6
	Protein tyrosine phosphatase, non-receptor type 18 (PTPN18)	BC031076		9.9	11.2
	Cardiac-MyBP-C associated Ca/CaM kinase (MLCK)	BC039103		8.5	9.4

	Acetyl-Coenzyme A carboxylase alpha (ACACA)	BC007115		6.5	6.4
	Deoxynucleotidyltransferase, terminal (DNTT)	AA585152		6.4	6.1
	GLI-Kruppel family member GLI3 (Greig cephalopolysyndactyly syndrome)(GLI3)	BC032660		6.2	6.4
	Insulin-like growth factor binding protein 2 (IGFBP2)	NM_000597		5.7	6.1
	Rho-guanine nucleotide exchange factor (RGNEF)	AB082529		5.1	4.7
	RAD51 homolog (RAD51)	AL833420		4.0	3.8
	Pre-B-cell leukemia transcription factor 1 (PBX1)	AL832146		4.0	4.6
	Leukemia inhibitory factor receptor (LIFR)	BC038371		3.8	4.1
	Glutamate receptor, metabotropic 3 (GRM3)	AI733361		3.4	3.6
	Pleckstrin homology, Sec7 and coiled-coil domains 1(cytohesin 1)(PSCD1)	CA442689		3.3	
	Cystatin C (CST3)	NM_000099		3.1	5.3
	CAMP responsive element binding protein 1 (CREB1)	AW945589		3.0	3.0
	Transforming growth factor, beta receptor II (TGFBR2)	AI809493		2.9	2.9
	Apoptosis antagonizing transcription factor (AATF)	AW471180		2.7	2.6
	Sterol regulatory element binding transcription factor 1 (SREBF1)	S66168		2.5	3.0
	Glutathione peroxidase 3 (GPX3)	AW149846		2.4	4.7
	Paired box gene 5 (B-cell lineage specific activator)(PAX5)	AW405428		2.3	2.2
	LIM homeobox 4 (LHX4)	AB055704		2.2	2.3
	Arginase (ARG1)	AV649309		2.1	2.0
	Aryl hydrocarbon receptor nuclear translocator (ARNT)	AL137290		2.1	2.4
	Fibroblast growth factor 12 (FGF12)	D60438		2.1	2.3
	RAR-related orphan receptor B (RORB)	R18374		2.1	2.1
	POU domain, class 1, transcription factor 1 (Pit1, growth hormone factor 1)(POU1F1)	NM_000306		1.9	1.9
	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1 (SERPINH1)	NM_004353		1.9	2.9
	Nodal homolog (NODAL)	AI670948		1.8	1.6
	Fibroblast growth factor 14 (FGF14)	BE221273		1.8	
	Forkhead box K2 (FOXK2)	AV763408		1.8	1.7
	Pantothenate kinase 2 (PANK2)	AI870137		1.8	1.7
	Fibroblast growth factor 10 (FGF10)	NM_004465		1.8	1.6
	Major histocompatibility complex, class I, B (HLA-B)	L07950		1.8	3.3
	Hepatocyte nuclear factor 4, gamma (HNF4G)	NM_004133		1.7	1.7
	Guanidinoacetate N-methyltransferase (GAMT)	NM_138924		1.7	2.0
	X (inactive)-specific transcript (XIST)	BE644917		2.0	
	Nuclear receptor subfamily 1, group H, member 2 (NR1H2)	NM_007121		1.6	1.6
	Pantothenate kinase 4 (PANK4)	NM_018216		1.6	2.1
	RAR-related orphan receptor C (RORC)	AI218580		1.6	
	Retinoid X receptor, beta (RXRB)	BF337038		1.5	1.6
	5'-3' exoribonuclease 1 (XRN1)	BC039314			12.4
	Homeodomain interacting protein kinase 1 (HIPK1)	BC036361			10.1
	LATS, large tumor suppressor, homolog 1 (LATS1)	BC015665			10.0
	CDC14 cell division cycle 14 homolog A (CDC14A)	U29111			9.4
	High mobility group AT-hook 2 (HMGA2)	U29113			8.1
	Mediator complex subunit 16 (MED16)	AA314406			7.1
	Thyroid hormone receptor associated protein 1 (THRAP1)	BC024236			4.0
	Insulin-like growth factor 2 mRNA binding protein 1 (IGF2BP1)	H93038			2.6
	HLA-G histocompatibility antigen, class I, G (HLA-G)	M90684			2.4
	NAD(P)H dehydrogenase, quinone 1 (NQO1)	BC000906			2.3
	Tubby like protein 4 (TULP4)	AL157491			2.3
	Major histocompatibility complex, class II, DR beta 1 (HLA-DRB1)	AJ297586			2.2
	X (inactive)-specific transcript (XIST)	BF223193		2.0	1.9
	Rho guanine nucleotide exchange factor (GEF)10 (ARHGEF10)	BC036809		2.2	1.9
	Major histocompatibility complex, class II, DQ beta 1 (HLA-DQB1)	M17565			1.6
	Acetyl-Coenzyme A carboxylase beta (ACACB)	AI057637			1.6
	Interleukin 10 receptor, beta (IL10RB)	BC001903			1.5

	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (ACE2)	NM_021804			1.5
Metabolism	Proprotein convertase subtilisin/kexin type 2 (PCSK2)	AL031664	19.4		
	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked (DDX3Y)	NM_004660	14.0		
	Thioredoxin domain containing 13 (TXNDC13)	BF572868	13.0		
	Collagen, type I, alpha 2 (COL1A2)	NM_000089	12.0		
	SEC24 related gene family, member A (SEC24A)	AJ131244	11.3		
	Aftiphilin (AFTPH)	BF055271	10.4		
	Fumarylacetoacetate hydrolase domain containing 1 (FAHD1)	AW070436	9.7		
	Glutamate decarboxylase 2 (GAD2)	NM_000818	8.8		
	SH3-domain GRB2-like 2 (SH3GL2)	NM_003026	6.0		
	Glutathione reductase (GSR)	AI888037	4.7		
	Matrix metallopeptidase 3 (MMP3)	NM_002422	4.4		
	Solute carrier family 22, member 5 (SLC22A5)	NM_003060	3.9	2.2	2.2
	Triosephosphate isomerase 1 (TPI1)	BF116254	3.8		
	Potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8)	NM_004982	3.3		
	CDP-diacylglycerol synthase (phosphatidate cytidylyltransferase) 2 (CDS2)	Y16521	3.3		
	Syntaxin 7 (STX7)	NM_003569	3.3		
	Sorbitol dehydrogenase (SORD)	L29008	3.1		
	Ras homolog gene family, member Q (RHOQ)	BF978689	3.0		
	Voltage-dependent anion channel 3 (VDAC3)	BC002456	2.8		
	Argininosuccinate synthetase 1(ASS1)	NM_000050	2.7		
	ATP-binding cassette, sub-family C, member 1 (ABCC1)	NM_004996	2.7		
	Prosaposin (PSAP)	NM_002778	2.7		
	Sodium channel, nonvoltage-gated 1 alpha (SCNN1A)	NM_001038	2.7		
	Potassium channel tetramerisation domain containing 10 (KCTD10)	AW073741	2.5		
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	AFFX-HUMGAPDH/ M33197_3	2.5		
	Pancreatic lipase-related protein 1 (PNLIPRP1)	BC005233	2.5		
	Pyruvate dehydrogenase kinase, isozyme 3 (PDK3)	NM_005391	2.4		
	Glutaredoxin (GLRX)	AF162769	2.4		
	Triosephosphate isomerase 1 (TPI1)	NM_000365	2.2		
	Ceruloplasmin (ferroxidase) (CP)	AI922198	2.2		
	Chloride intracellular channel 5 (CLIC5)	AL049313	2.1		
	Cytochrome b-245, alpha polypeptide (CYBA)	NM_000101	2.1		
	Steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)(SRD5A1)	BC006373	2.0		
	Carnitine palmitoyltransferase 1A (CPT1A)	BC000185	2.0		
	Sterol O-acyltransferase 2 (SOAT2)	AF059203	1.9		
	A kinase (PRKA) anchor protein 10 (AKAP10)	AA456929	1.9		
	Thioredoxin interacting protein (TXNIP)	AI439556	1.9		
	Acid phosphatase 1, soluble (ACP1)	BE872974	1.8		
	Glutathione S-transferase M3 (GSTM3)	AL527430	1.8		
	Glutathione S-transferase theta 1 (GSTT1)	NM_000853	1.8		
	Lectin, galactoside-binding, soluble, 2 (LGALS2)	NM_006498	1.8		
	Potassium inwardly-rectifying channel, subfamily J, member 15 (KCNJ15)	D87291	1.8		
	Amine oxidase, copper containing 3 (AOC3)	NM_003734	1.7		
	Chromosome 11 open reading frame 24 (C11orf24)	NM_022338	1.6		
	Phospholipase A2, group IIA (PLA2G2A)	NM_000300	1.5		
	Vacuolar protein sorting 13B (COH1)	BC016375		15.5	13.3
	Carboxyl ester lipase (CEL)	BC042510		12.8	10.9
	Potassium channel, subfamily K, member 1 (KCNK1)	AL833343		6.7	6.4
	Choline dehydrogenase (CHDH)	AA609488		6.0	6.2
	Tyrosinase (TYR)	BC027179		4.0	4.2
	Hydroxysteroid (17-beta) dehydrogenase 12 (HSD17B12)	BC012536		3.5	3.7
	NADPH oxidase 1 (NOX1)	AI826277		3.2	2.7

