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Transcriptional regulation of healthy and diabetic pancreatic β-cell gene expression

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Stockholm 2008

Published and printed by the Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden

ISBN 978-91-7409-182-3

To my mother

Dubito, ergo cogito, ergo sum

René Descartes

ABSTRACT

Hyperglycemia, deficient insulin secretion, and insulin resistance are the characteristic pathogenetic features of type 2 diabetes (T2D), hallmarked by functional and survival defects of the insulin-producing islet β-cell. The primary pathogenesis of T2D probably involves both genetic and environmental forces, and hyperglycemia and very commonly hyperlipidemia might further aggravate β-cell dysfunction. To gain a deeper understanding of the precise nature of the cellular and molecular defects responsible for T2D, it is necessary to pin down the molecular mechanisms by which glucotoxicity and lipotoxicity adversely impact rodent and human β-cells. This was the over-arching aim of the current work. Additionally, the influence of antidiabetic drugs, with alleged ability to protect and preserve β-cell function and viability, on the above pathways was addressed.

Paper I, II

Long term exposure to free fatty acids (FFAs) may alter β-cell signaling and gene expression, precipitating β-cell failure through impaired insulin synthesis and secretion in combination with apoptosis, a phenomenon commonly referred to as lipotoxicity. It is still unclear whether β-cell failure and apoptosis are induced by increased FFAs alone, in synergy with glucose, or if predisposing genetic factors are required. Also, the protective effects of the PPARγ agonist pioglitazone (a thiazolidinedione) and the GLP-1 receptor agonist exenatide (an incretin mimetic) against FFA-induced β-cell dysfunction and apoptosis are still debated.

We sought to investigate the long term effect of pioglitazone and exenatide on global alterations in gene expression, with/out palmitate to evoke β-cell lipotoxicity, in human and rat diabetic and non-diabetic islets. Microarray interrogation and qRT-PCR analyses revealed alterations in several functional categories, most importantly epigenetic regulation of gene expression that seem to regulate β-cell function and survival. Furthermore, FFAs seem to contribute and play an important role in the development of β-cell failure in synergy with hyperglycemia, in obese individuals, or in subjects who are genetically more predisposed to T2D. Considering that the nutritional state directly induces epigenetic modifications, pioglitazone and exenatide appear to normalize these epigenetic misregulations and may protect the β-cell from lipotoxic insult. The epigenetic modifications of the genome provide new promising targets for clinical diagnostics and also therapeutic purposes in T2D.

Paper III

Chronic hyperglycemia is believed to be an important determinant of β-cell dysfunction that may become irreversible over time (known as glucotoxicity). The objective of this study was to investigate the expression of glucose-regulated key genes in islets from spontaneously diabetic Goto-Kakizaki (GK) rats compared to normoglycemic Wistar rats, by using the microarray and qRT-PCR technology. 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. Our results identify key elements in glucose-regulated gene expression in β-cells, revealing substantial qualitative and quantitative differences in gene expression between healthy and diabetic β-cells, which may have implications for our understanding of the etiology and treatment of human T2D. Such markers 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.

Paper IV

Pancreatic and duodenal homeobox-1 (PDX-1) transcription factor is critical for embryonic development of the pancreas and normal islet function. In β-cells, PDX-1 regulates a number of genes involved in maintaining β-cell identity and function, including insulin. Glucose homeostasis requires tight regulation of insulin synthesis and secretion. The homeodomain of the PDX-1 protein plays a critical role in DNA binding and glucose-dependent regulation of insulin gene transcription. The molecular basis of PDX-1 nuclear translocation, however, remains poorly understood.

In this study, we aimed to identify amino acid sequences responsible for the nuclear translocation of mouse PDX-1 by using site-directed mutagenesis of putative phosphorylation sites and positively charged amino acid residues in putative nuclear localization signal (NLS) motifs of GFP-tagged PDX-1. We demonstrate that the NLS motif RRMKWKK is necessary, and in conjunction with the integrity of the ´helix 3` domain of the PDX-1 homeodomain is sufficient, for the nuclear import of PDX-1 in the β-cell.

