

Ex vivo transcriptional profiling of human pancreatic islets following chronic exposure to monounsaturated fatty acids

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Abstract

The aim of this study was to assess the effects of chronic free fatty acid (FFA) exposure on gene expression and the functional state of human pancreatic islets. Chronic exposure of islets to oleate (OA) resulted in a significant reduction in glucose-stimulated insulin secretion (GSIS) compared with control (466 ± 82 vs 234 ± 57 ng/ μ g DNA, $P < 0.05$). OA treatment also led to reduction in total insulin content of the islets ($17\,609 \pm 3816$ vs $10\,599 \pm 3876$ ng insulin/ μ g DNA) and to an increase in the rate of reactive oxygen species (ROS) generation. Interestingly, the suppressive effects of OA on biosynthesis and secretion of insulin were accompanied by alteration in the expression of 40 genes, as determined by microarray analysis and subsequent qPCR validation. The majority of genes regulated by OA encoded metabolic enzymes. The expression of enzymes

involved in oxidative defense was elevated, indicating a link between ROS generation and antioxidant defense activation. Additionally, pretreatment of human islets with OA led to a significant increase (30%) in the rate of oxidation of this fatty acid and to a significant decrease (75%) in glucose oxidation. Importantly, individual analysis of gene clusters from the islets of all donors revealed the induction of genes involved in inflammation and immunity, which provides further evidence that FFA are risk factors for the development of type 2 diabetes. In summary, our data indicate that chronic exposure of human islets to FFA activates inflammatory and metabolic pathways that lead to oxidative stress, reduced β -cell insulin content, and inhibition of GSIS.

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Introduction

Type 2 diabetes (T2D) is a multifactorial disease and its etiology is attributed to a combination of genetic and environmental factors (Haslam & James 2005). Defects in β -cell function are critical in the development of both T1D and T2D. The prevalence of obesity in modern societies has increased dramatically over the past few years and has reached epidemic proportions. Obesity is closely related to insulin resistance and hyperinsulinemia and the very frequent combination of obesity and T2D is characterized as 'diabesity' (Koulouridis 2004). A multitude of factors contribute to the pathogenesis of T2D including inflammatory cytokines, increased concentrations of free fatty acids (FFA), adipokines, impaired mitochondrial function through defects in mitochondrial lipid oxidation, endoplasmic reticulum (ER) stress, and chronic hyperglycemia (referred to as glucotoxicity; Stumvoll *et al.* 2005).

Since T2D is a heterogeneous clinical entity, determining its causes and consequences is a challenging issue. High-throughput

methods, such as microarrays, allow the screening of a large number of genes in a relatively short period of time (Stoughton 2005). To date, there are no published studies that have used microarrays to identify candidate genes and relevant metabolic and signaling pathways regulated by FFA in human pancreatic islets. This is primarily due to the scarcity of human tissue for research purposes (Haber *et al.* 2003, 2006, Newsholme *et al.* 2007b). Alterations in patterns of expression of multiple genes can offer new insights into regulatory mechanisms, biochemical pathways, and potentially aid in the development of unique therapeutic drug targets.

A plethora of studies has indicated that chronic exposure of β cells to elevated levels of FFA is accompanied by the loss of glucose-stimulated insulin secretion (GSIS) and a decrease in total insulin content (Paolisso *et al.* 1995, Mason *et al.* 1999, Lupi *et al.* 2002a, Oprescu *et al.* 2007). Additionally, the consensus from numerous studies indicates that monounsaturated as well as the majority of polyunsaturated fatty acids are well tolerated as opposed to long-chain saturated fatty acids that are the most toxic to β cells (Haber *et al.* 2003, 2006,

Newsholme *et al.* 2007b). Oleate (OA) has been shown to induce defects in GSIS in numerous pancreatic β -cell lines as well as rodent and human islets (Haber *et al.* 2006). However, its toxicity is minimal since no significant induction of apoptosis in β cells with this fatty acid has been reported (Newsholme *et al.* 2007b). Thus, for the purpose of our study, we used OA as the FFA of choice in order to eliminate possible toxic effects on pancreatic islets. Here, we used oligonucleotide microarrays and quantitative real-time PCR in order to examine the role of chronic FFA exposure in human islets on a genome-wide scale. In addition, we examined various pathways involved in β -cell function/physiology. Importantly, individual analysis of gene clusters from the islets of all donors revealed the induction of genes involved in reactive oxygen species (ROS) activity, inflammation, and immunity. This provides evidence that chronic exposure of human islets to FFA activates inflammatory and metabolic pathways that lead to oxidative stress, reduced β -cell insulin content, and inhibition of GSIS.

Materials and Methods

Fatty acid preparation

OA bound to fatty acid-free BSA was prepared by stirring 4 mmol/l OA with 5% (wt/vol) BSA in Krebs–Ringer HEPES buffer (KRBH) for 30 min at 50 °C. Quantitative determination of FFA in the resulting solution was achieved by the use of the NEFA C test kit assay (VWR International, Canada).

Human islet isolation and culture

Human islets were obtained from the Clinical Transplantation Laboratory at the University of Alberta from non-diabetic human cadavers. Human islets were isolated following the Edmonton Protocol (O’Gorman *et al.* 2005) and cultured in RPMI 1640 medium supplemented with 5.5 mmol/l glucose, 10% fetal bovine serum, 25 mmol/l HEPES, 2 mmol/l L-glutamine, 100 Units/ml penicillin, and 100 μ g/ml streptomycin, in a 37 °C humidified chamber. Human islets were incubated in RPMI 1640 containing 5.5 mmol/l glucose and treated for 48 h with either 0.5% (wt/vol) BSA or 0.4 mM OA in BSA. Human islet donor information is summarized in Supplemental Table 1, which can be viewed online at <http://joe.endocrinology-journals.org/content/vol196/issue3/>.

Insulin secretion experiments and insulin content determination

Human islets (20 islets/well) were preincubated for two sequential periods of 30 min in KRBH buffer (components (128.8 mmol/l NaCl, 4.8 mmol/l KCl, 1.2 mmol/l KH₂PO₄, 1.2 mmol/l MgSO₄, 2.5 mmol/l CaCl₂, 5 mmol/l NaHCO₃, and 10 mmol/l HEPES) containing

2.8 mmol/l glucose and 0.1% BSA in a 37 °C humidified air incubator. The islets were incubated in KRBH buffer containing low (2.8 mmol/l) or high (16.7 mmol/l) glucose concentrations for 1 h. Insulin in the supernatant was determined using an RIA kit (Linco Research Inc., St Charles, MO, USA). Insulin secretion measurements were normalized to total DNA content in the sample. The total cellular insulin (50 islets/well) content was extracted by sonication in buffer containing 75% ethanol/1.5% (vol/vol) HCl to ensure complete cellular lysis. The content was assessed by means of RIA as described previously (Wang *et al.* 2004). The DNA content of the human islets was measured from the ethanol/HCl solution using a spectrophotometer.

