Global expression profiling of glucose-regulated genes in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats

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Abstract

The spontaneously diabetic Goto-Kakizaki (GK) rat is frequently used as a model for human type 2 diabetes. Selective loss of glucose-sensitive insulin secretion is an early pathogenetic event in human type 2 diabetes, and such a defect also typifies islets from the GK rat. We investigated whether expression of specific glucose-regulated genes is disturbed in islets from GK rats when compared with Wistar rats. Large-scale gene expression analysis using Affymetrix microarrays and qRT-PCR measurements of mRNA species from normal and diabetic islets were performed after 48 h of culture at 3 or 20 mM glucose. Of the 2020 transcripts differentially regulated in diabetic GK islets when compared with controls, 1033 were up-regulated and 987 were down-regulated. We identified significant changes in islet mRNAs involved in glucose sensing, phosphorylation, incretin action, glucocorticoid handling, ion transport, mitogenesis, and apoptosis that clearly distinguish diabetic animals from controls. Such markers may provide clues to the pathogenesis of human type 2 diabetes and may be of predictive and therapeutic value in clinical settings in efforts aiming at conferring β-cell protection against apoptosis, impaired regenerative capacity and functional suppression occurring in diabetes.

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Introduction

In human type 2 diabetes, loss of glucose-sensitive insulin secretion is an early pathogenetic event (Malaisse 1994, Zimmet et al. 2001). The pancreatic islet β-cell serves as a unique fuel-sensing organ and is the sole producer of insulin, the key hormone in maintenance of normoglycemia, whose exocytosis is tightly controlled by the ambient plasma glucose concentrations (MacDonald 1990, Sjoholm 1998). In contrast to most other cells, in which signaling is controlled through plasma membrane receptor-operated processes, the β-cell senses subtle fluctuations in plasma glucose concentration and translates these into finely tuned changes in insulin exocytosis (MacDonald 1990, Sjoholm 1998).

As the lesions intrinsic to the β-cell causing the selective loss of glucose sensitivity remain elusive, the aim of this study was to investigate whether expression of specific genes regulated by glucose is disturbed in islets from spontaneously diabetic Goto-Kakizaki (GK) rats when compared with normoglycemic Wistar rats. The GK rat is a non-obese animal model for type 2 diabetes (Goto et al. 1976, 1988). This widely employed model exhibits diabetes-related phenotypes such as hyperglycemia, glucose intolerance, insulin resistance, and a deficient insulin response to glucose in vivo and invitro. There seems to be a selective loss of glucose-sensitive insulin secretion (Kimura et al. 1982, Goto et al. 1988, Portha et al. 1991, Ostenson et al. 1993, Abdel-Halim et al. 1994) due to multiple defects in the insulin stimulus-secretion coupling. (For a review, see Portha 2005.)

Our results reveal substantial qualitative and quantitative differences in glucose-regulated islet gene expression between healthy and diabetic rats that may have implications for our understanding of the etiology and treatment of human type 2 diabetes.

Materials and methods

Materials

Collagenase A was obtained from Roche. Culture medium RPMI-1640, fetal calf serum, t-glutamine, benzylpenicillin, and streptomycin were from Flow Laboratories (Irvine, UK). Rat insulin ELISA kit was from Mercodia (Uppsala, Sweden).

Islet preparation and insulin secretion

Pancreatic islets were isolated by collagenase digestion (Sandler et al. 1987) from male diabetic GK and control Wistar rats, ~3 months old, purchased from Taconic Europe (barrier EBU 202, Bomholt site, Ry, Denmark).
The GK inbred model was developed by Tohoku University in 1975. Aarhus University Hospital in Denmark received stock in 1994. M&B A/S (now Taconic Europe) received stock from Aarhus in 1997. Glucose at 3 mM was chosen as a low-glucose comparator, non-stimulatory to both Wistar and GK islets, used in studying high glucose effects on islet gene expression in both rat strains. At the end of the culture period, batches of 500 islets were transferred to Eppendorf tubes with 0.5 ml Trizol (Invitrogen), in which the islets were homogenized and then snap-frozen in liquid nitrogen.

Insulin secretion and islet insulin content were measured as described (Sandler et al. 1987).

### Monitoring of glycemia

Non-fasting glucose levels were monitored in intracardiac blood in normal Wistar and diabetic GK rats by measurement of glucose concentration using a glucose meter (Ascensia Contour, Bayer HealthCare), immediately after killing. The test principle used by this device is electrochemical biosensor technology using glucose oxidase. The strip uses the enzyme glucose oxidase to produce an electrical current that will stimulate a chemical reaction.

### Isolation of islet RNA

Total RNA was isolated from islet tissue that was stored in Trizol at -70°C using standard techniques. Briefly, tissues were homogenized, chloroform was added and the tubes were vigorously mixed for 15 s, and then allowed to sit for 2–3 min at room temperature before the tubes were centrifuged at 11 900 g for 15 min at 4°C. The upper aqueous layer was removed to a new tube and isopropanol alcohol was added, the tubes were mixed, incubated at room temperature for 10 min, and centrifuged at 11 900 g for 10 min at 4°C. The pellets were washed with 75% ethanol and centrifuged at 7500 g for 5 min at 4°C. The pellets were dried for 10 min, 100 μl RNase free water was added and incubated at 50°C for 10 min. Isolated total RNA was further purified using an RNeasy minicolumn (Qiagen 74104) by suspending the RNA pellet in 350 μl buffer RLT with 2-mercaptoethanol, followed by addition of 250 μl 100% ethanol. The samples were applied to the column and centrifuged for 15 s at ≥8000 g with this step repeated with the flow-through. The column was washed twice with 500 μl buffer RPE centrifugation, and then dried by centrifugation for 2 min at maximum speed. The bound RNA was eluted using 30 μl of 65°C RNase-free water and centrifugation.