LIST OF PUBLICATIONS

I. **Ghanaat-pour H**, Sjöholm Å.
Gene expression regulated by pioglitazone and exenatide in normal and diabetic rat islets exposed to lipotoxicity.
Diab/Metab Res Rev, in press.

II. **Ghanaat-pour H**, Sjöholm Å.
Exenatide and pioglitazone regulate fatty acid-induced gene expression in normal and diabetic human islets.
Manuscript.

III. **Ghanaat-pour H**, Huang Z, Lehtihet M, Sjöholm Å.
Global expression profiling of glucose-regulated genes in pancreatic islets of spontaneously diabetic Goto-Kakizaki rats.
J Mol Endocrinol 2007 Aug; 39(2):135-50.

IV. Moede T, Leibiger B, **Ghanaat-pour H**, Berggren P, Leibiger IB.
Identification of a nuclear localisation signal, RRMKWKK, in the homeodomain transcription factor PDX-1.
FEBS Lett 1999; 461:229-34.

CONTENTS

LIST OF ABBREVIATIONS

ACACA	Acetyl-Coenzyme A carboxylase alpha
ABCC8	ATP-binding cassette, sub-family C, member 8
APOE	Apolipoprotein E
ARNT	Aryl hydrocarbon receptor nuclear translocator
Bcl2l1	Bcl2-like 1
Bmf	Bcl2 modifying factor
CACNA1E	Calcium channel, voltage-dependent, R type, alpha 1E subunit
CCND1	Cyclin D1
CEL	Carboxyl ester lipase
DAD1	Defender against cell death 1
DHC	11-dehydrocorticosterone
EGFR	Epidermal growth factor receptor
Esr1	Estrogen receptor 1
ESTs	Expressed sequence tags
FFA	Free fatty acid
Foxa1	Forkhead box a1
GADD45B	Growth arrest and DNA-damage-inducible beta
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFP	Green fluorescent protein
GIPR	Gastric inhibitory polypeptide receptor
GK	Goto-Kakizaki
GLP-1	Glucagon-like peptide 1
HNF4α	Hepatocyte nuclear factor 4, alpha
HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1
IGFBP3	Insulin-like growth factor binding protein 3
Il1r1	Interleukin-1 (IL-1) receptor type I
Il1rn	IL-1 receptor antagonist
IR	Insulin receptor
ISL1	ISL LIM homeobox 1
KCNB1	Potassium voltage-gated channel, Shab-related subfamily, member 1
KCNJ10	Potassium inwardly-rectifying channel, subfamily J, member 10

KCNJ11	Potassium inwardly-rectifying channel, subfamily J, member 11
K_v	Voltage-activated K^+ channel
Neurod1	Neurogenic differentiation 1
NLS	Nuclear localization signal
PDK	Pyruvate dehydrogenase kinase
PDX-1	Pancreatic and duodenal homeobox-1
PEPCK	Phosphoenolpyruvate carboxykinase
PKA	Protein kinase A
PKC	Protein kinase C
PLCB1	Phospholipase C, beta 1
PRKAB1	Protein kinase, AMP-activated, beta 1 non-catalytic subunit
PRKAB2	Protein kinase, AMP-activated, beta 2 non-catalytic subunit
PPAR	Peroxisome proliferator-activated receptor
PPARβ/δ	peroxisome proliferator-activated receptor delta
PPARγ	peroxisome proliferator-activated receptor gamma
PPY	Pancreatic polypeptide
REG3A	Regenerating islet-derived 3 alpha
RT-PCR	Reverse transcriptase polymerase chain reaction
Rxra	Retinoid X receptor alpha
SGK1	Serum/glucocorticoid regulated kinase 1
T2D	Type 2 diabetes
TCF7L2	Transcription factor 7-like 2
TXNIP	Thioredoxin interacting protein
TZD	Thiazolidinedione
Xbp1	X-box binding protein 1