Intracellular ROS measurements

Human islets were treated for 48 h with 0.4 mmol/l OA and incubated in KRBH buffer containing 5.5 mmol/l glucose, 0.4 mmol/l OA, and 10 μ mol/l DCFH-DA for 30 min at 37 °C. Fluorescence was recorded using an Olympus BX51W1 fluorescent microscope fitted with a 10 \times /0.30 water immersion objective and cooled CCD camera equipped with a magnification changer (U-TVAC, Olympus, Carsen Group Inc., Markham Canada). Measurements were performed at 480 nm excitation and 510 nm emission using a xenon lamp-based DeltaRam high-speed monochromator and video camera. The collected data were analyzed using the ImageMaster 3 software (Photon Technology International Inc., London, ON, Canada).

RNA extraction and gene expression profiling

Total RNA was isolated from human islets (1000 islets) using TRIzol reagent (Invitrogen Inc.) and further purified by the RNeasy kit (Qiagen Inc.), according to the manufacturer’s instructions. For each Affymetrix GeneChip, total RNA was prepared for hybridization and subsequently hybridized according to the recommendations from Affymetrix (Santa Clara, CA, USA). In all, islet preparations from five donors were processed using the U133A Affymetrix GeneChips. The GeneChips for microarray analysis were processed on two separate occasions.

Primer design, reverse transcription, and quantitative real-time PCR

Primers were designed using the software Primer Express (PE Applied Biosystems, Perkin–Elmer, Foster City, CA, USA). Each 10 μ l reaction mix consisted of 6 μ l real-time PCR master mix and 4 μ l template. Real-time PCR mix contained 1 \times PCR buffer, 3 mmol/l MgCl₂, 0.2 mmol/l dNTP, 1 nmol/l forward and reverse primers, 1:50 dilution of ROX reference dye, 3:100 000 dilution of SYBR Green I, and 0.05 Units of Platinum Taq DNA polymerase. The real-time PCR was monitored and analyzed by the Sequence Detection

System ver. 2.0 (PE Applied Biosystems, Perkin–Elmer). All genes were normalized to the control gene TATA box binding protein. Primer sequences are shown in Supplemental Table 2, which can be viewed online at <http://joe.endocrinology-journals.org/content/vol196/issue3/>. All real-time PCR samples were processed simultaneously.

Microarray data analysis

The expression levels from microarrays along with the present/absent calls were calculated using GCOS ver. 1.2 (Affymetrix) with the statistical algorithm from MAS5. The setting used for GCOS analysis were the following: Alpha1: 0.05, Alpha2: 0.065, Tau: 0.015, Gamma1H: 0.0045, Gamma1L: 0.0045, Gamma2H: 0.006, Gamma2L: 0.006, Target: 500, NF: 1. Genes chosen for the analysis were of present calls in all samples analyzed by GCOS. Furthermore, GCOS analysis included paired samples treated with fatty acids from each individual donor eliminating variables such as age and sex. Novel biological patterns in our microarray data were identified using L2L, a database consisting of lists of differentially expressed genes compiled from the published mammalian microarray studies. With the L2L database, we systematically compared our own list of differentially expressed genes with a database of lists of differentially expressed genes that were derived from the published microarray data, and identified common expression patterns.

Glucose oxidation

Glucose oxidation was determined by measuring the formation of $^{14}\text{CO}_2$ from $[\text{U-}^{14}\text{C}]\text{glucose}$ (Gaidhu *et al.* 2006). Groups of 200 islets were incubated in 2 ml microtubes containing 500 μl KRBH (pH 7.4) containing 0.4 $\mu\text{Ci}/\text{ml}$ D- $[\text{U-}^{14}\text{C}]\text{glucose}$ plus non-radioactive glucose to a final concentration of either 2.8 or 16.7 mmol/l. The buffer was gassed with $\text{O}_2:\text{CO}_2$ (95:5%) for 30 min prior to the onset of the experiment. The microtubes containing islets were placed in standard 20 ml scintillation vials. The scintillation vials were then capped airtight using rubber stoppers and gassed with $\text{O}_2:\text{CO}_2$ (95:5%) for an additional 30 s. Subsequently, the vials were shaken continuously at 37 °C for 120 min. The assay was terminated by injecting 200 μl H_2SO_4 (4 mol/l) into the microtube. $^{14}\text{CO}_2$ liberated from the incubation medium was captured by a subsequent injection of 500 μl phenylethylamine–methanol (1:1) through the rubber stoppers into the scintillation vials. After 2 h at 37 °C (to allow the liberated $^{14}\text{CO}_2$ to be trapped by phenylethylamine–methanol), the cap was removed, and 10 ml scintillation fluid was added to each vial. Non-specific (NS) $^{14}\text{CO}_2$ produced was measured by incubating vials containing all buffers but lacking islets. The NS values were subtracted from all conditions. Radioactivity was measured in a liquid scintillation counter.

OA oxidation

OA oxidation was measured by the modification of a method described previously (Patane *et al.* 2000). In brief, groups of 200 islets were placed in 2 ml microtubes containing 500 μl KRBH (pH 7.4) containing 0.4 $\mu\text{Ci}/\text{ml}$ $[\text{1-}^{14}\text{C}]\text{OA}$ and non-labeled OA (final concentration 20 $\mu\text{mol}/\text{l}$), 5.5 mmol/l glucose, 2% BSA, and 0.8 mmol/l L-carnitine. The buffer was gassed with $\text{O}_2:\text{CO}_2$ (95:5%) for 30 min prior to the onset of the experiment. The microtubes were placed in standard 20 ml scintillation vials, gassed with $\text{O}_2:\text{CO}_2$ (95:5%) for an additional 30 s, and then capped airtight using rubber stoppers. The vials were then shaken continuously at 37 °C for 120 min. The assay was terminated by injecting 200 μl H_2SO_4 (4 mol/l) into the microtube. $^{14}\text{CO}_2$ liberated from the incubation medium was captured by a subsequent injection of 500 μl phenylethylamine–methanol (1:1) through the rubber stoppers into the scintillation vials. After 2 h at 37 °C (to allow the liberated $^{14}\text{CO}_2$ to be trapped by phenylethylamine–methanol), the cap was removed and 10 ml scintillation fluid was added to each vial. Control (NS) incubation-lacking islets were run with each series. The NS values were subtracted from all conditions. Radioactivity was measured in a liquid scintillation counter.

Statistical analysis

Data are expressed as means \pm S.E.M. Statistical analysis was performed using non-parametric tests. Insulin secretion data, insulin content, glucose/FFA oxidation, and ROS production measurements were analyzed using the Wilcoxon test. The real-time PCR data were analyzed using the Mann–Whitney *U* test. Differences were deemed significant when $P < 0.05$.