### Genome wide RNA transcript profiling

The integrity of total RNA was confirmed by a microfluidics lab-on-a-chip analysis using a RNA 6000 Nano LabChip device and an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). The GeneChip Rat Expression Array 230A (Affymetrix Inc., Santa Clara, CA, USA) was used, which contains 15 866 different probe sets interrogating primarily annotated genes. Processing of total RNA samples and GeneChip experiments were carried out essentially as recommended by Affymetrix. Ten micrograms of labeled cRNA was hybridized for ~16 h at 45°C to an expression probe array. The array was then washed, stained with streptavidin-R-phycocerythrin (SAPE, Molecular Probes; Eugene, OR, USA) and the signal amplified using a biotinylated goat anti-streptavidin antibody (Vector Laboratories, Burlingame, CA, USA) followed by a final staining with SAPE. Liquid handling steps were performed using Affymetrix GeneChip Fluidics Workstation 400. The array was then scanned twice using a confocal laser scanner (GeneArray Scanner 2500; Agilent Technologies) at an excitation wavelength of 488 nm and emission recorded at 570 nm, resulting in a scanned image. The scanned image was converted into numerical values of the signal intensity (Signal) and categorical expression level measurement (Absolute Call) using the Affymetrix Microarray Analysis Suite 5.0 software (MAS 5.0; Affymetrix Inc., Santa Clara, CA, USA). The software scaled the average intensity of all genes of each chip to a target intensity arbitrarily set at 150 units.

The quality control data for all microarrays used yielded values that were considered acceptable. High similarity of the scaling factors applied to each individual GeneChip array served as a first indicator of an optimal GeneChip experiment. Second, a background average value of ~65 indicated low unspecific binding. Third, a 3′ to 5′ signal ratio less or equal to 3 of the glyceraldehyde dehydrogenase gene or the β-actin gene indicated that the starting RNA had not undergone degradation and that the cDNA synthesis was optimal. Finally, the number of genes called ‘present’ was characteristic for the given tissue thus indicating an optimal recovery of the contained information.

To determine which genes were glucose responsive, genes were considered induced or suppressed if the average fold change was greater or equal to 1.1. The differentially regulated transcripts were annotated and then clustered according to functional annotation. A choice of twofold change limit of significance for the expressed transcripts could underestimate, and thereby miss, the true impact of small but biologically relevant and qualitatively important gene expression changes (Yao et al. 2004, Choe et al. 2005). Also, a twofold change, commonly used in the literature, is dubious to
Table 1 Glycemia in non-diabetic Wistar rats and diabetic GK rats

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic Wistar rats</td>
<td>8.4±0.3</td>
</tr>
<tr>
<td>Diabetic GK rats</td>
<td>17.2±3.1*</td>
</tr>
</tbody>
</table>

Non-fasting glucose levels were measured in intracardiac blood by glucose oxidase immediately after killing. Values are means±S.E.M. of six experiments. *Denotes P<0.05 for a chance difference versus non-diabetic Wistar rats using Student’s t-test.

generate optimal results, taking into account that a factor of 2 can have different significance influenced by expression levels (Baldi & Long 2001, Mutch et al. 2002). Nevertheless, by including a quite large number of arrays (6 and 9) we have reduced random errors when compared with similar experiments, in which fewer numbers of samples have been used. Cluster analyses were performed as described in GeneSpring (Agilent Technologies), NetAffx (Affymetrix) and other databases.

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), 10 mM deoxynucleoside triphosphate, 1 μl random hexamer primers (100 pmol/μl), 1.5 μl dithiothreitol (Amersham Pharmacia Biotech; 100 mmol/l), and 1 μl RT1 reverse transcriptase (200 U/μl; Invitrogen). 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 RT-PCR

Expression of selected genes (Table 13) was performed from total RNA using an ABI Prism 9400 PCR machine (PE Applied Biosystems, Foster City, CA, USA). These genes were selected based on fold change in expression, GO results, and/or potential roles in diabetes. The amount was adjusted to the endogenous reference gene β-actin. The selected genes and β-actin primers and hybridization probes (Perkin–Elmer Biosystems, Warrington, UK) were used. Probes were labeled at the 5′ end with the reporter dye molecule 6-carboxy-fluorescein and at the 3′ end with the quencher dye molecule 6-carboxytetramethylrhodamine. Results are expressed relative to β-actin with and the ratio of different groups (WH/WL, growth hormone/GL, and GL/WL). Therefore, the final value indicates an increase or decrease in mRNA for selected genes.

Statistical analysis

Results presented are derived from islets of individual rats, isolated on different days, unless otherwise stated. Means±S.E.M. were calculated and groups of data were compared using Student’s t-test for paired or unpaired data. Differences were considered statistically significant when P<0.05.

Results

Diabetic phenotype in GK rats

Non-fasting glucose levels monitored in intracardiac blood in normal Wistar and diabetic GK rats, as expected, were significantly higher in diabetic GK rats relative to normal Wistar rats (Table 1).

Loss of glucose-sensitive insulin secretion in islets from GK rats

As shown in Table 2, insulin secretion at a high glucose concentration (20 mM), relative to 3 mM glucose, was stimulated 4.8-fold in non-diabetic Wistar rat islets(P<0.001) but was not significantly augmented in islets from diabetic GK rats. In contrast, the islet insulin content was 40% higher in GK rats than in normal Wistar rats. This has been previously reported in GK islets after culture in widely different glucose concentrations (Metz et al. 1999).

Table 2 Glucose-sensitive insulin secretion in islets from non-diabetic Wistar rats and diabetic GK rats

<table>
<thead>
<tr>
<th></th>
<th>Insulin secretion (ng/islet per 60 min)</th>
<th>Insulin secretion (ng/islet per 60 min)</th>
<th>Islet insulin content (ng/islet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 mM glucose</td>
<td>20 mM glucose</td>
<td>Islet insulin content (ng/islet)</td>
</tr>
<tr>
<td>Non-diabetic Wistar rats</td>
<td>3±56 ±0.4</td>
<td>26±4 ±2.8</td>
<td>66±6 ±3.6</td>
</tr>
<tr>
<td>Diabetic GK rats</td>
<td>6±67 ±0.6</td>
<td>7±8 ±0.5†</td>
<td>93±2 ±2.1†</td>
</tr>
</tbody>
</table>

Isolated islets were preincubated for 45 min at 3 mM glucose and then exposed for 60 min to the indicated glucose concentrations. Insulin in incubation buffers and islet insulin contents were measured by ELISA. Values are means±S.E.M. of six experiments. *Denotes P<0.001 for a chance difference versus Wistar islets in low glucose. †Denotes P<0.001 for a chance difference versus Wistar islets in high glucose, ‡ denotes P<0.001 for a chance difference versus Wistar islets, using Student’s t-test.