	Lipocalin 2 (LCN2)	NM_005564		3.1	3.1
	Sucrase-isomaltase (alpha-glucosidase)(SI)	NM_001041		2.1	2.1
	Sorbin and SH3 domain containing 1 (SORBS1)	AI377221		2.1	1.8
	Proprotein convertase subtilisin/kexin type 2 (PCSK2)	AU158871		2.0	1.7
	Transferrin receptor (TFRC)	N76327		2.0	
	Solute carrier family 18, member 2 (SLC18A2)	NM_003054		1.9	2.1
	Paraoxonase 1 (PON1)	U53784		1.9	
	Mevalonate kinase (MVK)	AU150926		1.8	2.1
	Cystathionine-beta-synthase (CBS)	BE613178		1.8	1.9
	HLA-B associated transcript 3 (BAT3)	BC003133		1.6	1.5
	Alcohol dehydrogenase IB (class I), beta polypeptide (ADH1B)	M21692		1.5	
	Solute carrier family 6, member 15	BC039443			12.7
	DEAD box polypeptide 3, Y-linked (DDX3Y)	BC011022			12.1
	Protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform (PPP2R2B)	AF086066			5.0
	Recombination activating gene 1 (RAG1)	BC037344			4.8
	Lipid storage droplet protein 5 (LSDP5)	BC033570		4.6	4.6
	Transferrin receptor (TFRC)	N76327			2.0
	Hexosaminidase (glycosyl hydrolase family 20, catalytic domain) containing (HEXDC)	BM980844		2.4	2.4
	Paraoxonase 1 (PON1)	U53784			1.8
	Solute carrier family 14, member 2 (SLC14A2)	AL049328			1.7
	Farnesyl diphosphate synthase (FDPS)	AL022163			1.6
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	BF689355			1.5
Apoptosis	Defender against cell death 1 (DAD1)	NM_001344	9.0		
	Ras homolog gene family, member B (RHOB)	AI263909	9.4		
	Engulfment and cell motility 1 (ELMO1)	NM_014800	5.9		
	Pleckstrin homology-like domain, family A, member 1 (PHLDA1)	AI795908	5.2		
	Lectin, galactoside-binding, soluble, 1 (galectin 1)(LGALS1)	NM_002305	5.2		
	Serine/threonine kinase 4 (STK4)	BE222274	4.3		
	HIV-1 Tat interactive protein 2 (HTATIP2)	NM_006410	3.8		
	Caspase 10, apoptosis-related cysteine peptidase (CASP10)	NM_001230	2.9		
	NADH dehydrogenase 1 alpha subcomplex, 13 (NDUFA13)	NM_015965	2.8		
	Caspase recruitment domain family, member 10 (CARD10)	AY028896	2.5		
	Secreted phosphoprotein 1 (SPP1)	AB019562	2.4		
	Inhibitor of growth family, member 4 (ING4)	AA887083	2.3		
	Brain and reproductive organ-expressed (BRE)	AL566299	2.2		
	Cytoplasmic FMR1 interacting protein 2 (CYFIP2)	NM_030778	2.1		
	Nucleolar protein 3 (apoptosis repressor with CARD domain)	AI912351	2.0		
	Death-associated protein (DAP)	NM_004394	2.0		
	DNA fragmentation factor, beta polypeptide (DFFB)	NM_004402	2.0		
	Tumor necrosis factor superfamily, member 13 (TNFSF13)	AF114013	1.9		
	Mitogen-activated protein kinase kinase kinase 10 (MAP3K10)	NM_002446	1.8		
	TNF receptor-associated factor 2 (TRAF2)	NM_021138	1.7		
	Testis-specific kinase 2 (TESK2)	NM_007170	1.6		
	CD14 molecule (CD14)	NM_000591	1.6		
	Clusterin (CLU)	M25915	1.6		
	Growth arrest and DNA-damage-inducible, beta (GADD45B)	AF087853	1.6		
	Tumor necrosis factor receptor superfamily, member 12A (TNFRSF12A)	NM_016639	1.6	1.9	2.0
	TNF receptor-associated factor 5 (TRAF5)	BC032830		9.4	9.1
	Mitogen-activated protein kinase 1 (MAPK1)	AL833111		6.1	6.3
	Dedicator of cytokinesis 1 (DOCK1)	AI377320		4.0	3.6
	Serine/threonine kinase 17b (STK17B)	AI221707		3.7	3.9
	Programmed cell death 6 (PDCD6)	BC02055		3.6	3.0
	Adrenergic, alpha-1A-, receptor (ADRA1A)	BF221864		3.6	3.6
	BCL2/adenovirus E1B interacting protein like (BNIPL)	NM_138279		3.0	2.7
	Sphingosine kinase 1 (SPHK1)	NM_021972		2.3	2.4

	Protein phosphatase 1, regulatory (inhibitor) subunit 13B (PPP1R13B)	AK023188		1.7	1.5
	Tumor necrosis factor receptor superfamily, member 10a (TNFRSF10A)	W65310		1.6	
	Sterile alpha motif and leucine zipper containing kinase AZK (ZAK)	AI475164		1.5	2.0
	Clusterin (CLU)	M25915			4.1
	Apoptosis-inducing factor, mitochondrion-associated, 1(AIFM1)	NM_004208			1.7
	NLR family, pyrin domain containing 1 (NLRP1)	NM_021730			1.7
	Von Hippel-Lindau tumor suppressor (VHL)	BF972755			1.6
Response to stimulus	DnaJ (Hsp40) homolog, subfamily A, member 1 (DNAJA1)	NM_001539	12.6		
	Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3)	AI075407	7.5		
	Fusion (FUS)(CHOP)	NM_004960	3.6		
	Major histocompatibility complex, class I, E (HLA-E)	NM_005516	2.8		
	DnaJ (Hsp40) homolog, subfamily B, member 2 (DNAJB2)	NM_006736	2.6		
	CD55 molecule, decay accelerating factor for complement (CD55)	BC001288	2.5		
	CD58 molecule (CD58)	D28586	1.9		
	Interleukin 1 receptor antagonist (IL1RN)	U65590	1.9		
	Major histocompatibility complex, class I-related (MR1)	NM_001531	1.7		
	Paraoxonase 3 (PON3)	L48516	1.5		
	Activated leukocyte cell adhesion molecule (ALCAM)	BC041127		8.9	8.5
	Decay accelerating factor for complement (DAF)(CD55)	CA448665		3.5	8.8
	T cell receptor alpha locus (TRA@)	AE000659		1.8	1.9
	Lymphotoxin beta (TNF superfamily, member 3)(LTB)	BC018898		1.7	1.8
	Olfactory receptor, family 2, subfamily H, member 1 (OR2H1)	AJ459850			8.2
	Recombination activating gene 1 (RAG1)	BC037344			4.8
	Tyrosinase (TYR)	BC027179			4.2
	Lysozyme (LYZ)	U25677			3.0
	REV1 homolog (REV1)	W87784			2.7
	Paraoxonase 1 (PON1)	U53784			1.8
Signal transduction	Chemokine (C-X-C motif) ligand 2 (CXCL2)	M57731	10.7		
	TRAF family member-associated NFKB activator (TANK)	NM_004180	4.9		
	CD59 molecule (CD59)	BF983379	2.9		
	TBC1 domain family, member 4 (TBC1D4)	NM_014832	2.7		
	Urocortin (UCN)	NM_003353	1.7		
	c-mer proto-oncogene tyrosine kinase (MERTK)	NM_006343	1.7		
	Growth differentiation factor 15 (GDF15)	BC000529		4.3	1.9
	SH3 multiple domains 2 (SH3MD2)	AB062480		3.0	4.3
	Relaxin/insulin-like family peptide receptor 1(RXFP1)	NM_021634		3.7	3.3
	SH3-domain GRB2-like 3 (SH3GL3)	U79301		1.9	1.9
	Gastrin-releasing peptide (GRP)	BF110750		1.8	1.7
	Hypothetical LOC554175	BC006530			8.3
	Ras and Rab interactor 3 (RIN3)	BC034153			4.8
	GTPase, IMAP family member 1 (GIMAP1)	NM_130759			3.4
	Mitogen-activated protein kinase associated protein 1 (MAPKAP1)	NM_024117			1.8

Table 2: Transcripts up-regulated by palmitate, with or without pioglitazone or exenatide, in diabetic human islets.

pathogenesis. In the present study, we identify a subset of genes that seems to be involved in maladaptive metabolic alterations underlying the lipotoxic state leading to β-cell dysfunction and diabetes.