Results

The effects of OA exposure on insulin secretion and total islet insulin content

A large body of evidence deriving from both *in vitro* and *in vivo* studies in rodent models suggests that FFA impair insulin secretion and additionally saturated fatty acids can impair insulin gene expression (Newsholme *et al.* 2007b). The exposure of human islets to 0.4 mmol/l OA for 48 h did not significantly change basal insulin secretion (BSA: 98.3 ± 12.5 versus OA: 134.8 ± 31.7 ng insulin/ μg DNA). However, OA exposure resulted in a significant reduction in insulin secretion induced by glucose (466.8 ± 81.7 to 234.8 ± 57.3 ng insulin/ μg DNA; Fig. 1A). Along with the effects on insulin secretion OA also caused a significant decrease in total cellular insulin content, reducing it from $17\,608.9 \pm 3816.2$ to $10\,598.9 \pm 3876.1$ ng insulin/ μg DNA (Fig. 1B).

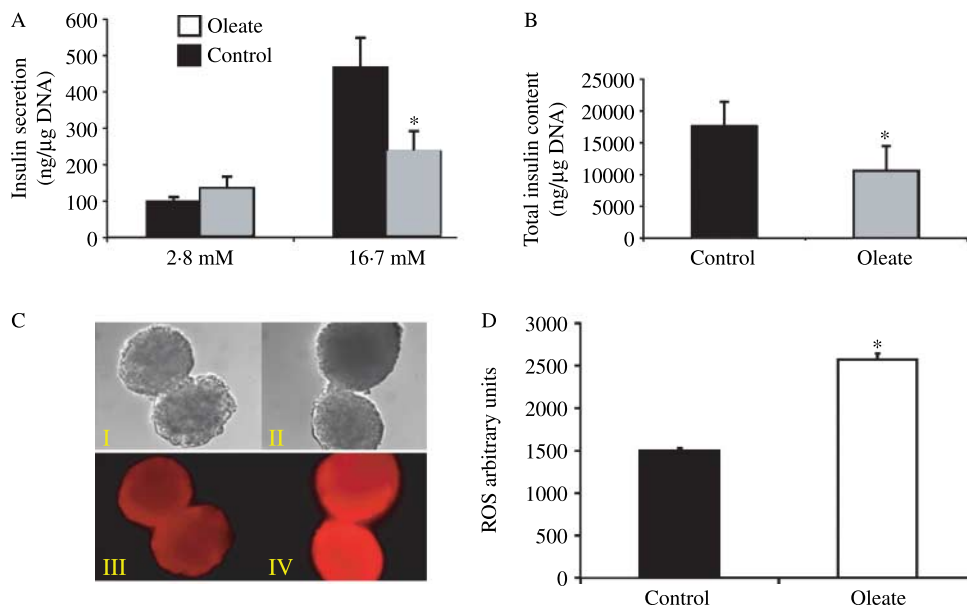


Figure 1 (A) The effect of 48-h treatment of human pancreatic islets with oleate (OA 0.4 mM+0.5% BSA) on basal (2.8 mmol/l) and glucose-stimulated insulin secretion (16.7 mM). * $P < 0.05$ for OA versus control (BSA) at 16.7 mmol/l glucose. Insulin secretion measurements were normalized to cellular DNA content. Results are the means of four separate experiments ($n=4$). Black bars represent human islets treated with BSA only, while white bars indicate human islets treated with OA. (B) The effect of 48-h treatment of human pancreatic islets with OA (0.4 mmol/l) on total insulin content. For each experiment, 50 human islets were treated with OA or BSA and subsequently total insulin content was determined. * $P < 0.05$ for OA versus control (BSA). Total insulin content measurements were normalized to the cellular DNA content. Results are the means of four separate experiments ($n=4$). (C) Microscopy images of human islets. Representative light microscopy images of human islets treated with: I) BSA and II) 0.4 mmol/l OA for 48 h. Fluorescent images (DCF fluorescence) of human islets treated with: III) BSA and IV) 0.4 mmol/l OA for 48 h. (D) Quantification of fluorescence (ROS arbitrary units) in BSA- and OA-treated human islets. OA (0.4 mmol/l) was added to the DCF containing medium and the measurements were performed at 5.5 mmol/l glucose. Results are the means of three separate experiments performed in triplicate.

The effects of OA on global gene expression in human pancreatic islets

The changes in gene expression profiles were identified as being increased or decreased by comparing fatty acid treatment with BSA control. As shown in Fig. 3G, out of the 14 500 genes or expressed sequence tags (ESTs) analyzed by gene microarray, OA decreased the expression of 601, 561, 139, 356, and 502 and increased the expression of 239, 100, 165, 194, and 968 genes or ESTs in five independent donor microarray analyses respectively. However, OA only altered the expression profiles of 40 genes consistently in five independent experiments. Out of these 40 genes, 27 were upregulated while 13 genes downregulated (Table 1). Table 1 shows the genes that were subsequently validated by means of real-time PCR. A total of 34 out of the 40 (85%) genes deemed significantly changed by the microarray analysis were further confirmed by the real-time PCR.

OA affected genes involved in metabolism

The development of obesity and associated insulin resistance involves a multitude of gene products, including proteins

involved in lipid synthesis and oxidation. Fatty acids have a very strong direct influence on the molecular events that govern transcription. In our study, the genes involved in metabolism comprised the largest functional cluster with 25% (10 out of 40) classified in this category (Table 1). Treatment with OA resulted in the upregulation of enzymes involved in fatty acid β -oxidation such as acyl-coenzyme A dehydrogenase, very long chain (2 ± 0.16 -fold); carnitine palmitoyltransferase 1 (CPT1; 3.77 ± 0.71); acetyl-coenzyme A acyltransferase 2 (1.64 ± 0.15); enoyl-coenzyme A hydratase, 1 (2.1 ± 0.06); and mitochondrial trifunctional protein (1.72 ± 0.16). Finally, a great upregulation in angiotensin-like protein 4 (over fourfold) was observed in both microarray analysis and qPCR validation. In order to further investigate the role of OA on β -oxidation, human islets were chronically treated with OA for 48 h as a model of chronic FFA exposure. Fatty acid oxidation was upregulated by 30% in islets treated with OA compared with BSA-treated control islets (Fig. 2A). This finding is in accordance with the human islet transcriptional profile demonstrating an upregulation of genes of fatty acid β -oxidation (Table 1). Additionally, as shown in Fig. 2B, OA pre-exposed islets showed a 75% reduction in their ability to