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Microarray analysis of global glucose-regulated gene expression changes in islets from normal Wistar and diabetic GK rats

To gain insight into the molecular events underlying the selective loss of glucose-sensitive insulin secretion in islets from GK rats, we performed genome-wide oligonucleotide microarray analyses of glucose-stimulated islets from normal Wistar and diabetic GK rats. Of the 15,866 transcripts analyzed by microarray, 2020 were differentially expressed by comparing samples prepared from diabetic GK rat islets and normal Wistar rats, cultured in either 3 mM or 20 mM glucose. The functional grouping of the differentially expressed transcripts shows that 1033 of these 2020 transcripts were up-regulated and 987 were down-regulated. A great proportion of these glucose responsive genes and expressed sequence tags are involved in metabolism, signaling, transport, apoptosis, transcription, proliferation, and immune response (Fig. 1). The experimental setup of the current work, two days in vitro culture, was deliberately designed in an attempt to minimize remaining influence of in vivo glucotoxicity or other manifestations of the diabetic state on islet function. However, we cannot completely exclude the possibility of remaining minor influences of in vivo glucotoxicity.

Figure 1 (A) Functional clusters of genes up-regulated by high glucose in diabetic GK rats vs. non-diabetic Wistar rats. (B) Functional clusters of genes up-regulated by low glucose in diabetic GK rats versus non-diabetic Wistar rats. (C) Functional clusters of genes down-regulated by high glucose in diabetic GK rats versus non-diabetic Wistar rats. (D) Functional clusters of genes down-regulated by low glucose in diabetic GK rats versus non-diabetic Wistar rats.
or other manifestations of the diabetic state on islet gene expression.

Detailed descriptions of a selection of the differentially expressed transcripts, classified according to function and average fold change, are listed in Tables 3–12.

### Differential expression of a subset of genes confirmed with qRT-PCR

In order to validate the qualitative changes in gene expression revealed by the microarray analyses, the expression of selected genes was also confirmed by real-time qRT-PCR. The same expression pattern as that identified by the microarray analyses was found by real-time qRT-PCR for 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) (NM_017080), serum- and glucocorticoid-inducible kinase 1 (SGK-1) (NM_019232), insulin-like growth factor binding protein 3 (IGFBP-3) (NM_012588), and ApoE (J02582; Table 13), whose expression levels were all significantly changed (P<0.05). Insulin-2 was not captured as differentially expressed in the microarray analyses, using the selected cut off values, but was significantly more up-regulated by high glucose in islets from normal Wistar than from diabetic GK rats in the qRT-PCR analysis. Microarrays seem to offer a smaller dynamic range when compared with other platforms. This means that changes observed in other platforms may not be accurately reflected by microarray expression levels. The poor correlation between microarray and qRT-PCR may be due to the inherent sensitivity limits of a PCR-based approach (qRT-PCR) in comparison with a hybridization-based approach.