1 INTRODUCTION

1.1 Type 2 diabetes

More than 100 million people worldwide have diabetes and will suffer a substantially reduced quality of life as a consequence. In fact, more than 80 % of diabetics will die early from cardiovascular diseases, renal failure, infection, or acute decompensation [1]. Previously viewed as a disease of the elderly, type 2 diabetes (T2D) now is seen in ever-younger age groups. In the U.S. half of all newly diagnosed diabetes in children and adolescents now is type 2, a horrifying scenario when considering the magnitude of premature cardiovascular and cerebrovascular morbidity in these individuals. Recent estimates by the Center for Disease Control indicate that the life-time risk of getting diabetes is not less than 40 % for people born in 2000 in the U.S., with certain ethnic groups being significantly overrepresented (www.cdc.gov/diabetes).

As the average life span is shortened by 10-15 years by diabetes, there will be a loss of workforce and economic productivity in society at large thus further increasing the number of people on sick leave and premature retirement. In times of reduced health care budgets, this unfortunate development will leave governments swamped with an enormous financial burden in providing care for the escalating number of diabetic patients. In the U.S. alone, the annual cost (in 2007) for diabetes care is an astonishing $174 billion, of which some 97 % is targeted to T2D (www.cdc.gov/diabetes).

Importantly, clinical studies in T2D have provided compelling evidence that β-cell function/numbers decline despite the use of traditional drug treatments to control glucose [2, 3], eventually leaving most patients reliant on exogenous insulin replacement therapy. Moreover, these treatments themselves can impair human β-cell function [4, 5] and precipitate life-threatening complications. Hence, it is immediately apparent that there is an

urgent need to find new molecular targets capable of preventing and treating diabetes, particularly agents and pathways that protect against the loss of β-cell mass.

T2D is a complex heterogeneous group of metabolic conditions characterized by hyperglycemia, caused by both impaired insulin secretion and resistance to insulin at its target cells. Furthermore, T2D arises from an early direct or indirect combination of defects in muscle, adipocytes, hepatocytes, β-cells, and possibly the central nervous system (CNS) [6]. Phenotypic differences of T2D are highly variable, which may reflect the diverse etiopathogeneses in human T2D, a classical polygenic disorder. Several mechanisms have been proposed to trigger insulin resistance and β-cell dysfunction, including increased proinflammatory cytokines, adipokines, endoplasmic reticulum stress, oxidative stress, glucotoxicity and lipotoxicity [7-9].

It is believed that T2D results from the complex interplay between a genetic predisposition and environmental factors (Fig. 1). Multiple lines of evidence, the spectrum of T2D prevalence in different ethnic groups [10], familial aggregation [11], twin studies [12-15], and heritability of intermediate phenotypes [16-19], collectively support the view that genetic components play an important role in the pathogenesis of T2D.

Identification of genes causal for multifactorial T2D by utilizing candidate gene and genome-wide scan approaches has proved challenging due to the complexity and heterogeneity of the disease. Consequently, a rather short list of genes have been reproducibly associated with T2D, including peroxisome proliferator activated receptor gamma (*PPARG*) [20], hepatocyte nuclear factor 4, alpha (*HNF4A*) [21], potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) [22], calpain 10 (*CAPN10*) [23], ectonucleotide pyrophosphatase phosphodiesterase (*ENPP1*) [24], and transcription factor 7-like 2 (*TCF7L2*) [25, 26].

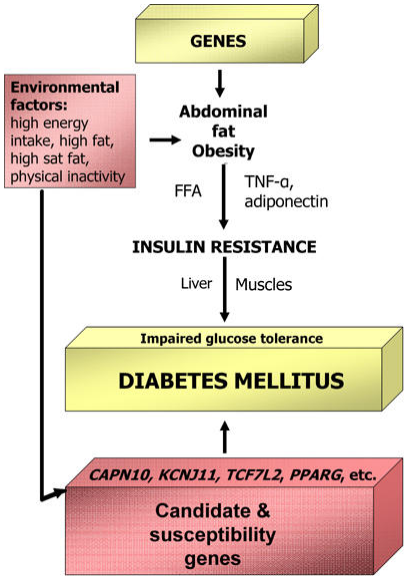

Figure 1. Interrelation between genes and environmental factors in type 2 diabetes.