Table 1 List of genes transcriptionally regulated by oleate (OA) treatment in human islets of five individual donors

| Gene | Microarray | Real-time PCR | Accession number |
|---|--------------|---------------|------------------|
| Fatty acid/glucose metabolism | | | |
| Acyl-coenzyme A dehydrogenase, very long chain (ACAVDL) | +1.62 ± 0.11 | +2 ± 0.16 | NM_000018 |
| Monoglyceride lipase (MGLL) | +1.26 ± 0.05 | +1.27 ± 0.08 | BC006230 |
| Enoyl coenzyme A hydratase 1 (ECH1) | +1.37 ± 0.08 | +2.1 ± 0.06 | NM_001398 |
| Carnitine palmitoyltransferase 1A, liver (CPT1A) | +2.2 ± 0.12 | +3.77 ± 0.71 | NM_001031847 |
| Mitochondrial trifunctional protein (HADHA) | +1.43 ± 0.05 | +1.72 ± 0.16 | NM_000182 |
| Adipose differentiation-related protein (ADFP) | +3.83 ± 1.09 | NC | NM_001122 |
| Acetyl-coenzyme A acyltransferase (ACAA2) | +1.51 ± 0.09 | +1.63 ± 0.15 | NM_006111 |
| PPAR γ angiopoietin-related protein (ANGPTL4) | +4.34 ± 1.10 | +4.1 ± 0.12 | NM_139314 |
| Aldo-keto reductase, family 1, member B10 (AKR1B10) | +1.31 ± 0.05 | +1.76 ± 0.17 | NM_020299 |
| Methylcrotonoyl-coenzyme A carboxylase 1 (MCCC1) | -0.80 ± 0.07 | -0.75 ± 0.10 | NM_020166 |
| Cellular transport | | | |
| Aquaporin 3 (AQP3) | 1.26 ± 0.11 | NC | NM_004925 |
| Glycolysis | | | |
| Aldolase B | -0.69 ± 0.06 | NC | NM_000035 |
| Cell proliferation | | | |
| Insulin-induced gene 1 (INSIG1) | -0.76 ± 0.06 | -0.75 ± 0.19 | NM_005542 |
| IGF-binding protein 3 (IGFBP3) | -0.74 ± 0.11 | NC | NM_000598 |
| Electron transport | | | |
| Cytochrome P450, family 1, subfamily B, polypeptide 1 (CYP1B1) | -0.79 ± 0.06 | NC | NM_000104 |
| Electron transfer flavoprotein, α -polypeptide (ETF α) | +1.49 ± 0.08 | +1.32 ± 0.05 | NM_000126 |
| Electron transfer flavoprotein, β -polypeptide (ETF β) | +1.35 ± 0.10 | +1.33 ± 0.05 | NM_001985 |
| CGI-44 protein; sulfide dehydrogenase like (SQRD1) | +1.28 ± 0.10 | +1.41 ± 0.11 | NM_021199 |
| Inflammatory response | | | |
| C-reactive protein, pentraxin related (CRP) | +1.35 ± 0.09 | +1.42 ± 0.05 | NM_000567 |
| Chemokine ligand 1, CX3CL1 (Fractalkine) | +2.08 ± 0.12 | +2.47 ± 0.11 | NM_002996 |
| Tumor necrosis factor superfamily, member 21 (TNFRSF21) | +1.51 ± 0.09 | +1.46 ± 0.08 | NM_014452 |
| Prostaglandin-endoperoxidase synthase 2 (PTGS2) | -0.85 ± 0.20 | NC | NM_000963 |
| Oxidative stress | | | |
| Catalase (CAT) | +1.43 ± 0.05 | +1.70 ± 0.10 | NM_001752 |
| Metallothionein 1F (MTF1) | +1.19 ± 0.09 | +1.49 ± 0.12 | NM_005949 |
| Sequestosome 1 (SQSTM1) | +1.41 ± 0.08 | +1.47 ± 0.08 | NM_003900 |
| Signal transduction | | | |
| Regulator of G-protein signaling 4 (RGS4) | -0.65 ± 0.12 | -0.75 ± 0.19 | NM_005613 |
| Growth differentiation factor 15 (GDF15) | +1.35 ± 0.16 | +1.45 ± 0.09 | NM_004864 |
| Neuromedin B (NMB) | -0.82 ± 0.11 | -0.82 ± 0.06 | NM_021077 |
| Cytolysis | | | |
| Monocyte to macrophage differentiation associated (MMD) | +1.55 ± 0.07 | +1.59 ± 0.13 | NM_012329 |
| Regulation of transcription | | | |
| Inhibitor of DNA binding 4 (ID4) | -0.76 ± 0.03 | -0.80 ± 0.09 | U16153 |
| v-myc myelocytomatosis viral oncogene homolog (avian; MYC) | +1.30 ± 0.10 | +1.54 ± 0.14 | NM_002467 |
| Cell motility | | | |
| Vanin 1 (VNN1) | -0.89 ± 0.10 | -0.84 ± 0.10 | NM_004666 |
| Cytoskeleton organization | | | |
| Carbohydrate sulfotransferase 6 (CHST6) | +1.37 ± 0.09 | +2.13 ± 0.21 | NM_021149 |
| Keratin 6B (KRT6B) | +1.69 ± 0.12 | +1.95 ± 0.10 | AI831452 |
| Cell cycle regulation | | | |
| Transforming growth factor- α (TGFA) | +1.33 ± 0.03 | +1.64 ± 0.13 | NM_003236 |
| Cell adhesion | | | |
| Galectin-4 (LGALS4) | +1.55 ± 0.22 | +1.40 ± 0.11 | NM_006149 |
| Transport | | | |
| Transcobalamin 1 (TCN1) | -0.68 ± 0.08 | -0.67 ± 0.10 | NM_001062 |
| Other | | | |
| C10 open reading frame 10 (C10orf10) | -0.79 ± 0.04 | -0.74 ± 0.06 | NM_007021 |
| Phosphodiesterase 10A (PDE10A) | +1.55 ± 0.22 | +2.56 ± 0.31 | NM_006661 |
| Glomerular epithelial protein 1 (GLEPP1) | -0.82 ± 0.08 | -0.82 ± 0.10 | U20489 |

Both microarray and real-time PCR analyses are shown for the genes of interest. NC, no change (indicates genes that were not validated by real-time PCR analysis).

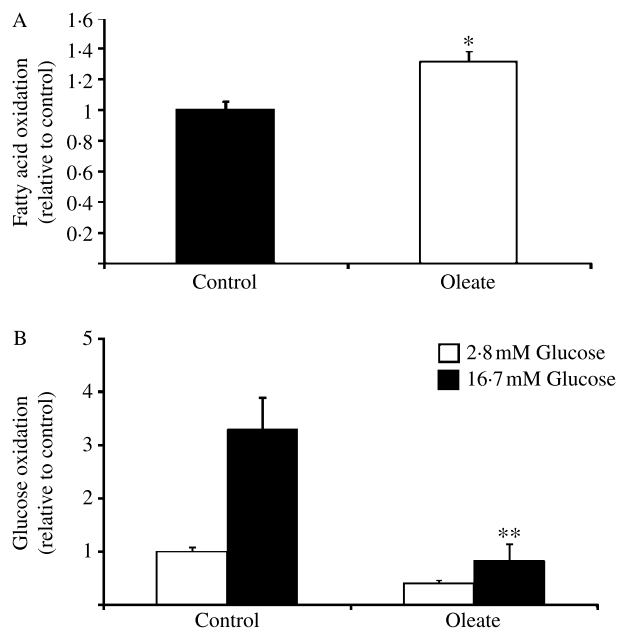


Figure 2 (A) Fatty acid oxidation in human pancreatic islets exposed for 48 h to 0.4 mmol/l oleate. [^{14}C]oleic acid was added at the end of the culture period. * $P < 0.05$ for OA versus control (BSA) at high glucose. (B) Glucose oxidation at 2.8 or 16.7 mmol/l glucose in human pancreatic islets cultured either with or without 0.4 mmol/l OA as indicated. ** $P < 0.01$ for OA versus control (BSA) at 16.7 mmol/l glucose.

oxidize glucose, whereas no changes were observed in glucose oxidation under basal conditions.