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Gene name</th>
<th>Function</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB052294</td>
<td>ATP-binding cassette, sub-family C (CFTR/MRP), member 8 (Abcc8)</td>
<td>Transport</td>
<td>1.5</td>
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<tr>
<td>U44897</td>
<td>Potassium inwardly rectifying channel, subfamily J, member 11 (Kcnj11)</td>
<td>Transport</td>
<td>1.5</td>
</tr>
<tr>
<td>NM_053685</td>
<td>Hyperpolarization-activated cyclic nucleotide-gated potassium channel 3 (Hcn3)</td>
<td>Transport</td>
<td>1.4</td>
</tr>
<tr>
<td>NM_013186</td>
<td>Potassium voltage gated channel, Shaw-related subfamily, member 1 (Kcnb1)</td>
<td>Transport</td>
<td>1.8</td>
</tr>
<tr>
<td>X92069</td>
<td>Purinergic receptor P2X, ligand-gated ion channel, 5 (P2x5)</td>
<td>Transport</td>
<td>1.6</td>
</tr>
<tr>
<td>AA685184</td>
<td>Sodium channel, voltage-gated, type III, beta (Scn3b)</td>
<td>Transport</td>
<td>1.5</td>
</tr>
<tr>
<td>AW532988</td>
<td>Hyperpolarization activated cyclic nucleotide-gated potassium channel 2 (Hcn2)</td>
<td>Transport</td>
<td>1.4</td>
</tr>
<tr>
<td>NM_012878</td>
<td>Surfactant associated protein D (Sftpd)</td>
<td>Transport</td>
<td>1.5</td>
</tr>
<tr>
<td>AI547447</td>
<td>Potassium voltage gated channel, Shaw-related subfamily, member 3 (Kcn3c)</td>
<td>Transport</td>
<td>1.8</td>
</tr>
<tr>
<td>NM_012663</td>
<td>Vesicle-associated membrane protein 2 (Vamp2)</td>
<td>Transport</td>
<td>1.6</td>
</tr>
<tr>
<td>M83681</td>
<td>RAB3D, member RAS oncogene family (Rab3d)</td>
<td>Transport</td>
<td>1.6</td>
</tr>
<tr>
<td>AA945569</td>
<td>Synaptotagmin 1 (Syt1)</td>
<td>Transport</td>
<td>1.3</td>
</tr>
<tr>
<td>NM_031344</td>
<td>Fatty acid desaturase 2 (Fads2)</td>
<td>Metabolism</td>
<td>1.4</td>
</tr>
<tr>
<td>AA848820</td>
<td>15-hydroxyprostaglandin dehydrogenase (Hpgd)</td>
<td>Metabolism</td>
<td>1.7</td>
</tr>
<tr>
<td>BI277460</td>
<td>Phosphoenolpyruvate carboxykinase 1 (Pck1)</td>
<td>Metabolism</td>
<td>1.4</td>
</tr>
<tr>
<td>Y11321</td>
<td>Forkhead box E1 (thyroid transcription factor 2) (Foxe1)</td>
<td>Metabolism</td>
<td>1.5</td>
</tr>
<tr>
<td>L23863</td>
<td>POU domain, class 2, transcription factor 3 (Pou2f3)</td>
<td>Metabolism</td>
<td>1.5</td>
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<tr>
<td>AI639457</td>
<td>GTP cyclohydrolase 1 (Gch)</td>
<td>Metabolism</td>
<td>1.5</td>
</tr>
<tr>
<td>AW143805</td>
<td>Guanine nucleotide binding protein, alpha q polypeptide (Gnaq)</td>
<td>Metabolism</td>
<td>1.4</td>
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<tr>
<td>AW533214</td>
<td>Rabaptin 5 (Rabep1)</td>
<td>Metabolism</td>
<td>1.3</td>
</tr>
<tr>
<td>NM_012714</td>
<td>Gastric inhibitory polypeptide receptor (Gipr)</td>
<td>Signal transduction</td>
<td>2.3</td>
</tr>
<tr>
<td>NM_053777</td>
<td>Mitogen activated protein kinase kinase 6 interacting protein (Mapk8ip)</td>
<td>Signal transduction</td>
<td>1.2</td>
</tr>
<tr>
<td>X92069</td>
<td>Purinergic receptor P2X, ligand-gated ion channel, 5 (P2x5)</td>
<td>Signal transduction</td>
<td>1.6</td>
</tr>
<tr>
<td>AI234096</td>
<td>Protein kinase, cAMP dependent regulatory, type I, alpha (Prkar1a)</td>
<td>Signal transduction</td>
<td>1.1</td>
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<tr>
<td>AA799421</td>
<td>Protein kinase C, epsilon (Prkce)</td>
<td>Signal transduction</td>
<td>1.2</td>
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<tr>
<td>NM_053856</td>
<td>Secretogranin III (Scg3)</td>
<td>Signal transduction</td>
<td>1.7</td>
</tr>
<tr>
<td>NM_133522</td>
<td>Somatostatin receptor 3</td>
<td>Signal transduction</td>
<td>1.3</td>
</tr>
<tr>
<td>NM_019128</td>
<td>Internexin, alpha (Inexa)</td>
<td>Development</td>
<td>1.9</td>
</tr>
<tr>
<td>NM_012630</td>
<td>Prolactin receptor (Prl)</td>
<td>Development</td>
<td>1.3</td>
</tr>
<tr>
<td>AW143798</td>
<td>Cyclin D1 (Cnd1)</td>
<td>Development</td>
<td>1.2</td>
</tr>
<tr>
<td>AF106659</td>
<td>Ubiquitin specific protease 2 (Usp2)</td>
<td>Catalytic activity</td>
<td>1.3</td>
</tr>
<tr>
<td>L07578</td>
<td>Casein kinase 1, delta (Csnk1d)</td>
<td>Catalytic activity</td>
<td>1.4</td>
</tr>
<tr>
<td>NM_019349</td>
<td>Serine/threonine kinase 2 (Slik)</td>
<td>Catalytic activity</td>
<td>1.5</td>
</tr>
</tbody>
</table>
(microarray). Also, differences in sequence, their placement along the target transcript and feature design may all contribute to discrepancies in results obtained by the two methods used. All these reasons could explain why the insulin-2 gene was not captured as differently expressed in the microarray data but in qRT-PCR analysis.

**Discussion**

In human type 2 diabetes, loss of glucose-sensitive insulin secretion is an important pathogenetic event (Malaisse 1994, Zimmet et al. 2001). The change in β-cell phenotype involves a selective loss of glucose-stimulated insulin secretion even in the very earliest stages of disease progression. The molecular basis for this functional defect remains elusive, but also constitutes an inviting target for attempts to intervene against β-cell failure and outbreak of type 2 diabetes. In the present work, we have attempted to identify such targets using genome-wide transcription profiling of glucose-regulated genes in isolated pancreatic islets from normal Wistar rats and diabetic GK rats. In this substrain of GK rats of this age, β-cell density and relative volume of islet endocrine cells are not different from age- and sex-matched Wistar rats (Ohneda et al. 1993, Guenifi et al. 1995). Our results reveal substantial qualitative and quantitative differences in glucose-regulated islet gene expression between healthy and diabetic rats that may have implications for our understanding of the etiology of human type 2 diabetes.

Key elements in steroid metabolism were abnormally expressed in diabetic GK islets (Table 7); e.g. 11β-HSD-1 (NM_017080; Davani et al. 2000, Duplomb et al. 2004, Ortsater et al. 2005) and SGK-1 (NM_019232; Ullrich et al. 2005) which are important effectors of glucocorticoid handling in the β-cell and known to influence glucose-sensitive insulin secretion. In rodents, 11β-HSD-1 (NM_017080) converts inactive 11-dehydrocorticosterone into active corticosterone. The mRNA and activity of 11β-HSD-1 (NM_017080) have been shown to be up-regulated in islets from hyperglycemic mice (Davani et al. 2000, Ortsater et al. 2005) and the Zucker Diabetic Fatty rat (Duplomb et al. 2004) when compared with their normoglycemic counterparts. The β cell may be extra susceptible to glucocorticoid excess, since both iatrogenic Cushing syndrome and steroid-induced diabetes in animal