Pancreatic polypeptide (*PPY*) belongs to the *NPY* family and is expressed in the pancreatic islets of Langerhans. Moreover, *PPY* is released from the pancreas in response to a meal, specially by fat [24,25]. *PPY* decreases food intake and increases energy expenditure [26,27]. In this study, *PPY* expression was up-regulated in non-diabetic islets by pioglitazone and exenatide in the presence of palmitate (Table 4), but not by palmitate alone (epigenetic gene regulation pathway). This induction of *PPY* gene expression could influence pancreatic and gastric hormone release, and modulate different physiological processes. Interestingly, palmitate had no effect alone on *PPY* gene expression, possibly due to the absence of obesity, considering that obese subjects have lower serum levels of *PPY* [28,29]. Also, the *PPY* gene could be silenced by palmitate in non-diabetic islets. On the other hand, *PPY* was up-regulated by palmitate (Table 2) and this was reduced/normalized by pioglitazone in diabetic islets (Table 1). The

Function	Gene Title	Public ID	Fold	change	
			Palmitate	Palmitate + pioglitazone	Palmitate + exenatide
Regulation of gene expression, epigenetic	Fibroblast growth factor receptor 2 (FGFR2)	NM_022975	1.7	3.4	2.4
	Calreticulin (CALR)	AA910371	1.6		
	Aldolase B, fructose-bisphosphate (ALDOB)	BC005314		6.5	5.4
	Tropomyosin 3, 4 (TPM3, TPM4)	AF362887		4.9	7.4
	Insulinoma-associated 1 (INSM1)	BF108585		2.5	2.1
	YY1 transcription factor (YY1)	AA748649		2.5	
	Mitogen-activated protein kinase kinase 3 (MAP2K3)	AA780381		2.4	1.7
	Acetyl-Coenzyme A carboxylase beta (ACACB)	AI970898		1.9	1.8
	GLIS family zinc finger 3 (GLIS3)	AW025602		1.7	
	Caveolin 2 (CAV2)	AA150110		1.6	1.7
	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit (CACNA1A)	AA769818		1.6	
	Aryl hydrocarbon receptor nuclear translocator (ARNT)	AL137290			2.0
	Sp1 transcription factor (SP1)	AU121035			1.9
	Cysteine-rich, angiogenic inducer, 61 (CYR61)	NM_001554			1.8
	ATPase, Cu++ transporting, alpha polypeptide (ATP7A)	NM_000052			1.6
	Transcription factor 4 (TCF4)	BF592782			1.6
Metabolism	Voltage-dependent anion channel 3 (VDAC3)	U90943		2.3	2.4
	Ras homolog gene family, member Q (RHOQ)	BF978689		2.0	2.3
	Solute carrier family 22, member 5 (SLC22A5)	NM_003060		1.8	1.7
	ATP-binding cassette, sub-family C (CFTR/MRP), member 1 (ABCC1)	NM_004996		1.6	
	ATPase, H+ transporting, lysosomal 9kDa, V0 subunit e (ATP6V0E)	NM_003945		5.1	4.2
	Transferrin receptor (TFRC)	N76327			1.6
	Transient receptor potential cation channel, subfamily C, member 1(TRPC1)	U31110			1.6
	Alcohol dehydrogenase IB (class I), beta polypeptide (ADH1B)	M21692			1.5
	Nuclear factor related to kappaB binding protein (NFRKB)	AI869532			1.5
	Syntaxin 7 (STX7)	NM_003569			1.5
Apoptosis	Lectin, galactoside-binding, soluble, 1 (galectin 1)(LGALS1)	NM_002305	1.9		
	Caspase recruitment domain family, member 10 (CARD10)	AY028896	1.6		
	Serine/threonine kinase 4 (STK4)	BE222274	1.5		
	Pleckstrin homology-like domain, family A, member 1 (PHLDA1)	AI795908	1.7		
	Apolipoprotein E (APOE)	N33009		2.5	1.5
	Secreted phosphoprotein 1 (SPP1)	AB019562		4.8	3.0
	Tumor necrosis factor receptor superfamily, member 10a (TNFRSF10A)	W65310		2.5	
	Ras homolog gene family, member B (RHOB)	AI263909		2.4	2.1
	Neuronal thread protein AD7c-NTP (AD7C-NTP)	AF010144		1.6	1.6
	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D (SEMA4D)	NM_006378		1.5	
	DNA fragmentation factor, 40kDa, beta polypeptide (caspase-activated DNase) (DFFB)	NM_004402		1.5	
	Protein phosphatase 1, regulatory (inhibitor) subunit 13B (PPP1R13B)	AK023188			1.8
	Programmed cell death 6 (PDCD6)	AI692169			1.6
Signal transduction	Growth differentiation factor 15 (GDF15)	BC000529	1.9	7.4	7.5
	Striatin, calmodulin binding protein 4 (STRN4)	NM_013403	1.6		
	Protein tyrosine kinase 9 (PTK9)	N25562		3.1	1.7
	Chemokine (C-X-C motif) ligand 2 (CXCL2)	M57731		2.3	2.0

Table 3: Transcripts down-regulated by palmitate, with or without pioglitazone or exenatide, in non-diabetic human islets.

linkage of the region containing *PPY* [30] and also genetic association of this gene with type 2 diabetes [31-33], suggest that *PPY* may be pathogenetically and therapeutically relevant in diabetes.

Transcription factor 7-like 2 (*TCF7L2*/*TCF4*), identified as the most important type 2 diabetes susceptibility gene to date [34], is involved in Wnt signaling induced β-cell proliferation [35,36]. The expression

Function	Gene Title	Public ID	Fold	change	
			Palmitate	Palmitate + pioglitazone	Palmitate + exenatide
Regulation of gene expression, epigenetic	Hydroxyprostaglandin dehydrogenase 15-(NAD)(HPGD)	NM_000860	2.3		
	Vav 3 oncogene (VAV3)	AF118887	1.8		
	Major histocompatibility complex, class II, DR alpha (HLA-DRA)	M60333	1.8	2.7	3.8
	Alpha-2-glycoprotein 1, zinc-binding (AZGP1)	D90427	1.8	1.8	2.5
	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 (NFATC3)	U85430	1.7	1.7	2.3
	Glutathione peroxidase 3 (GPX3)	AW149846	1.7		
	Glutamate-ammonia ligase (GLUL) (glutamine synthetase)	U08626	1.7		
	Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	AF182273	1.6		
	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 (ACE2)	AK026461	1.6	2.4	
	Paired box gene 8 (PAX8)	BF056746	2.5	2.5	2.2
	Ribosomal protein S5 (RPS5)	NM_001009		13.2	13.8
	Ribosomal protein L29 (RPL29)	NM_000992		11.4	14.8
	Pancreatic polypeptide (PPY)	M15788		9.9	7.7
	Ribosomal protein L28 (RPL28)	NM_000991		8.6	10.1
	Heat shock 27kDa protein 1 (HSPB1)	NM_001540		8.2	12.7
	Glutathione peroxidase 1(GPX1)	NM_000581		7.8	10.4
	Colipase, pancreatic (CLPS)	NM_001832		6.2	8.7
	Major histocompatibility complex, class II, DR beta 1 (HLA-DRB1)	NM_002125		5.8	8.5
	Major histocompatibility complex, class II, DQ beta 1 (HLA-DQB1)	AI583173		5.4	6.0
	Glutathione peroxidase 2 (gastrointestinal)	NM_002083		4.7	5.9
	Glutathione S-transferase A1 (GSTA1)	NM_000846		4.6	3.5
	Aldolase A, fructose-bisphosphate (ALDOA)	AK026577		4.2	4.9
	Y box binding protein 1 (YBX1)	BE966374		4.1	3.5
	Epidermal growth factor receptor (EGFR)	BE878463		4.0	2.2
	Protease, serine, 2 (trypsin 2)(PRSS2)	NM_002770		3.7	4.7
	Cytochrome P450, family 3, subfamily A, polypeptide 5 (CYP3A5)	X90579		3.6	4.5
	Amylase, alpha 2A (AMY2A)	NM_004038		3.4	2.7
	Regenerating islet-derived 3 alpha (REG3A)	NM_002580		3.3	3.6
	Major histocompatibility complex, class II, DR beta 5 (HLA-DRB5)	NM_021983		3.1	3.9
	Eukaryotic translation initiation factor 4E family member 2 (EIF4E2)	AF047695		3.2	4.0
	Eukaryotic translation initiation factor 4B (EIF4B)	NM_001417		3.0	3.0
	ADAM metallopeptidase domain 28 (ADAM28)	NM_021778		3.0	2.7
	Major histocompatibility complex, class II, DM alpha (HLA-DMA)	X76775		2.9	3.0
	Interleukin 32 (IL32)	NM_004221		2.8	3.0
	Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	AF182273		2.7	2.4
	Bone morphogenetic protein 2 (BMP2)	NM_001200		2.7	2.3
	Solute carrier family 3, member 2 (SLC3A2)	NM_002394		2.7	4.2
	Vav 3 oncogene (VAV3)	AF118887		2.6	2.2
	Eukaryotic translation initiation factor 2-alpha kinase 1 (EIF2AK1)	NM_014413		2.6	2.7