OA upregulated genes involved in inflammation, oxidative stress, and transcription

Whether there is a clear role for inflammation in the development of T2D at the level of the β cell has not been ascertained. In our study, OA treatment upregulated the expression of genes involved in the generation of inflammation (Table 1) such as tumor necrosis factor receptor superfamily member 21 (1.47 ± 0.08), fractalkine (*CX3CL1*; 2.47 ± 0.11), and C-reactive protein (CRP; 1.42 ± 0.05). An upregulation of enzymes involved in oxidative stress/defense was also elicited by OA treatment with the induction of catalase (1.7 ± 0.1), metallothionein 1F (1.49 ± 0.12), and sequestosome 1 (1.47 ± 0.08). Interestingly, fatty acid treatment resulted in the upregulation of aldose reductase (*AKR1B10*; 1.76 ± 0.17), an enzyme that has been implicated in the pathogenesis of T2D (Srivastava *et al.* 2005; Table 1). OA treatment resulted in the upregulation of *cMYC* (1.54 ± 0.14), a member of the *MYC* family of oncogenes that code for nuclear phosphoproteins that appear to promote cell growth and transformation by regulating the transcription of target genes required for proliferation (Table 1). Fatty acids have been implicated in the formation of ROS in many models of lipotoxicity. Furthermore, increased oxidative stress has been reported to be associated with the

diabetic condition in several experimental and clinical settings (Robertson *et al.* 2004, Del Guerra *et al.* 2005). In the present study, we further explored the role of oxidative stress because oxidative markers were upregulated in our microarray analysis. The exposure of human islets to OA treatment (0.4 mmol/l) for 48 h caused a significant increase in ROS production (approximately twofold, $P < 0.05$) as shown in Fig. 1C and D.

Effects of OA on glucose sensing, insulin signaling, and exocytosis

We used the real-time PCR to test for the involvement of chronic FFA exposure on the regulation of genes involved in glucose sensing, insulin signaling, fatty acid metabolism, mitochondrial function, transcription, and cell survival. Although these genes were not changed by microarray analysis, we used PCR, a more sensitive detection method that favors the detection of low-abundance genes. Supplemental Fig. 1, which can be viewed online at <http://joe.endocrinology-journals.org/content/vol196/issue3/> shows the results demonstrating that OA treatment lead to an increase in the mRNA level for uncoupling protein 2 (UCP2). In addition, we observed a modest reduction in the level of *GLUT2* mRNA (0.74 ± 0.22 -fold), as well as a modest increase in the exocytotic SNARE protein syntaxin-4 (1.18 ± 0.24 -fold) mRNA levels that were not statistically significant.

Other genes unaffected by OA treatment

T2D is attributed to various factors affecting β -cell function. The transcriptional regulation of mature onset diabetes of the young genes, pancreatic hormones, and glucose metabolism proteins was examined, as well as important islet transcription factors including the members of the family of bHLH/PAS transcription factors. Our findings revealed that OA treatment did not significantly change the mRNA levels of any of these genes (Supplemental Fig. 2, which can be viewed online at <http://joe.endocrinology-journals.org/content/vol196/issue3/>).

GO annotation of gene expression among donor islets

We compared the overlap among the lists of differentially expressed genes in all five donors (Fig. 3) using the L2L Microarray Analysis Tool (Seattle, WA, USA). There are subsets of genes that are highly regulated by FFA treatment in all five donors independently of the donor-specific parameters. These subsets include signal transducers, cell growth/cell survival regulators, metabolic regulators, and importantly, regulators of immune, inflammatory, and oxidative stress responses indicating significant similarities against fatty acid-induced changes in gene transcription.

Discussion

Most of the studies addressing the effects of fatty acids on β cells have employed rat, mouse, or hamster-derived