### Table 4 Islet transcripts down-regulated by high versus low glucose in diabetic GK rats

<table>
<thead>
<tr>
<th>Accession no.</th>
<th>Gene name</th>
<th>Function</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_019370</td>
<td>Ectonucleotide pyrophosphatase/phosphodiesterase 3 (Enpp3)</td>
<td>Metabolism</td>
<td>1.6</td>
</tr>
<tr>
<td>NM_132398</td>
<td>Glycoprotein (transmembrane) nmb (Gpnmmb)</td>
<td>Metabolism</td>
<td>1.6</td>
</tr>
<tr>
<td>NM_012907</td>
<td>Apolipoprotein B editing complex 1 (Apoeb1)</td>
<td>Metabolism</td>
<td>2.5</td>
</tr>
<tr>
<td>NM_053963</td>
<td>Matrix metallopeptidase 12 (Mmp12)</td>
<td>Metabolism</td>
<td>2.5</td>
</tr>
<tr>
<td>AA849399</td>
<td>Cathepsin Z (Ctaz)</td>
<td>Metabolism</td>
<td>2</td>
</tr>
<tr>
<td>NM_017320</td>
<td>Cathepsin S (cathepsin S)</td>
<td>Metabolism</td>
<td>2.5</td>
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<tr>
<td>L12458</td>
<td>Lysozyme (Lyz)</td>
<td>Metabolism</td>
<td>2.5</td>
</tr>
<tr>
<td>NM_019232</td>
<td>Serum- and glucocorticoid-inducible kinase 1 (Sgk)</td>
<td>Apoptosis</td>
<td>1.6</td>
</tr>
<tr>
<td>NM_133416</td>
<td>B-cell leukemia/lymphoma 2 related protein A1 (Bcl2a1)</td>
<td>Apoptosis</td>
<td>2</td>
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<tr>
<td>NM_012580</td>
<td>Heme oxygenase (decycling) 1 (Hmox1)</td>
<td>Apoptosis</td>
<td>1.3</td>
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<tr>
<td>J02582</td>
<td>Apolipoprotein E (Apoe)</td>
<td>Apoptosis</td>
<td>2</td>
</tr>
<tr>
<td>BG666928</td>
<td>BH3 interacting domain death agonist (Bid)</td>
<td>Apoptosis</td>
<td>1.3</td>
</tr>
<tr>
<td>AF279911</td>
<td>Bcl2-associated death promoter (Bad)</td>
<td>Apoptosis</td>
<td>1.1</td>
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<td>NM_133307</td>
<td>Protein kinase C, delta (Prkcd)</td>
<td>Apoptosis</td>
<td>1.3</td>
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<tr>
<td>NM_053619</td>
<td>Complement component 5, receptor 1 (C5r1)</td>
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<td>AI003808</td>
<td>Ectonucleoside triphosphate diphosphohydrolase 1 (Entpd1)</td>
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<td>NM_013185</td>
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<td>NM_130421</td>
<td>Lymphocyte cytosolic protein 2 (Lcp2)</td>
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<td>AI178808</td>
<td>Interleukin 2 receptor, gamma (severe combined immuno-deficiency) (Il2rg)</td>
<td>Signal transduction</td>
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models are associated with loss of glucose-stimulated insulin secretion, and steroid immunosuppressive treatment adversely affects islet transplantation outcome (Shapiro et al. 2000). Glucocorticoids decrease insulin gene expression (Philippe et al. 1992), increase glucose-6-phosphatase, phosphoenolpyruvate carboxykinase (PEPCK) activity and glucose cycling (Ling et al. 1998, Davani et al. 2004), and induce β-cell apoptosis (Weinhaus et al. 2000), all events occurring in GK rat islets (Portha et al. 2000). Glucocorticoids decrease insulin secretion normally evoked by dexamethasone negatively, since the suppression of glucose-sensitive insulin secretion is instrumental in directly regulating insulin secretion 

β-cell susceptibility to glucocorticoids may also be relevant in the natural unfolding of diabetes, since mice overexpressing the glucocorticoid receptor restricted to the β-cell develop early β-cell failure, glucose intolerance and later in life overt diabetes (Ling et al. 1998, Davani et al. 2004). Humans with impaired β-cell function (low insulin responders) are predisposed to become overly diabetic during glucocorticoid therapy (Wajngot et al. 1992).

Another gene involved in transducing glucocorticoid effects, which was found to be abnormally regulated by glucocorticoids, is SGK-1 (NM_019232; Table 7). SGK-1, whose expression is increased by glucocorticoids (Ullrich et al. 2005), appears to be instrumental in directly regulating insulin secretion negatively, since the suppression of glucose-sensitive insulin secretion normally evoked by dexamethasone was lost in islets from SGK-1 (NM_019232) knockout mice (Ullrich et al. 2005). In islets, SGK-1 (NM_019232) up-regulates the activity of voltage-activated K⁺ channels (Kᵥ) thereby reducing Ca²⁺ entry and insulin secretion (Ullrich et al. 2005). Since intracellular Ca²⁺ handling appears intrinsically disturbed in GK rat islets (Kato et al. 1996, Varadi et al. 1996), and can be deranged by glucocorticoid treatment (Lambillotte et al. 1997), it seems that SGK-1 (NM_019232) also may be of both pathogenetic and therapeutic interest in type 2 diabetes and steroid diabetes.

As for the differential expression of Kᵥ in our present study, it was found that the delayed rectifier Kᵥ2.1 (Kcnb1; NM_013186) subtype was preferentially up-regulated by high glucose in islets from diabetic GK rats (Table 3). Since this is the dominant Kᵥ in rodent and human islets, contributing to some 85% of their steady-state outward current, it negatively regulates Ca²⁺ dynamics and insulin secretion (MacDonald & Wheeler 2003). Hence, the up-regulation of Kᵥ2.1 in diabetic GK islets, is SGK-1 (NM_019232; Table 7). SGK-1, whose expression is increased by glucocorticoids (Ullrich et al. 2005), appears to be instrumental in directly regulating insulin secretion negatively, since the suppression of glucose-sensitive insulin secretion normally evoked by dexamethasone was lost in islets from SGK-1 (NM_019232) knockout mice (Ullrich et al. 2005). In islets, SGK-1 (NM_019232) up-regulates the activity of voltage-activated K⁺ channels (Kᵥ) thereby reducing Ca²⁺ entry and insulin secretion (Ullrich et al. 2005). Since intracellular Ca²⁺ handling appears intrinsically disturbed in GK rat islets (Kato et al. 1996, Varadi et al. 1996), and can be deranged by glucocorticoid treatment (Lambillotte et al. 1997), it seems that SGK-1 (NM_019232) also may be of both pathogenetic and therapeutic interest in type 2 diabetes and steroid diabetes.