Genes in combination with environmental factors may lead to obesity, insulin resistance and finally diabetes. Environmental factors may also act in concert together with diabetes candidate and susceptibility genes to trigger the pathogenesis of type 2 diabetes directly.

Different environmental stresses, population differences in activity, and different diets clearly cause some genes to manifest as a disease phenotype. Considering the role of environment in the current diabetes pandemic, the *thrifty phenotype* hypothesis suggests that T2D may arise due to early environmental effects (possibly even *in utero*), whose influence to cause disease is only expressed much later in life and is maintained throughout the lifetime of the individual [27, 28] (Fig. 2). Moreover, under the influence of environmental factors and nutritional state, epigenetic mechanisms allow an organism to alter stable changes in gene expression, which may contribute to the development of abnormal phenotypes [29]. Epigenetics are defined as

stably maintained mitotically heritable patterns of gene expression that occur without changes in DNA sequence. It seems that a healthy intrauterine environment cannot overcome the effects of the genetic predisposition and the maternal epigenetic inheritance [30]. Interestingly, nutrients and epigenetic drugs appear to restore the epigenetic regulation of gene expression that is reversible, in contrast to genetics.

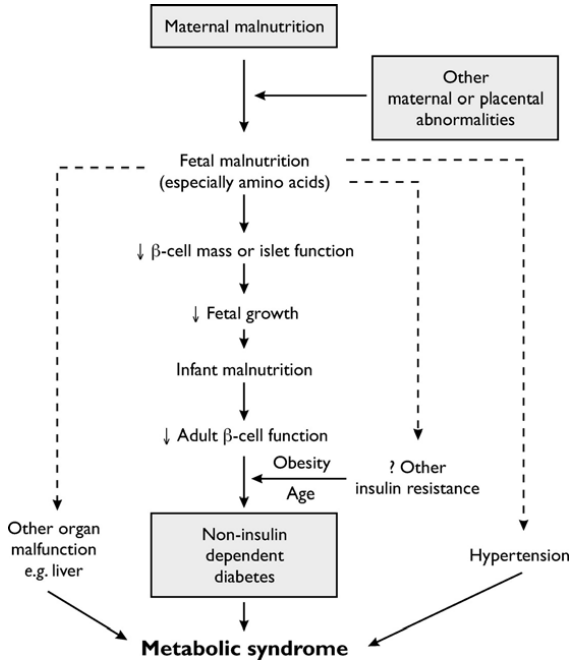

Figure 2. The original diagrammatic representation of the thrifty phenotype hypothesis.

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1.2 The pancreatic β-cell

The islets of Langerhans contain four major types of endocrine cells in clusters where the core of the islets consists of insulin-producing β-cells, whereas the other three islet cell types, α, δ, and PP cells, are localized around the islet mantle [32-34] (Fig. 3). Healthy human adults have approximately 2 million islets, with a weight about 2 % of the pancreas. β-cells, normally comprising 65-80 % of the islet cells, synthesize and secrete the hormone insulin in response to nutrients, hormones, and nervous stimuli, to maintain glucose homeostasis [35].

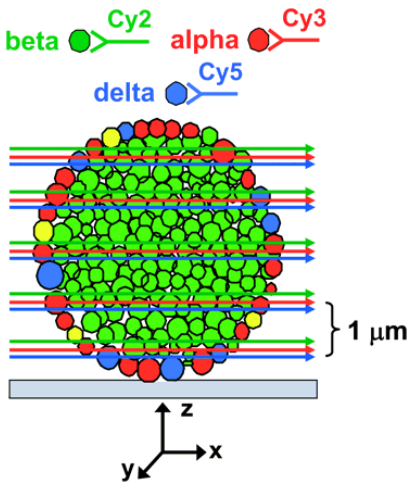

Figure 3. Schematic representation of optical sectioning of isolated islets by confocal laser scanning microscopy.