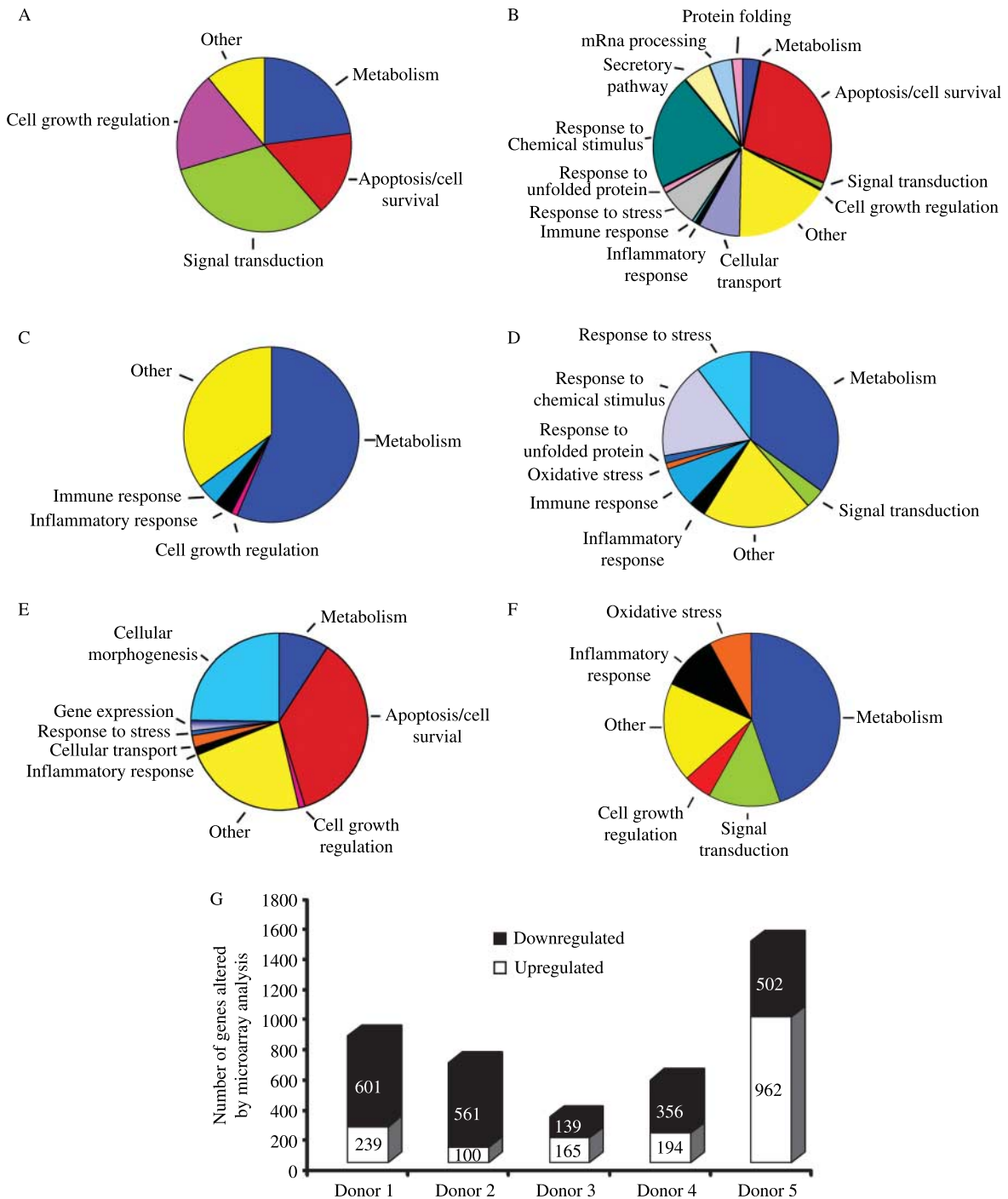


Figure 3 Genes transcriptionally regulated by OA. Clustering was based on their known biological function for all five donors examined. Results for each individual cluster are shown as percentages of the total genes deemed significantly altered by microarray analysis. The gene ontology analysis was performed using the L2L Microarray Analysis Tool (<http://depts.washington.edu/l2l/>), a database of published human microarray gene expression data, and a software tool for comparing the published data to a particular microarray results set. (A–E) Donors 1–5, (F) all five donors combined, and (G) number of genes or EST upregulated and downregulated following treatment with OA (0.4 mmol/l) for 48 h in five independent experiments. The Microarray Suite 5.0 (MAS5) method (Affymetrix) was used to obtain probe set summaries.

insulinoma β -cell lines or islets for *in vitro* studies. Although the commonly used rodent β -cell lines have provided a wealth of information and valuable insights into β -cell function in normal and pathological states, controversies still exist about the effects of increased plasma FFA concentration on human β -cell function *in vivo*. In order to address this issue, we have used DNA microarray technology to study the effects of OA on human pancreatic islet physiology. We compared global gene profiles of control islets (exposed to BSA only) versus islets chronically exposed to OA. Here, we provide novel evidence that OA treatment of human islets regulates multiple gene networks involved in metabolism, apoptosis/cell survival, signal transduction, response to stress, response to inflammation, immunity, and ER stress.

T2D is primarily characterized by impaired insulin secretion and action. One of the early indications of the defect in insulin secretion is selective impairment in GSIS. The great majority of type 2 diabetic individuals are obese and often have elevated plasma levels of FFA (Boden 1997), which seems to be a consequence of their expanded (Bjorntorp *et al.* 1969) and more lipolytically active (Rebuffe-Scrive *et al.* 1990) adipose tissue. It is well established that FFA can impair insulin action (Delarue & Magnan 2007) in peripheral tissues. However, it is less clear whether FFA can also impair β -cell function and play an important role in the defect in GSIS that characterizes T2D. OA and palmitate are the most abundant circulating fatty acids, representing 41 and 21% of the total amount of fatty acid circulating in the blood respectively (Richieri & Kleinfeld 1995). Our results indicate that in human islets *ex vivo*, chronic exposure to OA causes selective loss of glucose responsiveness as shown by a marked reduction (50%) in GSIS. These results are in line with other human islet *ex vivo* studies that reported decreased GSIS in human islets cultured for 48 h with either 0.125 mmol/l palmitate or OA (Zhou & Grill 1995). Similar results were also reported by Lupi *et al.* (2002b) and Dubois *et al.* (2004) showing suppression of GSIS from human islets after exposure for 48 h to a 2 mmol/l mixture of OA and palmitate. Although the above studies clearly indicate the loss of glucose responsiveness as a result of FFA pre-exposure, they do not differentiate the effect of each FFA subtype, i.e., mono-unsaturated versus saturated. In addition, since the concentration of FFA in normal human plasma *in vivo* generally ranges between 0.1 and 0.5 mmol/l (Rodén 2004), the majority of the aforementioned studies have used supra-physiological concentrations usually found in diabetic individuals. Our findings are unique since they are the first to investigate the effect of OA on human islet insulin secretion under physiological glucose concentrations in non-obese and non-diabetic donors. Pre-exposure to OA also reduced islet insulin content by 40% in accordance with the previously published results (Zhou & Grill 1995, Wang *et al.* 2004). The reduction of insulin content by OA in our study did not occur at the level of transcription, since insulin mRNA levels were unchanged as assessed by microarray analysis. This raises the possibility that modulation of insulin content by OA may

occur at the posttranscriptional level. Moreover, OA may also have translation-independent effects on insulin content by modulation of posttranslation pathways and/or degradation of insulin.

Fatty acid-induced derangements in GSIS involve a multitude of factors including: triglyceride accumulation (Muoio & Newgard 2006), disruption of glucose metabolism (Shoelson *et al.* 2007), increased UCP2 expression (Joseph *et al.* 2002), mitochondrial fuel metabolism, and generation of secretion coupling factors via pyruvate cycling pathways (Newsholme *et al.* 2007a), increases in CPT1 expression (Xiao *et al.* 2001), increases in the level of ROS in β cells (Koshkin *et al.* 2003, Oprescu *et al.* 2007), and most recently the GPR40 receptor (a putative FFA receptor; Steneberg *et al.* 2005). In our study, we observed a significant upregulation of genes involved in the β -oxidation of FFA (see Table 1) including CPT1 (~ 2.2 -fold), which is in accordance with the previous studies (Dubois *et al.* 2004). CPT1 represents a rate-limiting step for the import of long-chain fatty acids (LCFAs) into the mitochondria for β -oxidation. Activation of CPT1 may be seen either as a positive (McGarry & Dobbins 1999, Unger & Zhou 2001) or negative (Randle *et al.* 1963, Roduit *et al.* 2004) phenomenon for pancreatic β -cell function. When activated, CPT1 facilitates oxidation of LCFAs, which could prevent their accumulation as long-chain fatty acyl-CoA or other lipid metabolites in the cytosol and exert an antilipotoxic effect in β cells (Unger & Zhou 2001). Alternatively, increased oxidation of LCFAs by CPT1 activation could lead to the impairment of glucose metabolism through substrate competition, as postulated previously by the glucose-fatty acid cycle (Randle *et al.* 1963), and affect the ability of β cells to sense glucose and secrete insulin. Interestingly, in our experiments, OA oxidation was significantly increased ($\sim 30\%$) after exposure of human pancreatic islets to this fatty acid for 48 h. Even though this may have contributed to minimize the lipotoxic effect induced by chronic exposure to OA, it was not sufficient to prevent the impairment of GSIS induced by this fatty acid. In this context, it is important to consider that glucose is the most potent nutrient secretagogue for pancreatic β cells and its metabolism is essential for coupling to occur between glucose sensing and release of insulin by β cells (Roduit *et al.* 2004). It is widely accepted that an increase in the cytosolic ATP-to-ADP ratio, as a result of glucose metabolism, is an important event in this process. An increase in this ratio closes ATP-sensitive K^+ channels, causing depolarization of the plasma membrane. This, in turn, opens voltage-gated Ca^{2+} channels, causing influx of Ca^{2+} and exocytosis of insulin secretory granules (MacDonald *et al.* 2005). Previous studies have shown that lipotoxicity in human islets leads to selective loss of glucose responsiveness with no effect on insulin secretion in response to arginine (Lupi *et al.* 2002a). This suggests that FFA selectively impair the release of insulin mediated by glucose, possibly by interfering with its metabolism. In our study, we tested this hypothesis by measuring glucose oxidation in islets