As for the differential expression of Kᵥ in our present study, it was found that the delayed rectifier Kᵥ2.1 (Kcnb1; NM_013186) subtype was preferentially up-regulated by high glucose in islets from diabetic GK rats (Table 3). Since this is the dominant Kᵥ in rodent and human islets, contributing to some 85% of their steady-state outward current, it negatively regulates Ca²⁺ dynamics and insulin secretion (MacDonald & Wheeler 2003). Hence, the up-regulation of Kᵥ2.1
in GK islets is likely to contribute to a more hyperpolarized state of the diabetic β-cells, thereby suppressing glucose-stimulated insulin secretion. Since this is exactly what typifies the GK rat islets (Table 2; Hughes et al. 1998), it is possible that Kv2.1 overexpression is an intrinsic β-cell defect that contributes to its loss of glucose sensitivity in the GK rat. To our knowledge, this is the first report describing Kv2.1 (Kcnb1; NM_013186) overexpression in diabetic islets and makes Kv2.1 an attractive target for antidiabetic drugs.

Among other ion transporters of strong pathophysiological and therapeutic interest that were aberrantly expressed in GK rat islets, were the sulfonylurea receptor (SUR1/Abcc8 (AB052294)) and its associated inwardly rectifying ATP-sensitive K⁺ channel (Kir6.2/Kcnj11 (U44897)). Both were found to be up-regulated by high glucose in GK rat islets (Table 3) but not in Wistar, again features that would be expected to contribute to attenuation of glucose-sensitive insulin secretion in GK rat islets. Consistent with such a scenario are also previous reports describing that nonsense mutations in the SUR1/Abcc8 (AB052294) or Kir6.2/Kcnj11 (U44897) genes result in hyperinsulinism and hypoglycemia due to unregulated over-secretion of insulin (Nestorowicz et al. 1997, Verkarre et al. 1998).

Among other genes dysregulated in GK islets, we found up-regulation of the membrane receptor CD36 (NM_031561; Table 11). This facilitates the major fraction of long-chain FA uptake in a variety of tissues, including the β-cell (Noushmehr et al. 2005). In human β-cells, CD36 was found to specifically mediate the inhibitory effects of FA on insulin secretion (Noushmehr et al. 2005). Interestingly, CD36 (NM_031561) was also recently found to be up-regulated by high glucose and associated with accelerated atherosclerosis in humans (Griffin et al. 2001) thus forming a possible link between diabetes and atherosclerosis. CD36 (NM_031561) functions as a transporter for oxidized lipids in GK islets.

### Table 6 Islet transcripts down-regulated by high glucose in diabetic GK rats versus high glucose in non-diabetic Wistar rats

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<td>Liver glycogen phosphorylase (Pygl)</td>
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<td>Hair and enhancer of split 2 (Drosophila) (Hes2)</td>
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LDL-cholesterol (Okajima et al. 2005). These findings, in conjunction with previous reports showing that oxidized LDL-cholesterol suppresses insulin gene transcription and promotes β-cell death (Cnop et al. 2002, Okajima et al. 2005), raises the possibility that CD36 (NM_031561) up-regulation might be involved in β-cell dysfunction. Although GK rats are not hyperlipidemic (Zhou et al. 1995), the increased CD36 (NM_031561) expression may promote functional suppression and possibly lipoapoptosis as part of glucolipotoxicity (Koyama et al. 1998, Prentki et al. 2002). In accordance with such a scenario, previous reports indicate that endogenous FA catabolism is greater in GK islets than in Wistar islets (Sener et al. 1993), and that high-fat feeding impedes glucose-sensitive insulin secretion in islets from GK rats while not affecting Wistar islets (Briaud et al. 2002).

Several genes regulating glucose metabolism were also found to be dysregulated in GK rat islets. In Wistar islets, several transcripts encoding glycolytic enzymes were up-regulated by glucose (Table 9), thus confirming findings in islet cell lines (Roche et al. 1997) indicating an intact islet adaptive response to hyperglycemia. In contrast, these glycolytic genes were down-regulated by high glucose in diabetic islets (Table 6), suggesting impaired glycolytic signaling that may contribute to β-cell failure in GK rats (Mertz et al. 1996). These genes were also found to be down-regulated in islets from patients with type 2 diabetes (Gunton et al. 2005). Impaired glucose metabolism might negatively impact other islet functions than solely secretion, such as insulin gene expression and β-cell proliferation, both events strongly induced by glucose (Nielsen et al. 1985, Sjoholm 1997).

## Table 7

<table>
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<th>Function</th>
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<td>V-maf musculoaponeurotic fibrosarcoma oncogene family, protein G (avian; Mafg)</td>
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these islets (Ostenson et al. 1993). Interestingly, PEPCK/PCK1 (BI277460) was recently proposed as a candidate gene for human type 2 diabetes (Cao et al. 2004).

Several important effectors of protein phosphorylation and exocytosis were also dysregulated in GK islets. The AMP-activated protein kinase (AMPK; Prkab2, Prkab1; NM_022627, NM_031976), proposed as a novel β-cell glucose sensor (da Silva Xavier et al. 2003, Tsuboi et al. 2003, Leclerc & Rutter 2004) was down-regulated in diabetic islets (Table 1), consistent with reports of reduced AMPK (Prkab2, Prkab1; NM_022627, NM_031976) activity in human islets from type 2 diabetic subjects (Del Guerra et al. 2005). Protein kinase C (PKC) isoforms were also differentially expressed; PKC-d/Prkcd (NM_133307) being down-regulated by high glucose in GK islets (Table 4), whereas PKC-e/Prkce (AA799421) showed the opposite response (Table 3). PKC-d/Prkcd (NM_133307) has previously been linked to β-cell apoptosis (Carpenter et al. 2002, Eitel et al. 2003), so its suppression in GK islets (recently confirmed; Warwar et al. 2006) could represent a defence effort against β-cell demise. Conversely, the up-regulation in GK islets of PKC-e/Prkce (AA799421), known to be required for short-term insulin secretion (Hoy et al. 2003; Mendez et al. 2003), may represent an effort to compensate for the impaired insulin secretion by the diabetic islets. Phospholipase C b1 (PLC-b1; BE097028) was up-regulated by high glucose in GK (but not Wistar) islets (Table 5), an event that could contribute to impaired insulin secretion in GK islets since overexpression of PLC-b1 (BE097028) in insulin-secreting cells reportedly inhibits insulin release (Ishihara et al. 1999). Somatostatin receptor subtype 3, whose expression has been reported in normal rat islets (Ludvigsen et al. 2004), was found to be up-regulated by high glucose in diabetic GK islets (Table 3). This could indicate an enhanced sensitivity of diabetic islets to the suppressive influences of paracrine somatostatin on insulin secretion.