Islet cell types are illustrated in four different colors: ß cells, green; α cells, red; δ cells, blue; PP cells, yellow. Antibodies applied to islet hormones for islet cell labeling are shown schematically at the top. Red, green, and blue arrows represent image overlay of α, ß, and δ cells in a single focal plane (optical slice). x,y,z refer to axis. Optical slices through islet were acquired by moving focal plane (x,y) along z-axis from the bottom to the top of the islet at 1-µm increments. Using image analysis software, individual optical sections were assembled into a three-dimensional (3-D) stack and projected as a 0° view with respect to the y-axis (head-on projection).

In adult rodents, new β-cell formation is maintained either by slow replication of existing β-cells or by differentiation of new cells from ductal precursors or stem cells, a process that is estimated to occur over a period of 3 months [36, 37]. In contrast to rodents, the mechanisms governing human β-cell growth, differentiation, replication and death remain much more elusive and largely rely on *post mortem* examinations.

It is now well recognized that progressive deterioration of β-cell function is an early and important lesion in the chain of events leading to T2D in adult individuals, and plays a key role in the development of hyperglycemia [38, 39]. In healthy subjects, insulin sensitivity and β-cell function are inversely and proportionally related [40], whereby changes in insulin sensitivity are mirrored by changes in insulin secretion to keep glycemia within a narrow range [41]. In human T2D, loss of glucose-sensitive insulin secretion is an important pathogenetic event that is also of significance in early type 1 diabetes and in the context of islet transplantation. 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 T2D. A reduction in β-cell mass also seems to contribute to insulin deficiency in T2D [42] (Fig. 4). In healthy subjects, under conditions of insulin resistance (*e.g.,* obesity, pregnancy, *etc.*), β-cells respond to the increased functional demand by enhancing their function and expanding their mass [42]. The β-cell mass is increased in obese non-diabetic individuals, and progressively declines with the onset of T2D due to an imbalance between β-cell apoptosis and neogenesis (by proliferation and differentiation) [42, 43]. A predisposing genetic background, together with the degree of glucotoxicity and lipotoxicity, seems to exacerbate and accelerate this deleterious process [44-47].

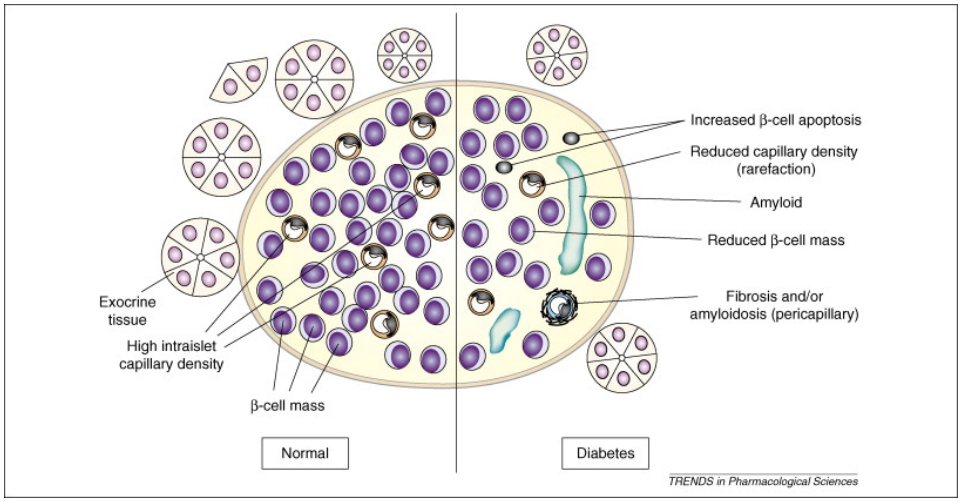

Figure 4. A cartoon showing islet structure in type 2 diabetes.

In normal subjects, highly vascularized islets are dispersed throughout the pancreas. Type 2 diabetes is characterized by several abnormalities in islet morphology. There is a decrease in β-cell mass and an increased prevalence of apoptotic β-cells. Although islet amyloidosis is usually reported as a characteristic feature of type 2 diabetes, islet fibrosis is also reported and can be considered to be a sign of inflammation. In addition, there are microvascular changes, with islet capillary rarefaction and pericapillary fibrosis or amyloidosis.