pre-exposed to OA. We observed a marked reduction in the ability of islets to oxidize glucose at low (2.8 mol/l) and high (16.7 mmol/l) concentrations of this substrate, which is compatible with the inhibition of GSIS induced by OA. Our findings support the hypothesis that OA-induced inhibition of GSIS may be related, at least in part, to impaired glucose metabolism by substrate competition, which is a plausible mechanism by which OA caused the suppression of GSIS in our study.

There is also compelling evidence that an increase in UCP2 expression results in the suppression of GSIS, whereas the absence of UCP2 enhances GSIS (Chan *et al.* 2004). Upregulation of UCP2 expression inhibits ATP synthesis. FFA have been shown to increase UCP2 mRNA and protein levels in β cells. As a result, ATP synthesis is reduced and GSIS is blunted (Zhang *et al.* 2001). Furthermore, UCP2 knockout mice have lower fasting glucose levels and elevated circulating insulin levels when fed a high-fat diet compared with wild-type littermates (Joseph *et al.* 2002). Exposure of wild-type mouse islets to palmitate reduces GSIS, whereas UCP2^{-/-} islets show enhanced GSIS (Chan *et al.* 2004). Our study demonstrated an increase in mRNA levels of UCP2 in human islets following OA treatment by means of real-time PCR, which is consistent with the notion that UCP2 negatively correlates with GSIS.

Oxidative stress has been shown to decrease GSIS induced by prolonged exposure to fatty acids and glucose (Oprescu *et al.* 2007, Tang *et al.* 2007). The inability of β cells to respond to a glucose challenge in the presence of ROS might represent an adaptive response (also known as lipoadaptation) in an attempt to further limit ROS generation, thereby allowing the β cell to resist apoptosis. Here, we demonstrate for the first time a stimulatory effect of OA on ROS production in human islets, a finding that is in line with the previously published studies (Carlsson *et al.* 1999, Oprescu *et al.* 2007). Additionally, increased ROS may represent a potential source of pancreatic β -cell deregulation, given the low expression of antioxidant enzymes in islets (Robertson *et al.* 2005).

Several lines of evidence have established a link between inflammation and the development of obesity and T2D (Wellen & Hotamisligil 2005). In our analysis, several genes implicated in the generation of inflammation were upregulated. These include tumor necrosis factor receptor, fractalkine, and CRP suggesting that fatty acids could act via this pathway to accelerate deterioration of β -cell function. Additionally, one important study implicates fatty acids in the induction of inflammation using microarray analysis in the murine pancreatic β -cell line MIN6 (Busch *et al.* 2002). This study clearly demonstrates that palmitate and OA lead to an upregulation of transcripts involved in the inflammatory response with the former eliciting a more pronounced response than the latter.

In summary, our study of human islets chronically exposed to OA recapitulated some of the functional abnormalities observed in patients with T2D. These include: an impairment of GSIS, a reduction of total insulin content, and elevated

production of ROS. In addition, our study is the first to demonstrate a direct mechanism by which elevated FFA diminish GSIS in human islets. This mechanism entails increased FFA oxidation with a concomitant decrease in glucose oxidation. Finally, our results also suggest that chronic exposure to FFA treatment of human islets mediates alterations in genes implicated in inflammation and immunity. Taken together, our findings have identified several candidate molecules for further investigation into the mechanism of lipotoxicity in human pancreatic islets.

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References

- Bjorntorp P, Bergman H, Varnauskas E & Lindholm B 1969 Lipid mobilization in relation to body composition in man. *Metabolism* **18** 840–851.
- Boden G 1997 Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46** 3–10.
- Busch AK, Cordery D, Denyer GS & Biden TJ 2002 Expression profiling of palmitate- and oleate-regulated genes provides novel insights into the effects of chronic lipid exposure on pancreatic beta-cell function. *Diabetes* **51** 977–987.
- Carlsson C, Borg LA & Welsh N 1999 Sodium palmitate induces partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets *in vitro*. *Endocrinology* **140** 3422–3428.
- Chan CB, Saleh MC, Koshkin V & Wheeler MB 2004 Uncoupling protein 2 and islet function. *Diabetes* **53** (Suppl 1) S136–S142.
- Delarue J & Magnan C 2007 Free fatty acids and insulin resistance. *Current Opinion in Clinical Nutrition and Metabolic Care* **10** 142–148.
- Dubois M, Kerr-Conte J, Gmyr V, Bouckenoghe T, Muharram G, d'Herbomez M, Martin-Ponthieu A, Vantyghem MC, Vandewalle B & Pattou F 2004 Non-esterified fatty acids are deleterious for human pancreatic islet function at physiological glucose concentration. *Diabetologia* **47** 463–469.
- Gaidhu MP, Fediuc S & Ceddia RB 2006 5-Aminoimidazole-4-carboxamide-1- β -D-ribofuranoside-induced AMP-activated protein kinase phosphorylation inhibits basal and insulin-stimulated glucose uptake, lipid synthesis, and fatty acid oxidation in isolated rat adipocytes. *Journal of Biological Chemistry* **281** 25956–25964.
- Del Guerra S, Lupi R, Marselli L, Masini M, Bugliani M, Sbrana S, Torri S, Pollera M, Boggi U, Mosca F *et al.* 2005 Functional and molecular defects of pancreatic islets in human type 2 diabetes. *Diabetes* **54** 727–735.
- Haber EP, Ximenes HM, Procopio J, Carvalho CR, Curi R & Carpinelli AR 2003 Pleiotropic effects of fatty acids on pancreatic beta-cells. *Journal of Cell Physiology* **194** 1–12.