Several critical factors controlling cell proliferation and apoptosis were also aberrantly expressed in GK islets. Cyclin D1 (Ccdn1; X75207), driving cells from G1 into cell cycle S-phase, was down-regulated in diabetic

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### Table 8 Islet transcripts down-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats

<table>
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<tr>
<td>Cell cycle</td>
<td>NM_017066</td>
<td>Pleiotrophin (Ptn)</td>
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<td>BE108911</td>
<td>DNA primase, p49 subunit</td>
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<tr>
<td></td>
<td>X75207</td>
<td>Cyclin D1 (Ccdn1)</td>
<td>1.2</td>
</tr>
<tr>
<td>Development</td>
<td>NM_012676</td>
<td>Troponin T2, cardiac (Tnnt2)</td>
<td>1.4</td>
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<td></td>
<td>NM_017086</td>
<td>Pleiotrophin (Ptn)</td>
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<td></td>
<td>AA801238</td>
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<td></td>
<td>BG673439</td>
<td>Claudin 11 (Cldn11)</td>
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<tr>
<td></td>
<td>NM_057208</td>
<td>Tropomyosin 3, gamma (Tpm3)</td>
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<tr>
<td></td>
<td>BG374290</td>
<td>Microtubule-associated protein 2 (Mtap2)</td>
<td>1.6</td>
</tr>
<tr>
<td>Response to stimulus</td>
<td>NM_012591</td>
<td>Interferon regulatory factor 1 (Irf1)</td>
<td>1.2</td>
</tr>
</tbody>
</table>
GK islets as compared with non-diabetic controls (Tables 8 and 12). Since cyclin D1 (X75207) is stimulated by known β-cell mitogens (Friedrichsen et al. 2006), it appears essential for postnatal β-cell growth (Kushner et al. 2005) and its overexpression increases human β-cell proliferation by tenfold (Cozar-Castellano et al. 2004); increase in D-type cyclin activity can be harnessed to advantage in promoting β-cell proliferation that is inadequate in islets from GK rats (Portha 2005) and type 2 diabetic patients (Bonner-Weir & Weir 2005; Butler et al. 2003). Caspase-6, involved in β-cell apoptosis (Thomas et al. 2001), was up-regulated in GK islets as compared with Wistar islets (Table 11). IGFBP-3 (NM_012588), recently proposed as a novel mediator of β-cell apoptosis (Shim et al. 2004), was up-regulated by high glucose (Table 9) and might contribute to β-cell glucoparosis. Apolipoprotein E (ApoE; J02582), a known constituent of islet amyloid deposits in both rodents and man (Powell et al. 2003), was up-regulated in GK islets (Table 7). ApoE (J02582) has been suggested to promote amyloidogenesis by stabilizing amyloid fibrils (Kahn et al. 1999), found in 90% of type 2 diabetic patients’ post-mortem (Opie 1901) and also in GK islets (Leckstrom et al. 1996). An association between ApoE (J02582) gene polymorphisms and type 2 diabetes has also been noted (Vidal et al. 2003). It is thus possible that all these proteins up-regulated (caspase-6, IGFBP-3, and ApoE)

### Table 9: Islet transcripts up-regulated by high versus low glucose in non-diabetic Wistar rats

<table>
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<th>Fold change</th>
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<td>U13253</td>
<td>Fatty acid binding protein 5, epidermal (Fabp5)</td>
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<td>AA901341</td>
<td>Solute carrier family 2 (facilitated glucose transporter), member 3 (Sic2a3)</td>
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<tr>
<td>BI283882</td>
<td>Glucose phosphate isomerase (Gpi)</td>
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<tr>
<td>BI294137</td>
<td>Hexokinase 2 (Hk2)</td>
<td>1.3</td>
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<tr>
<td>AI713204</td>
<td>Monoglyceride lipase (Mgl)</td>
<td>1.6</td>
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<tr>
<td>NM_012497</td>
<td>Aldolase C, fructose-biphosphate (Aldoc)</td>
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<td>NM_133298</td>
<td>Glycoprotein (transmembrane) nmb (Gpnmb)</td>
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<tr>
<td>NM_012580</td>
<td>Heme oxygenase (decycling) 1 (Hmxo1)</td>
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<td>NM_020308</td>
<td>A disintegrin and metalloproteinase domain 15 (metarginid; Adam15)</td>
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<td>D83508</td>
<td>Early growth response 2 (Egr2)</td>
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<td>Tissue inhibitor of metalloproteinase 2 (Timp2)</td>
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<td>Insulin-like growth factor binding protein 3 (Igfbp3)</td>
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<td>NM_022207</td>
<td>Unc-5 homolog B (C. elegans; Unc5b)</td>
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<td>Interleukin 6 signal transducer (Il6st)</td>
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<td>Phosphoglucomutase 1 (Pgm1)</td>
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<td>NM_031648</td>
<td>FXYD domain-containing ion transport regulator 1 (Fxyd1)</td>
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### Table 10: Islet transcripts down-regulated by high versus low glucose in non-diabetic Wistar rats