	DNA-damage-inducible transcript 3 (DDIT3)	BC003637		2.6	3.5
	Glutamate-ammonia ligase (GLUL) (glutamine synthetase)	U08626		2.6	3.0
	Aldolase B, fructose-bisphosphate (ALDOB)	BC005314		2.5	2.3
	S100 calcium binding protein A8 (S100A8)	NM_002964		2.5	2.3
	Interleukin 10 receptor, beta (IL10RB)	BC001903		2.4	3.7
	V-set domain containing T cell activation inhibitor 1 (VTCN1)	NM_024626		2.3	3.0
	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1 (SERPINH1)	NM_004353		2.3	4.0
	X (inactive)-specific transcript (XIST)	AV646597		2.3	3.3
	Major histocompatibility complex, class I, B (HLA-B)	L07950		2.3	4.8
	MAX-like protein X (MLX)	N40555		2.3	2.1
	Phosphofructokinase, muscle (PFKM)	U24183		2.3	2.2
	Carboxypeptidase M (CPM)	AW663908		2.2	2.8
	Islet cell autoantigen 1(ICA1)	L21181		2.1	3.9
	Pantothenate kinase 4 (PANK4)	NM_018216		2.1	2.8
	Fibrinogen beta chain (FGB)	NM_005141		2.1	
	Activating transcription factor 6 (ATF6)	NM_007348		2.0	
	Ectonucleotide pyrophosphatase/ phosphodiesterase 2 (ENPP2) (autotaxin)	L35594		2.0	2.3
	Retinoic acid receptor responder 2 (RARRES2)	BC000069		2.0	3.6
	CD53 molecule (CD53)	NM_000560		1.9	2.0
	Ghrelin/obestatin preprohormone (GHRL)	AB035700		1.9	1.9
	Serpin peptidase inhibitor, clade F, member 1 (SERPINF1)	NM_002615		1.9	2.0
	Regulator of G-protein signalling 4 (RGS4)	AL514445		1.9	1.9
	HLA-G histocompatibility antigen, class I, G (HLA-G)	M90685		1.8	3.0
	Major histocompatibility complex, class I, C (HLA-C)	U62824		1.7	2.6
	keratin 20 (KRT20)	AI732381		1.7	1.6
	NAD(P)H dehydrogenase, quinone 1 (NQO1)	BC000906		1.7	3.4
	HLA-G histocompatibility antigen, class I, G (HLA-G)	M90684		1.7	3.0
	Nuclear receptor subfamily 1, group H, member 3 (NR1H3)	NM_005693		1.6	
	Kruppel-like factor 5 (KLF5)	AB030824		1.6	1.8
	PHD finger protein 15 (PHF15)	AI735639		1.5	
	Rho guanine nucleotide exchange factor (GEF) 12 (ARHGEF12)	AI807672		1.5	1.8
	Cystatin C (CST3)	NM_000099		1.8	3.9
	Major histocompatibility complex, class II, DR beta 1 (HLA-DRB1)	NM_002125		3.1	3.7
	Zinc finger protein 148 (ZNF148)	L04282		2.6	3.2
	Major histocompatibility complex, class II, DM alpha (HLA-DMA)	X76775		2.9	3.0
	CDK5 regulatory subunit associated protein 1(CDK5RAP1)	NM_016408			2.7
	Cytochrome P450, family 3, subfamily A, polypeptide 4 (CYP3A4)	AF182273		2.7	2.4
	Forkhead box A3 (FOXA3)	R99562			2.2
	Spleen tyrosine kinase (SYK)	NM_003177			2.0
	Serpin peptidase inhibitor, clade H (heat shock protein 47), member 1 (SERPINH1)	NM_004353		2.3	2.0

	cAMP responsive element binding protein 1 (CREB1)	M34356			2.0
	General transcription factor IIH, polypeptide 1, 62kDa (GTF2H1)	NM_005316			1.9
	Major histocompatibility complex, class I, A (HLA-A)	AI923492			1.8
	Lamin B2 (LMNB2)	M94363			1.7
	Guanine nucleotide binding protein (G protein), beta 5 (GNB5)	NM_016194			1.6
	Eukaryotic translation initiation factor 4E family member 2 (EIF4E2)	AF047695		3.2	1.6
	MAX-like protein X (MLX)	N40555		1.7	1.6
	Lymphoid-restricted membrane protein (LRMP)	NM_006152			1.6
	Growth arrest-specific 6 (GAS6)	L13720			1.5
Metabolism	Butyrobetaine (gamma), 2-oxoglutarate dioxygenase (gamma-butyrobetaine hydroxylase) 1 (BBOX1)	NM_003986	2.6		
	Phosphogluconate dehydrogenase (PGD)	NM_002631	1.5	2.0	3.0
	Pancreatic lipase-related protein 1 (PNLIPRP1)	BC005233	1.5	4.4	4.9
	Thioredoxin interacting protein (TXNIP)	AI439556	1.5	2.7	3.2
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	AFFX-HUMGAPDH/ M33197_3		8.5	12.8
	Voltage-dependent anion channel 3 (VDAC3)	BC002456		5.6	5.9
	Esterase D/formylglutathione hydrolase (ESD)	BC001169		4.4	4.5
	Glutathione S-transferase M3 (GSTM3)	AL527430		3.8	
	Argininosuccinate synthetase 1(ASS1)	NM_000050		3.7	3.5
	Coenzyme Q9 homolog (COQ9)	AC004382		3.3	4.2
	Acid phosphatase 1, soluble (ACP1)	BE872974		3.0	3.3
	Potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8)	NM_004982		2.5	2.2
	Aminocarboxymuconate semialdehyde decarboxylase (ACMSD)	AW025340		2.4	2.4
	Potassium inwardly-rectifying channel, subfamily J, member 15 (KCNJ15)	D87291		2.4	2.5
	Glutathione S-transferase theta 1 (GSTT1)	NM_000853		2.2	2.2
	Lipocalin 2 (LCN2)	NM_005564		2.1	2.5
	Triosephosphate isomerase 1 (TPI1)	BF116254		2.1	3.0
	Ceruloplasmin (ferroxidase) (CP)	AI922198		2.0	2.1
	Syntaxin 17 (STX17)	AW014619		2.0	3.1
	Cytochrome b-245, alpha polypeptide (CYBA)	NM_000101		2.0	2.1
	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)(KMO)	AI074145		1.9	
	Glutaredoxin (GLRX)	AF162769		1.9	1.8
	Triosephosphate isomerase 1 (TPI1)	NM_000365		1.9	
	Carboxyl ester lipase (bile salt-stimulated lipase)(CEL)	BC042510		1.9	1.6
	Lectin, galactoside-binding, soluble, 2 (LGALS2)	NM_006498		1.8	
	Matrix metallopeptidase 3 (MMP3)	NM_002422		1.8	1.8
	Glutathione S-transferase A3 8 GSTA3)	NM_000847		1.7	1.7
	Ceruloplasmin (ferroxidase) (CP)	AI922198		1.7	
	SCO cytochrome oxidase deficient homolog 1 (SCO1)	AF183424		1.6	3.0
	A kinase (PRKA) anchor protein 10 (AKAP10)	AA456929		1.5	1.9
	Proprotein convertase subtilisin/kexin type 2 (PCSK2)	AL031664		1.5	1.5

	Ferritin, light polypeptide (FTL)	BG538564		8.4	18.1
	Ribosomal protein, large, P0 (RPLP0)	AA555113		8.1	12.7
	Esterase D/formylglutathione hydrolase (ESD)	BC001169		4.4	4.5
	Glutathione S-transferase M3 (GSTM3)	AL527430		3.8	3.8
	Vesicle-associated membrane protein 5 (VAMP5)	NM_006634		1.9	3.2
	Triosephosphate isomerase 1 (TPI1)	BF116254		2.1	3.0
	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1 (SULT1A1)	NM_001055			2.2
	Potassium inwardly-rectifying channel, subfamily J, member 8 (KCNJ8)	NM_004982		2.5	2.2
	HLA-B associated transcript 3 (BAT3)	BC003133			1.8
	Chromosome 11 open reading frame 24 (C11orf24)	NM_022338			1.7
	Glutamate dehydrogenase 2 (GLUD2)	AC006144			1.6
	Prosaposin (PSAP)	NM_002778			1.6
	Sorbin and SH3 domain containing 1 (SORBS1)	AI377221			1.5
Apoptosis	Apoptosis-inducing factor, mitochondrion-associated, 1 (AIFM1)	NM_004208	1.8	1.8	2.2
	Apolipoprotein E (APOE)	N33009	1.7		1.7
	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)(MIF)	NM_002415		9.3	10.3
	Lectin, galactoside-binding, soluble, 1 (galectin 1)(LGALS1)	NM_002305		8.2	12.3
	Defender against cell death 1(DAD1)	NM_001344		7.3	6.4
	Clusterin (CLU)	M25915		3.9	6.3
	NADH dehydrogenase 1 alpha subcomplex, 13 (NDUFA13)	NM_015965		3.3	4.1
	CD14 molecule (CD14)	NM_000591		2.7	2.4
	Growth arrest and DNA-damage-inducible, beta (GADD45B)	AF087853		2.6	4.3
	Tumor necrosis factor receptor superfamily, member 12A (TNFRSF12A)	NM_016639		2.2	2.7
	Death-associated protein (DAP)	NM_004394		1.9	3.9
	HIV-1 Tat interactive protein 2 (HTATIP2)	NM_006410		1.9	2.7
	Inhibitor of growth family, member 4 (ING4)	AA887083		1.8	
	NLR family, pyrin domain containing 1 (NLRP1)	NM_021730		1.6	1.9
	Brain and reproductive organ-expressed (BRE)	AL566299		1.6	
	Sphingosine kinase 1(SPHK1)	NM_021972		1.5	1.9
	Engulfment and cell motility 1 (ELMO1)	NM_014800			2.3
	Tumor necrosis factor (ligand) superfamily, member 13 (TNFSF13)	AF114013			1.7
	caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)(CASP1)	U13699			1.7
	Cell division cycle 2-like 2 (CDC2L2)	AF067524			1.5
	Programmed cell death 5 (PDCD5)	NM_004708			1.5
Response to stimulus	Interferon induced transmembrane protein 2 (1-8D)(IFITM2)	NM_006435		6.4	7.8
	Interferon-induced protein with tetratricopeptide repeats 3 (IFIT3)	AI075407		4.2	5.3
	CD59 molecule, complement regulatory protein (CD59)	BF983379		3.3	2.6
	CD55 molecule, decay accelerating factor for complement (CD55)	BC001288		2.6	3.6