- Haber EP, Procopio J, Carvalho CR, Carpinelli AR, Newsholme P & Curi R 2006 New insights into fatty acid modulation of pancreatic beta-cell function. *International Review of Cytology* **248** 1–41.
- Haslam DW & James WP 2005 Obesity. *Lancet* **366** 1197–1209.
- Joseph JW, Koshkin V, Zhang CY, Wang J, Lowell BB, Chan CB & Wheeler MB 2002 Uncoupling protein 2 knockout mice have enhanced insulin secretory capacity after a high-fat diet. *Diabetes* **51** 3211–3219.
- Koshkin V, Wang X, Scherer PE, Chan CB & Wheeler MB 2003 Mitochondrial functional state in clonal pancreatic beta-cells exposed to free fatty acids. *Journal of Biological Chemistry* **278** 19709–19715.
- Koulouridis E 2004 Insulin and human obesity. *Pediatric Endocrinology Reviews* **1** (Suppl 3) 438–442.
- Lupi R, Del Guerra S, Fierabracci V, Marselli L, Novelli M, Patane G, Boggi U, Mosca F, Piro S, Del Prato S *et al.* 2002a Lipotoxicity in human pancreatic islets and the protective effect of metformin. *Diabetes* **51** (Suppl 1) S134–S137.
- Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C, Patane G, Boggi U, Piro S, Anello M *et al.* 2002b Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. *Diabetes* **51** 1437–1442.
- MacDonald PE, Joseph JW & Rorsman P 2005 Glucose-sensing mechanisms in pancreatic beta-cells. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **360** 2211–2225.
- Mason TM, Goh T, Tchiphashvili V, Sandhu H, Gupta N, Lewis GF & Giacca A 1999 Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose *in vivo* in rats. *Diabetes* **48** 524–530.
- McGarry JD & Dobbins RL 1999 Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* **42** 128–138.
- Muoio DM & Newgard CB 2006 Obesity-related derangements in metabolic regulation. *Annual Review of Biochemistry* **75** 367–401.
- Newsholme P, Haber E, Hirabara S, Rebelato E, Procopio J, Morgan D, Oliveira-Emilio H, Carpinelli A & Curi R 2007a Diabetes associated cell stress and dysfunction - role of mitochondrial and non-mitochondrial ROS production and activity. *Journal of Physiology* **583** 9–24.
- Newsholme P, Keane D, Welters HJ & Morgan NG 2007b Life and death decisions of the pancreatic beta-cell: the role of fatty acids. *Clinical Science* **112** 27–42.
- O’Gorman D, Kin T, Murdoch T, Richer B, McGhee-Wilson D, Ryan EA, Shapiro JA & Lakey JR 2005 The standardization of pancreatic donors for islet isolations. *Transplantation* **80** 801–806.
- Oprescu AI, Bikopoulos G, Naassan A, Allister EM, Tang C, Park E, Uchino H, Lewis GF, Fantus IG, Rozakis-Adcock M *et al.* 2007 Free fatty acid-induced reduction in glucose stimulated insulin secretion evidence for a role of oxidative stress *in vitro* and *in vivo*. *Diabetes* **56** 2927–2937.
- Paolisso G, Gambardella A, Amato L, Tortoriello R, D’Amore A, Varricchio M & D’Onofrio F 1995 Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. *Diabetologia* **38** 1295–1299.
- Patane G, Piro S, Rabuazzo AM, Anello M, Vigneri R & Purrello F 2000 Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. *Diabetes* **49** 735–740.
- Randle PJ, Garland PB, Hales CN & Newsholme EA 1963 The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* **1** 785–789.
- Rebuffe-Scrive M, Anderson B, Olbe L & Bjorntorp P 1990 Metabolism of adipose tissue in intraabdominal depots in severely obese men and women. *Metabolism* **39** 1021–1025.
- Richieri GV & Kleinfeld AM 1995 Unbound free fatty acid levels in human serum. *Journal of Lipid Research* **36** 229–240.
- Robertson RP, Harmon J, Tran PO & Poyntout V 2004 Beta-cell glucose toxicity, lipotoxicity, and chronic oxidative stress in type 2 diabetes. *Diabetes* **53** (Suppl 1) S119–S124.
- Robertson RP, Tanaka Y, Takahashi H, Tran PO & Harmon JS 2005 Prevention of oxidative stress by adenoviral overexpression of glutathione-related enzymes in pancreatic islets. *Annals of the New Academy of Sciences* **1043** 513–520.
- Roden M 2004 How free fatty acids inhibit glucose utilization in human skeletal muscle. *News in Physiological Sciences* **19** 92–96.
- Roduit R, Nolan C, Alarcon C, Moore P, Barbeau A, Delghingaro-Augusto V, Przybykowski E, Morin J, Masse F, Massie B *et al.* 2004 A role for the malonyl-CoA/long-chain acyl-CoA pathway of lipid signaling in the regulation of insulin secretion in response to both fuel and nonfuel stimuli. *Diabetes* **53** 1007–1019.
- Shoelson SE, Herrero L & Naaz A 2007 Obesity, inflammation, and insulin resistance. *Gastroenterology* **132** 2169–2180.
- Srivastava SK, Ramana KV & Bhatnagar A 2005 Role of aldose reductase and oxidative damage in diabetes and the consequent potential for therapeutic options. *Endocrine Reviews* **26** 380–392.
- Steneberg P, Rubins N, Bartoov-Shifman R, Walker MD & Edlund H 2005 The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse. *Cell Metabolism* **1** 245–258.
- Stoughton RB 2005 Applications of DNA microarrays in biology. *Annual Review of Biochemistry* **74** 53–82.
- Stumvoll M, Goldstein BJ & van Haefen TW 2005 Type 2 diabetes: principles of pathogenesis and therapy. *Lancet* **365** 1333–1346.
- Tang C, Han P, Oprescu AI, Lee SC, Gyulkhandanyan AV, Chan GN, Wheeler MB & Giacca A 2007 Evidence for a role of superoxide generation in glucose-induced beta-cell dysfunction *in vivo*. *Diabetes* **56** 2722–2731.
- Unger RH & Zhou YT 2001 Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* **50** (Suppl 1) S118–S121.
- Wang X, Li H, De Leo D, Guo W, Koshkin V, Fantus IG, Giacca A, Chan CB, Der S & Wheeler MB 2004 Gene and protein kinase expression profiling of reactive oxygen species-associated lipotoxicity in the pancreatic beta-cell line MIN6. *Diabetes* **53** 129–140.
- Wellen KE & Hotamisligil GS 2005 Inflammation, stress, and diabetes. *Journal of Clinical Investigation* **115** 1111–1119.
- Xiao J, Gregersen S, Kruhoffer M, Pedersen SB, Orntoft TF & Hermansen K 2001 The effect of chronic exposure to fatty acids on gene expression in clonal insulin-producing cells: studies using high density oligonucleotide microarray. *Endocrinology* **142** 4777–4784.
- Zhang CY, Baffy G, Perret P, Krauss S, Peroni O, Grujic D, Hagen T, Vidal-Puig AJ, Boss O, Kim YB *et al.* 2001 Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes. *Cell* **105** 745–755.
- Zhou YP & Grill V 1995 Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. *Journal of Clinical Endocrinology and Metabolism* **80** 1584–1590.

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