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<td>AF166267</td>
<td>Kinesin light chain 3 (Klc3)</td>
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<td>BG664123</td>
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<td>NM_031599</td>
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<td>BE108911</td>
<td>DNA primase, p49 subunit</td>
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<tr>
<td>BM385445</td>
<td>Topoisomerase (DNA) 2 alpha (Top2a)</td>
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<td>BI705393</td>
<td>Fragile X mental retardation syndrome 1 homolog (Fmr1)</td>
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<td>NM_053358</td>
<td>Single stranded DNA binding protein 3 (Sdp3)</td>
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<td>BF290483</td>
<td>General transcription factor II I repeat domain-containing 1 (Gtf2rid1)</td>
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<tr>
<td>NM_017077</td>
<td>Forkhead box A3 (Foxa3)</td>
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### Table 11 Islet transcripts up-regulated in diabetic GK rats versus non-diabetic Wistar rats

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<td>Transport</td>
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<td>AF202115</td>
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<td>Transmembrane protein 9 (predicted; Tmem9_predicted)</td>
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<tr>
<td></td>
<td>Metabolism</td>
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<td>BI295900</td>
<td>Dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex; Dlat)</td>
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<td>ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d (Atp5d)</td>
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<td>Apoptosis</td>
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<td>AF051335</td>
<td>Reticulon 4 (Rtn4)</td>
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<td>Caspase 6 (Casp6)</td>
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<td>MAD homolog 2 (Drosophila; Madh2)</td>
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<td>Signal transduction</td>
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### Table 12 Islet transcripts down-regulated in diabetic GK rats versus non-diabetic Wistar rats

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<td>Transport</td>
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<td>Aldolase C, fructose-biphosphate (Aldoc)</td>
<td>1.2</td>
</tr>
<tr>
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<td>Phosphoglycerate kinase 1 (Pgk1)</td>
<td>1.1</td>
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<td>U03389</td>
<td>Prostaglandin-endoperoxide synthase 2 (Ptgs2)</td>
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<td>High mobility group AT-hook 1 (Hmga1)</td>
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<td>SNF1-like kinase (Snf11k)</td>
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<td>Cyclin D1 (Cnd1)</td>
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<td>Casein kinase 1, alpha 1 (Csnk1a1)</td>
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<td>Cell adhesion</td>
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<tr>
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<td>Contactin 3 (Ctnn3)</td>
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Table 13 Validation by qRT-PCR of glucose-regulated islet transcripts identified by microarray analysis

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Fold change</th>
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<tr>
<td>Serum- and glucocorticoid-inducible kinase 1 (Sgk)</td>
<td>Up-regulated by high versus low glucose in non-diabetic Wistar rats</td>
</tr>
<tr>
<td>Apolipoprotein E (ApoE)</td>
<td>Up-regulated by high versus low glucose in non-diabetic Wistar rats</td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 3 (Igfbp3)</td>
<td>Up-regulated by high versus low glucose in diabetic GK rats</td>
</tr>
<tr>
<td>Insulin-2</td>
<td>Down-regulated by high glucose in diabetic GK rats</td>
</tr>
<tr>
<td>Insulin-2</td>
<td>Down-regulated by high glucose in diabetic GK rats versus high glucose in non-diabetic Wistar rats</td>
</tr>
<tr>
<td>11β-hydroxysteroid dehydrogenase type 1 (Hsd11b1)</td>
<td>Up-regulated by low glucose in diabetic GK rats versus low glucose in non-diabetic Wistar rats</td>
</tr>
</tbody>
</table>

Results are means ± S.E.M. of three experiments. *Denotes \( P < 0.05 \) for a chance difference versus controls using Student’s unpaired \( t \)-test. †Denotes correlation between microarray and qRT-PCR is significant at the \( P < 0.01 \) level.

may contribute to the increased apoptosis known to occur in β-cells from GK rats (Koyama et al. 1998; Portha 2005) and patients with type 2 diabetes (Federici et al. 2001, Butler et al. 2003).

Expression of the glucose-dependent insulinotropic polypeptide receptor (GIPr; NM_012714) was approximately twofold lower in GK low glucose when compared with GK high glucose. This suggests a tonic reduction of incretin signaling in GK islets, potentially a major contributor to the islet defect in this diabetes model. Since the incretin signaling/sensing is an important physiologic regulator of glucose-stimulated insulin secretion and β-cell development, this impairment would be consistent with the reduced cyclin D1 (X75207) and increased apoptosis genes. The insulinotropic response to GIP (NM_012714) in β-cells is also grossly impaired in diabetic patients (Vilsboll et al. 2003).

Recent studies have suggested an inflammatory contribution to the pathogenesis of type 2 diabetes (Donath et al. 2005, Kolb & Mandrup-Poulsen 2005, Homo-Delarche et al. 2006). Major histocompatibility complex, class II gene (MHC class II; AI171966, BI301490) is an immune gene associated with type 2 diabetes (Acton et al. 1994). This gene was up-regulated in GK islets when compared with Wistar islets at 3 mM glucose and down-regulated by high glucose (20 mM) in GK islets in our study (Tables 4 and 7).

As expected, the insulin gene was robustly up-regulated (11.7-fold) in response to high glucose in islets from non-diabetic rats (Table 13), whereas glucose failed to influence insulin gene expression in islets from GK rats. Similar results were obtained in Psammomysobesus, another animal model of type 2 diabetes, by Leibowitz et al. (2002). However, the insulin content was significantly higher in islets from diabetic rats when compared with normal rats, suggesting that glucose-induced proinsulin biosynthesis might predominantly be controlled at the translational level and also by stabilization of proinsulin mRNA rather than at the transcriptional level (Webb et al. 2000).

In conclusion, we identified significant changes in several islet mRNAs involved in glucose sensing, phosphorylation, incretin action, glucocorticoid handling, ion transport, mitogenesis, and apoptosis that clearly distinguish diabetic animals from controls. Such markers may provide clues to the pathogenesis of human type 2 diabetes and may be of predictive and therapeutical value in clinical settings in efforts aiming at conferring β-cell protection against apoptosis, impaired regenerative capacity, and functional suppression occurring in diabetes.

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