	C-mer proto-oncogene tyrosine kinase (MERTK)	NM_006343		2.4	2.2
	Paraoxonase 3 (PON3)	L48516		2.3	2.6
	Major histocompatibility complex, class I-related (MR1)	NM_001531		2.2	2.0
	Major histocompatibility complex, class I, E (HLA-E)	NM_005516		2.0	2.3
	DnaJ (Hsp40) homolog, subfamily A, member 1 (DNAJA1)	NM_001539		1.5	2.3
	Calcium binding atopy-related autoantigen 1 (CBARA1)	AK022697			1.8
Signal transduction	Mitogen-activated protein kinase associated protein 1 (MAPKAP1)	NM_024117		2.5	3.1
	TRAF family member-associated NFKB activator (TANK)	NM_004180		2.2	4.7
	Docking protein 2, 56kDa (DOK2)	AI828929			1.6

Table 4: Transcripts up-regulated by palmitate, with or without pioglitazone or exenatide, in non-diabetic human islets.

of *TCF7L2/TCF4* transcript was induced by palmitate in diabetic islets in this study (Table 2), whereas in non-diabetic islets it remained unchanged. In addition, exenatide reduced the gene expression of *TCF7L2/TCF4* in both diabetic (Table 1) and non-diabetic (Table 3) islets incubated with palmitate. Moreover, pioglitazone down-regulated *TCF7L2/TCF4* expression in the presence of palmitate in diabetic islets (Table 1). The up-regulated level of *TCF7L2/TCF4* gene expression by palmitate noticed in our diabetic islets is in agreement with previous result where *TCF7L2/TCF4* was overexpressed in type 2 diabetic human islets in response to a variety of stimulus [37]. Moreover, according to Lyssenko et al. overexpression of *TCF7L2/TCF4* in the β-cell reduces insulin secretion, suggesting that its up-regulation by palmitate might be involved in β-cell dysfunction evoked by the fatty acid and perhaps also relevant in diabetic lipotoxicity in patients in whom β-cell dysfunction occur. The down-regulation of the palmitate-induced *TCF7L2/TCF4* elevation by exenatide and pioglitazone observed in our human islets is also very interesting as it might contribute to the beneficial long-term influence by these drugs on β-cell function and viability.

Regenerating islet-derived 3 alpha (*REG3A*) is a pancreatic secretory protein that seems to be involved in β-cell proliferation and differentiation [38] via a cell surface Reg receptor [39]. In the current study, *REG3A* expression was up-regulated by palmitate in diabetic islets (Table 2). Taking into account this and also another study, where similar effect has been seen for the other members of the REG family in the presence of hyperglycemia [40], it may indicate the β-cells attempt to ameliorate lipoapoptosis through stimulation of β-cell proliferation. Furthermore, elevated expression of *REG3A* that results in islet neogeneis, growth, and/or protection has also been reported by others [38,41]. Both pioglitazone and exenatide induced *REG3A* expression in non-diabetic islets exposed to palmitate (Table 4), and reduced *REG3A* expression in diabetic islets exposed to palmitate (Table 1). Possibly, pioglitazone and exenatide contribute to maintenance of β-cell mass through induction of *REG3A* when islets become exposed to lipotoxicity and so signaling through the *REG3A* pathway might be one mechanism by which these drugs exert their beneficial influences on the β-cell.

Growth arrest and DNA-damage-inducible beta (*GADD45B*) is a member of the GADD45 nuclear protein family that is involved in β-cell growth arrest, DNA repair, and apoptosis [42]. In this study, the expression of *GADD45B* was slightly up-regulated by palmitate in diabetic islets (Table 2). A similar effect in response to the cytotoxic IL-1β in INS-1E cells has recently been reported [43]. Both pioglitazone and exenatide reduced *GADD45B* expression in diabetic islets exposed to palmitate (Table 1). This may indicate that pioglitazone and exenatide, by normalizing the overexpression of *GADD45B* by palmitate, attempt to restore the balance between apoptosis and proliferation/survival in these islets. Considering that GADD45 family proteins are coactivators for RXR or PPAR activation [44], the modulation of *GADD45B* expression could influence nuclear hormone receptor activity and their involvment in fatty acid metabolism, apoptosis/cell survival, and insulin production/secretion.

Thioredoxin interacting protein (*TXNIP*) regulates the cellular redox state and mediates oxidative stress [45-49]. Our results show that *TXNIP* was up-regulated by palmitate in both diabetic (Table 2) and non-diabetic (Table 4) islets. Previous reports have shown *TXNIP* up-regulation by glucose [50,51], and it appears now that FFAs impose similar effects in the β-cell. Considering that *TXNIP* overexpression induces apoptosis in β-cells in response to glucose [50], and impairs glucose-induced insulin secretion [52], this could also be the ultimate scenario for β-cells exposed to FFAs. These results underline the importance of *TXNIP* for β-cell survival and insulin secretion. Our results reveal that *TXNIP* expression was reduced by both pioglitazone and exenatide in diabetic islets exposed to palmitate (Table 1), whereas it was induced by these drugs in the presence of palmitate in non-diabetic islets (Table 4). It seems that both pioglitazone and exenatide were able to reduce and normalize the overexpression of *TXNIP* induced by palmitate in diabetic islets and it is conceivable that this mechanism might in part explain the positive impact of these drugs in terms of β-cell funtion and insulin secretion possibly due to existence of diabetes (a certain genetic background) and also hyperglycemia in diabetic subjects. This effect of exenatide has been reported previously in pancreatic islets [53].

Aryl hydrocarbon receptor nuclear translocator (*ARNT*), a.k.a. hypoxia-inducible factor (*HIF*), form heterodimeric complexes that directly regulate the expression of genes involved in glucose transport and glucose metabolism [54]. Moreover, the *ARNT* gene is located in a chromosomal region that shows strong linkage to type 2 diabetes [55]. Our present results show that *ARNT* gene expression was reduced by palmitate in diabetic islets (Table 1). *ARNT* expression has previously been reported to be significantly decreased in islets from type 2 patients, *ARNT* gene silencing in isolated β-cells was found to grossly impair glucose-stimulated insulin secretion, and *ARNT* knock-out animals showed severe glucose intolerance [56]. Our findings suggest

Gene Name		Fold Change
Growth arrest and DNA-damage-inducible, beta (GADD45B)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	2.7 ± 0.001 * †
	Down-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	-1.8 ± 0.002 * †
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	8.4 ± 0.001 * †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in non-diabetic human islets	2.0 ± 0.001 * †
	Down-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	-7.1 ± 0.001 * †
Pancreatic polypeptide (PPY)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	4.1 ± 0.002 * †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in non-diabetic human islets	4.7 ± 0.001 * †
	Up-regulated by palmitate *vs.* control in diabetic human islets	6.6 ± 0.001 * †
Regenerating islet-derived 3 alpha (REG3A)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	2.1 ± 0.002* †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in non-diabetic human islets	5.2 ± 0.001* †
	Down-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	-8.4 ± 0.001* †
	Up-regulated by palmitate *vs.* control in diabetic human islets	10.3 ± 0.002* †
Thioredoxin interacting protein (TXNIP)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	12.6 ± 0.002 * †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in non-diabetic human islets	16.0 ± 0.002* †
	Down-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	-16.6 ± 0.002* †
Carboxyl ester lipase (CEL)	Up-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	6.7 ± 0.001 * †
	Up-regulated by exenatide & palmitate *vs.* palmitate in diabetic human islets	7.2 ± 0.001 *†
Aryl hydrocarbon receptor nuclear translocator (ARNT)	Down -regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	-14.9 ± 0.001 * †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	14.7 ± 0.001 * †
	Up-regulated by exenatide & palmitate *vs.* palmitate in diabetic human islets	3.2 ± 0.002 *†
ISL1 transcription factor, LIM/homeodomain (ISL1)	Down-regulated by exenatide & palmitate *vs.* palmitate in diabetic human islets	-3.1 ± 0.001 *†
	Up-regulated by palmitate *vs.* control in diabetic human islets	2.3 ± 0.001* †
Transcription factor 4 (TCF4/TCF7L2)	Down-regulated by pioglitazone & palmitate *vs.* palmitate in diabetic human islets	-1.9 ± 0.002 * †
	Down-regulated by exenatide & palmitate *vs.* palmitate in diabetic human islets	-2.0 ± 0.002 *†
	Up-regulated by palmitate *vs.* control in diabetic human islets	5.1 ± 0.002* †
Defender against cell death 1 (DAD1)	Up-regulated by exenatide & palmitate *vs.* palmitate in non-diabetic human islets	7.9 ± 0.002 * †
	Up-regulated by pioglitazone & palmitate *vs.* palmitate in non-diabetic human islets	4.1 ± 0.002 * †
	Up-regulated by palmitate *vs.* control in diabetic human islets	2.1 ± 0.001* †

Results are means ± SEM of 2 experiments. * denotes $P<0.05$ for a chance difference *vs* controls using Student's unpaired *t*-test. † denotes that the correlation between micoarray and qRT-PCR is significant at the $P<0.01$ level.

Table 5: Validation by qRT-PCR of differently regulated human islet transcripts identified by microarray analysis.

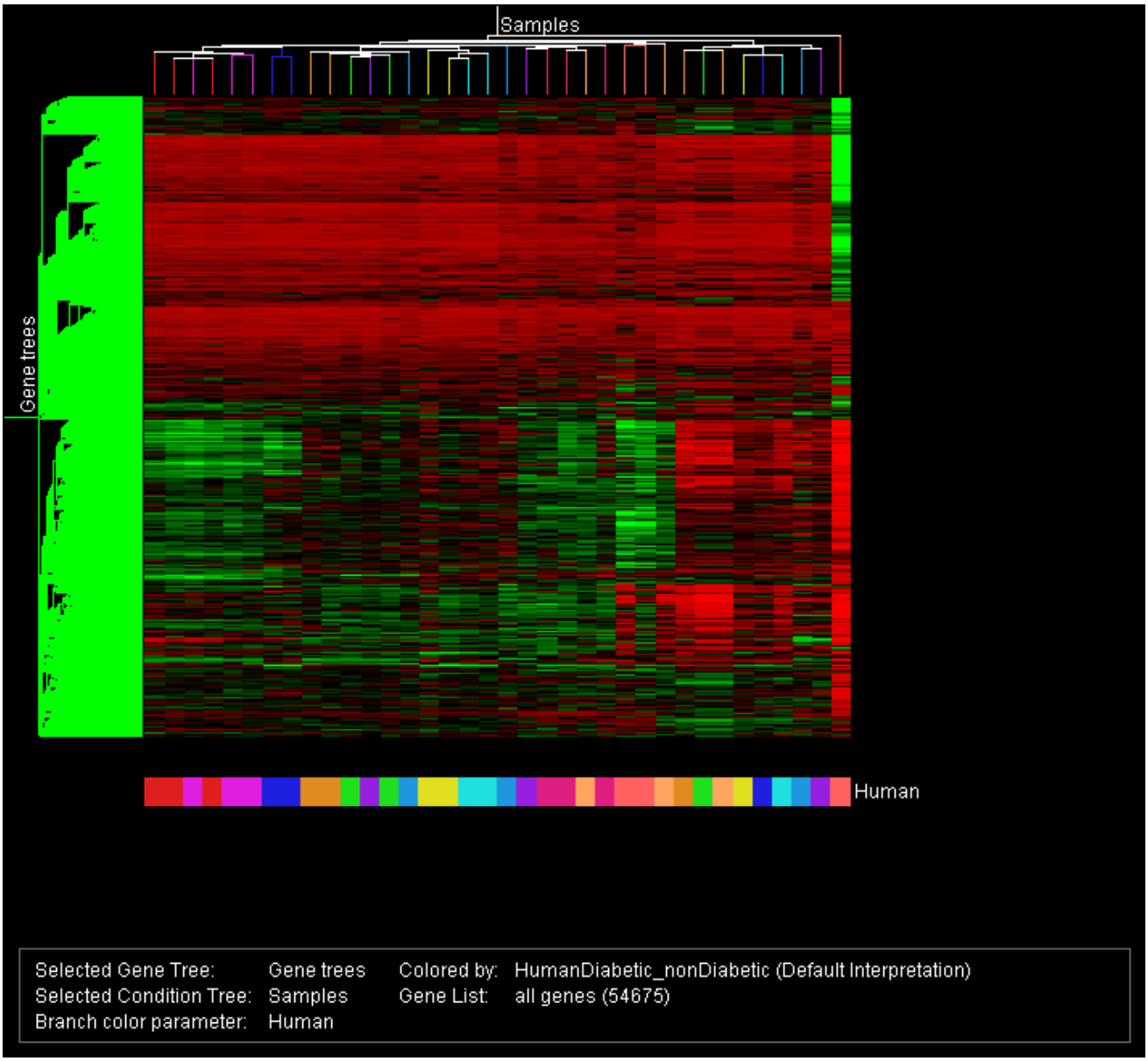

Figure 1: Hierarchical clustering. Each gene is represented by a single row of colored bars. The red color indicates down-regulation, black color shows unchanged and the green color denotes up-regulation of gene expression compared to controls.

that palmitate, which is in excess in diabetic patients' serum due to their diabetic dyslipidemia, might be one factor that contributes to this *ARNT* down-regulation in diabetic islets. More importantly, *ARNT* down-regulation by palmitate was normalized by both pioglitazone and exenatide in our diabetic islets (Table 2). This may indicate one important molecular target for pioglitazone and exenatide in islets, by which these drugs maintain optimal insulin secretion and β-cell function.

Epidermal growth factor receptor (*EGFR*) is involved in cellular differentiation, proliferation, and survival in many cell types of epithelial origin through interactions with EGF [57-59]. Also, *EGFR* transactivation in GLP-1–induced β-cell proliferation has been reported [60]. The gene expression of *EGFR* in our study was slightly up-regulated by palmitate in diabetic islets (Table 2), whereas in non-diabetic islets it remained unchanged. This may reflect the β-cell's attempt to diminish the apoptotic effect of palmitate in diabetic situations by stimulating the proliferation and β-cell survival trough induction of *EGFR* gene expression. On the other hand, the unchanged level of *EGFR* expression in non-diabetic islets could be due to the silencing effect of palmitate on the gene expression of *EGFR*. The expression of *EGFR* was reduced by pioglitazone in diabetic islets (Table 1), whereas it was induced by both pioglitazone and exenatide in non-diabetic islets (Table 4) exposed to palmitate. The latter may reflect the protective effects conferred by pioglitazone and exenatide in β–cells through maintaining the balance between their apoptosis and proliferation.

Carboxyl ester lipase (*CEL*), a lipolytical enzyme, highly expressed and secreted from pancreas, contributes to the digestion of dietary fat

[61,62]. As exocrine pancreatic dysfunction is common in diabetes, considerable interest for *CEL* in islet biology arose when it was discovered that *CEL* mutations occur in a hereditary syndrome of diabetes and exocrine pancreatic dysfunction [63]. *CEL* mutations are overrepresented in MODY patients with β–cell failure (63). In the current study, *CEL* expression was not altered by palmitate in diabetic or non-diabetic islets, possibly due to silencing effect of palmitate. However, the *CEL* gene expression was up-regulated by pioglitazone and exenatide in both diabetic (Table 2) and non-diabetic (Table 4) islets exposed to palmitate. As studies of the role of *CEL* in islet biology are still in their infancy, it seems warranted to pursue this track in future efforts to determine whether the *CEL* upregulation observed in islets exposed to pioglitazone and exenatide is causally linked to protection by these drugs against β–cell dysfunction in lipotoxicity.

Acetyl-Coenzyme A carboxylase alpha (*ACACA*) regulates fatty acid biosynthesis [64,65]. In the current study, *ACACA* gene expression was down-regulated by palmitate in diabetic islets (Table 1), thus confirming results in the INS-l β-cell line [66]. This indicates stimulation of fatty acid oxidation to produce energy at the expense of lipid synthesis and contributing to impaired glucose-sensitive insulin secretion [66]. Previous studies have shown similar results when polyunsaturated fatty acids reduced the transcriptional rates of lipogenic enzymes [67]. On the other hand, studies on the INS-l β-cell line [68], and also rats fed with high-carbohydrate diet [67], have indicated glucose as a potent inducer of *ACACA* gene expression. Interestingly, both pioglitazone and exenatide up-regulated *ACACA* gene expression suppressed by palmitate in diabetic islets (Table 2). This indicates the normalizing effect by these drugs on palmitate-induced imbalance between fatty acid breakdown and synthesis by increasing lipogenesis, thereby probably positively impacting long term β-cell function and survival by ameliorating lipotoxicity and restoring glucose-sensitivity. Commensurate with such a role, previous findings using *ACAC* gene silencing have revealed an essential role for *ACAC* in nutrient-induced insulin secretion [69,70].

Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is a classical glycolytic enzyme involved in β-cell fuel sensing and energy production that yields high-energy phosphate molecules [71]. Moreover, *GAPDH* is also involved in several other pathways, including apoptosis by its translocation into the nucleus, protein phosphotransferase/kinase reactions, translational control of gene expression, DNA replication and repair [72-74]. In this study, *GAPDH* gene expression was up-regulated by palmitate in diabetic islets (Table 4), whereas it remained unchanged in non-diabetic islets. This may reflect an attempt of the diabetic β-cell to sustain ATP production to endure lipotoxicity, whereas in non-diabetic islets *GAPDH* transcription seems to be silenced. Furthermore, the influence of lipotoxicity on transcriptional regulation of *GAPDH* gene expression appear to be different from the translational regulation, considering that palmitoyl-CoA has been reported to inhibit *GAPDH* enzyme activity [75]. Both pioglitazone and exenatide enhanced *GAPDH* gene expression in non-diabetic islets exposed to palmitate (Table 4). Possibly the *GAPDH* gene that was silenced by palmitate in these normal islets became activated by the drugs to cope with lipotoxicity, thus promoting β-cell survival by stimulating energy production. On the other hand, exenatide up-regulated (Table 2) while pioglitazone down-regulated (Table 1) *GAPDH* gene expression in diabetic islets exposed to palmitate. It is difficult to speculate why these drugs act differently in this scenario, but one conceivable explanation could be that they might act through different pathways (as mentioned above) to cope with lipotoxicity and to protect the β-cell.

We noticed small differences between the diabetic and non-diabetic samples when looking at the genes involved in the inflammatory process, as previously suggested [76]. This could perhaps suggest the presence of a mild inflammation in islets from T2D patients compared to normal samples. In agreement with a previous study [77], in our study Chemokine (C-X-C motif) ligand 2 (CXCL2) gene expression was induced (10.7 fold) by palmitate in diabetic islets (Table 2). Whether the induction of the inflammatory genes in islets from T2D patients could be the result of *in vivo* exposure to saturated NEFA and whether this inflammation causes loss of functional β-cell mass and thereby T2D remains to be elucidated and needs to be further investigated.

In this study, the expression of a number of key genes altered by palmitate, pioglitazone and/or exenatide were classified into epigenetic regulation of gene expression, which also belong to major pathways involved in lipid metabolism. We identified epigenetic targets with known significance in β-cell biology that are up-regulated, down-regulated or silenced by palmitate, pioglitazone and/or exenatide. The function and survival of the human β-cell seem to be regulated directly through epigenetic control of expression of genes involved in growth, proliferation, and differentiation rather than a direct effect on the apoptotic pathway. Since regulation of gene expression seems to be tightly under the epigenetic control and individually programmed, it might be different in different individuals and not totally be comparable to each other/generalized, which needs to be addressed in future investigations. FFAs seem to contribute and play an important role in the development of β-cell failure in synergy with hyperglycemia, in obese individuals, or in subjects who are genetically more predisposed to type 2 diabetes. Modern antidiabetic drugs such as pioglitazone and exenatide appear to normalize these epigenetic misregulations induced by lipotoxicity in the human β-cell. These identified β-cell genes may be harnessed to advantage in the development of novel diagnostic biomarkers or targeted therapeutics for type 2 diabetes based upon promotion of human β-cell function, survival, and proliferation.

Acknowledgments

Financial support was provided by the Amylin-Lilly Alliance, Takeda Pharmaceuticals North America, Stiftelsen Olle Engkvist Byggmästare, the Janne Elgqvist Family Foundation, the Swedish Society of Medicine, the Sigurd and Elsa Golje Memorial Foundation, Svenska Försäkringsföreningen, Svenska Diabetesstiftelsen, Berth von Kantzow's Foundation, Trygg-Hansa´s Research Foundation, and the Research Center at Södersjukhuset, Stockholm.

References

1. Boden G (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 46: 3-10.
2. Lewis GF, Carpentier A, Adeli K, Giacca A (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev 23: 201-229.
3. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, et al. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52: 102-110.
4. Gepts W, Lecompte PM (1981) The pancreatic islets in diabetes. Am J Med 70: 105-115.
5. Saito K, Yaginuma N, Takahashi T (1979) Differential volumetry of A, B and D cells in the pancreatic islets of diabetic and nondiabetic subjects. Tohoku J Exp Med 129: 273-283.
6. Kloppel G, Lohr M, Habich K, Oberholzer M, Heitz PU (1985) Islet pathology and the pathogenesis of type 1 and type 2 diabetes mellitus revisited. Surv Synth Pathol Res 4: 110-125.
7. Elks ML (1993) Chronic perifusion of rat islets with palmitate suppresses glucose-stimulated insulin release. Endocrinology 133: 208-214.
8. Sako Y, Grill VE (1990) A 48-hour lipid infusion in the rat time-dependently

inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. Endocrinology 127: 1580-1589.

9. Jacqueminet S, Briaud I, Rouault C, Reach G, Poitout V (2000) Inhibition of insulin gene expression by long-term exposure of pancreatic beta cells to palmitate is dependent on the presence of a stimulatory glucose concentration. Metabolism 49: 532-536.

10. Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V (2001) Lipotoxicity of the pancreatic beta-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. Diabetes 50: 315-321.

11. Harmon JS, Gleason CE, Tanaka Y, Poitout V, Robertson RP (2001) Antecedent hyperglycemia, not hyperlipidemia, is associated with increased islet triacylglycerol content and decreased insulin gene mRNA level in Zucker diabetic fatty rats. Diabetes 50: 2481-2486.

12. Briaud I, Kelpe CL, Johnson LM, Tran PO, Poitout V (2002) Differential effects of hyperlipidemia on insulin secretion in islets of langerhans from hyperglycemic versus normoglycemic rats. Diabetes 51: 662-668.

13. Zeender E, Maedler K, Bosco D, Berney T, Donath MY, et al. (2004) Pioglitazone and sodium salicylate protect human beta-cells against apoptosis and impaired function induced by glucose and interleukin-1beta. J Clin Endocrinol Metab 89: 5059-5066 .

14. Cnop M, Hannaert JC, Pipeleers DG (2002) Troglitazone does not protect rat pancreatic beta cells against free fatty acid-induced cytotoxicity. Biochem Pharmacol 63: 1281-1285.

15. Park S, Dong X, Fisher TL, Dunn S, Omer AK, et al. (2006) Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. J Biol Chem 281: 1159-1168.

16. Lupi R, Mancarella R, Del Guerra S, Bugliani M, Del Prato S, et al. (2008) Effects of exendin-4 on islets from type 2 diabetes patients. Diabetes Obes Metab 10: 515-519.

17. Xu G, Stoffers DA, Habener JF, Bonner-Weir S (1999) Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. Diabetes 48: 2270-2276.

18. Tourrel C, Bailbe D, Lacorne M, Meile MJ, Kergoat M, et al. (2002) Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the beta-cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. Diabetes 51: 1443-1452.

19. Gedulin BR, Nikoulina SE, Smith PA, Gedulin G, Nielsen LL, et al. (2005) Exenatide (exendin-4) improves insulin sensitivity and {beta}-cell mass in insulin-resistant obese fa/fa Zucker rats independent of glycemia and body weight. Endocrinology 146: 2069-2076.

20. Sorhede Winzell M, Ahren B (2004) Glucagon-like peptide-1 and islet lipolysis. Horm Metab Res 36: 795-803.

21. Yaney GC, Civelek VN, Richard AM, Dillon JS, Deeney JT, et al. (2001) Glucagon-like peptide 1 stimulates lipolysis in clonal pancreatic beta-cells (HIT). Diabetes 50: 56-62.

22. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ (2003) Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. J Biol Chem 278: 471-478.

23. Li L, El-Kholy W, Rhodes CJ, Brubaker PL (2005) Glucagon-like peptide-1 protects beta cells from cytokine-induced apoptosis and necrosis: role of protein kinase B. Diabetologia 48: 1339-1349.

24. Feinle-Bisset C, Patterson M, Ghatei MA, Bloom SR, Horowitz M (2005) Fat digestion is required for suppression of ghrelin and stimulation of peptide YY and pancreatic polypeptide secretion by intraduodenal lipid. Am J Physiol Endocrinol Metab 289: E948-953.

25. Miazza B, Palma R, Lachance JR, Chayvialle JA, Jonard PP, et al. (1985) Jejunal secretory effect of intraduodenal food in humans. A comparison of mixed nutrients, proteins, lipids, and carbohydrates. Gastroenterology 88: 1215-1222.

26. Batterham RL, Le Roux CW, Cohen MA, Park AJ, Ellis SM, et al. (2003) Pancreatic polypeptide reduces appetite and food intake in humans. J Clin Endocrinol Metab 88: 3989-3992.

27. Asakawa A, Inui A, Ueno N, Fujimiya M, Fujino MA, Kasuga M (1999) Mouse pancreatic polypeptide modulates food intake, while not influencing anxiety in mice. Peptides 20: 1445-1448.

28. Marco J, Zulueta MA, Correas I, Villanueva ML (1980) Reduced pancreatic polypeptide secretion in obese subjects. J Clin Endocrinol Metab 50: 744-747.

29. Lassmann V, Vague P, Vialettes B, Simon MC (1980) Low plasma levels of pancreatic polypeptide in obesity. Diabetes 29: 428-430.

30. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Wet al. 2006 The human obesity gene map: the 2005 update. Obesity (Silver Spring) 14: 529-644.

31. Nordman S, Ding B, Ostenson CG, Karvestedt L, Brismar K, et al. (2005) Leu7Pro polymorphism in the neuropeptide Y (NPY) gene is associated with impaired glucose tolerance and type 2 diabetes in Swedish men. Exp Clin Endocrinol Diabetes 113: 282-287.

32. Torekov SS, Larsen LH, Glumer C, Borch-Johnsen K, Jorgensen T, et al. (2005) Evidence of an association between the Arg72 allele of the peptide YY and increased risk of type 2 diabetes. Diabetes 54: 2261-2265.

33. Ma L, Tataranni PA, Hanson RL, Infante AM, Kobes S, et al (2005) Variations in peptide YY and Y2 receptor genes are associated with severe obesity in Pima Indian men. Diabetes 54: 1598-1602.

34. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A,et al. (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38: 320-323.

35. Smith U (2007) TCF7L2 and type 2 diabetes--we WNT to know. Diabetologia 50: 5-7.

36. Liu Z, Habener JF (2008) Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta-cell proliferation. J Biol Chem 283: 8723-8735.

37. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Aet al. (2007) Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 117: 2155-2163.

38. Suzuki Y, Yonekura H, Watanabe T, Unno M, Moriizumi S, et al. (1994) Structure and expression of a novel rat RegIII gene. Gene 144: 315-316.

39. Kobayashi S, Akiyama T, Nata K, Abe M, Tajima M,et al. (2000) Identification of a receptor for reg (regenerating gene) protein, a pancreatic beta-cell regeneration factor. J Biol Chem 275: 10723-10726.

40. Qiu L, List EO, Kopchick JJ (2005) Differentially expressed proteins in the pancreas of diet-induced diabetic mice. Mol Cell Proteomics 4: 1311-1318.

41. Lu Y, Ponton A, Okamoto H, Takasawa S, Herrera PL, Liu JL (2006) Activation of the Reg family genes by pancreatic-specific IGF-I gene deficiency and after streptozotocin-induced diabetes in mouse pancreas. Am J Physiol Endocrinol Metab 291: E50-58.

42. Eizirik DL, Bjorklund A, Cagliero E (1993) Genotoxic agents increase expression of growth arrest and DNA damage--inducible genes gadd 153 and gadd 45 in rat pancreatic islets. Diabetes 42: 738-745.

43. Larsen CM, Dossing MG, Papa S, Franzoso G, Billestrup N, et al. (2006) Growth arrest- and DNA-damage-inducible 45beta gene inhibits c-Jun N-terminal kinase and extracellular signal-regulated kinase and decreases IL-1beta-induced apoptosis in insulin-producing INS-1E cells. Diabetologia 49: 980-989.

44. Yi YW, Kim D, Jung N, Hong SS, Lee HS, et al. (2000) Gadd45 family proteins are coactivators of nuclear hormone receptors. Biochem Biophys Res Commun 272: 193-198.

45. Nishiyama A, Matsui M, Iwata S, Hirota K, Masutani H,et al. (1999) Identification of thioredoxin-binding protein-2/vitamin D(3) up-regulated protein 1 as a negative regulator of thioredoxin function and expression. J Biol Chem 274: 21645-21650.

46. Yamanaka H, Maehira F, Oshiro M, Asato T, Yanagawa Y, et al. (2000) A possible interaction of thioredoxin with VDUP1 in HeLa cells detected in a yeast two-hybrid system. Biochem Biophys Res Commun 271: 796-800.

47. Junn E, Han SH, Im JY, Yang Y, Cho EW, et al. (2000) Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. J Immunol 164: 6287-6295.

48. Chen J, Saxena G, Mungrue IN, Lusis AJ, Shalev A (2008) Thioredoxin-interacting protein: a critical link between glucose toxicity and beta-cell apoptosis. Diabetes 57: 938-944.

49. Corbett JA (2008) Thioredoxin-interacting protein is killing my beta-cells! Diabetes 57: 797-798.

50. Minn AH, Hafele C, Shalev A (2005) Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. Endocrinology 146: 2397-2405.

51. Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, et al. (2002) Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. Endocrinology 143: 3695-3698.

52. Minn AH, Pise-Masison CA, Radonovich M, Brady JN, Wang P, et al. (2005) Gene expression profiling in INS-1 cells overexpressing thioredoxin-interacting protein. Biochem Biophys Res Commun 336: 770-778.

53. Chen J, Couto FM, Minn AH, Shalev A (2006) Exenatide inhibits beta-cell apoptosis by decreasing thioredoxin-interacting protein. Biochem Biophys Res Commun 346: 1067-1074.

54. Semenza GL (1999) Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 15: 551-578.

55. Das SK, Elbein SC (2007) The search for type 2 diabetes susceptibility loci: the chromosome 1q story. Curr Diab Rep 7: 154-164.

56. Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, et al. (2005) Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. Cell 122: 337-349.

57. Shilo BZ (2003) Signaling by the Drosophila epidermal growth factor receptor pathway during development. Exp Cell Res 284:140-149

58. Sternberg PW, Horvitz HR (1991) Signal transduction during C. elegans vulval induction. Trends Genet 7: 366-371.

59. Wong RW (2003) Transgenic and knock-out mice for deciphering the roles of EGFR ligands. Cell Mol Life Sci 60: 113-118.

60. Buteau J, Foisy S, Joly E, Prentki M (2003) Glucagon-like peptide 1 induces pancreatic beta-cell proliferation via transactivation of the epidermal growth factor receptor. Diabetes 52: 124-132.

61. Howles PN, Carter CP, Hui DY (1996) Dietary free and esterified cholesterol absorption in cholesterol esterase (bile salt-stimulated lipase) gene-targeted mice. J Biol Chem 271: 7196-7202.

62. Wang CS, Hartsuck JA (1993) Bile salt-activated lipase. A multiple function lipolytic enzyme. Biochim Biophys Acta 1166: 1-19.

63. Raeder H, Johansson S, Holm PI, Haldorsen IS, Mas E, et al. (2006) Mutations in the CEL VNTR cause a syndrome of diabetes and pancreatic exocrine dysfunction. Nat Genet 38: 54-62.

64. McGarry JD, Foster DW (1980) Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem 49: 395-420.

65. Kim KH (1997) Regulation of mammalian acetyl-coenzyme A carboxylase. Annu Rev Nutr 17: 77-99

66. Brun T, Assimacopoulos-Jeannet F, Corkey BE, Prentki M (1997) Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic beta-cell line INS-1. Diabetes 46: 393-400.

67. Iritani **N (**1992) Nutritional and hormonal regulation of lipogenic-enzyme gene expression in rat liver. Eur J Biochem 205: 433-442.

68. Brun T, Roche E, Kim KH, Prentki M (1993) Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic beta-cell line (INS-1). J Biol Chem 268: 18905-18911.

69. Zhang S, Kim KH (1996) Acetyl-CoA carboxylase is essential for nutrient-induced insulin secretion. Biochem Biophys Res Commun 229: 701-705

70. Zhang S, Kim KH (1998) Essential role of acetyl-CoA carboxylase in the glucose-induced insulin secretion in a pancreatic beta-cell line. Cell Signal 10: 35-42.

71. Roche E, Assimacopoulos-Jeannet F, Witters LA, Perruchoud B, Yaney G, et al. (1997) Induction by glucose of genes coding for glycolytic enzymes in a pancreatic beta-cell line (INS-1). J Biol Chem 272: 3091-3098.

72. Sirover MA (1999) New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. Biochim Biophys Acta 1432: 159-184.

73. Ishitani R, Tanaka M, Sunaga K, Katsube N, Chuang DM (1998) Nuclear localization of overexpressed glyceraldehyde-3-phosphate dehydrogenase in cultured cerebellar neurons undergoing apoptosis. Mol Pharmacol 53: 701-707.

74. Nakazawa M, Uehara T, Nomura Y (1997) Koningic acid (a potent glyceraldehyde-3-phosphate dehydrogenase inhibitor)-induced fragmentation and condensation of DNA in NG108-15 cells. J Neurochem 68: 2493-2499.

75. Yang J, Gibson B, Snider J, Jenkins CM, Han X, et al. (2005) Submicromolar concentrations of palmitoyl-CoA specifically thioesterify cysteine 244 in glyceraldehyde-3-phosphate dehydrogenase inhibiting enzyme activity: a novel mechanism potentially underlying fatty acid induced insulin resistance. Biochemistry 44: 11903-11912.

76. Donath MY, Schumann DM, Faulenbach M, Ellingsgaard H, Perren A, et al. (2008) Islet inflammation in type 2 diabetes: from metabolic stress to therapy. Diabetes Care 31 Suppl 2: S161-S164.

77. Igoillo-Esteve M, Marselli L, Cunha DA, Ladrière L, Ortis F, et al. (2010) Palmitate induces a pro-inflammatory response in human pancreatic islets that mimics CCL2 expression by beta cells in type 2 diabetes. Diabetologia 53: 1395-1405.