In contrast to most other cells, in which signaling is controlled through plasma membrane receptor-operated processes, the β-cell serves as a unique fuel-sensing organ that senses subtle fluctuations in glycemia and translates these into finely tuned induction of a unique set of genes and the suppression of others [49, 50]. The lesions intrinsic to the β-cell causing the selective loss of glucose sensitivity remain elusive, however. Genome-wide expression profiling has been utilized as a powerful tool to provide important insights into differences in expression patterns of genes, in specific tissues under healthy and pathological conditions. For instance, gene expression profiling has been studied in the MIN-6 cell line [51], rat β-cells [49], and in human islets isolated from organ donors [52-55]. Monitoring gene expression in

the study of β-cell dysfunction of diabetes revealed expression of multiple genes involved in energy metabolism, the regulated insulin biosynthetic/secretory pathway, membrane transport, intracellular signaling, gene transcription, and protein synthesis/degradation.

1.3 Glucotoxicity, lipotoxicity, and glucolipotoxicity

Glucotoxicity

Glucose regulates insulin gene expression under normal circumstances through a network of transcription factors and co-activators to the insulin promoter, and also prolongs the half-life of insulin mRNA (Fig 5). Chronic hyperglycemia has been reported to impair glucose-induced insulin secretion, insulin gene expression, and to aggravate insulin resistance [56]. Prolonged exposure to elevated glucose, so called glucotoxicity, incurs irreversible damage to cellular components of insulin production over time [57, 58]. Thereby defects in insulin biosynthesis and secretion evolve, and eventually, reduction in the β-cell mass by apoptosis [59, 60]. Continuous over-stimulation of the β-cell by glucose could eventually lead to depletion of insulin stores, worsening of hyperglycemia, and finally deterioration of β-cell function. Although the exact biochemical mechanisms mediating this effect remain elusive, a growing body of evidence emphasizes the role of oxidative stress as an important mechanism for glucose toxicity [61, 62], and also the involvement of a posttranscriptional defect in pancreas duodenum homeobox-1 (PDX-1) mRNA maturation [63, 64].

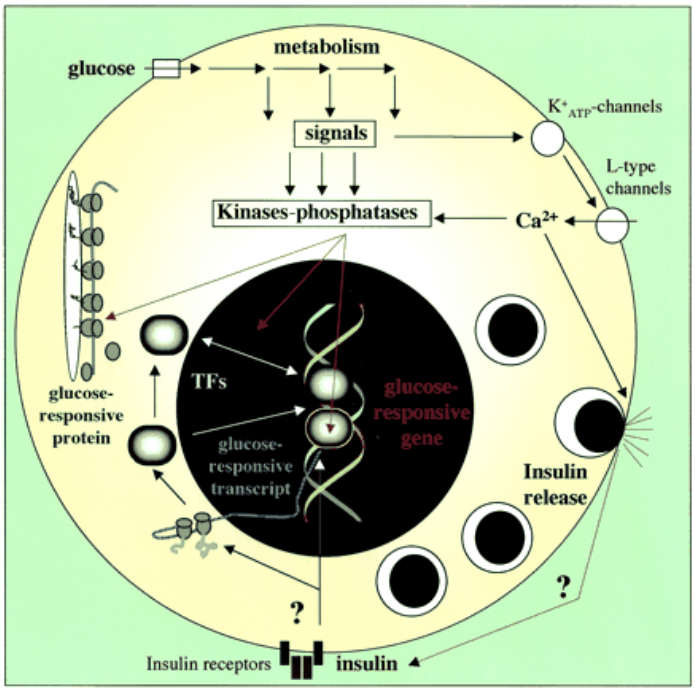

Figure 5. Molecular pathways of glucose-regulated gene expression.

Multiple regulatory pathways can alter the concentration and/or the activity of transcription factors (TFs) when glucose stimulates β-cells. For an explanation, see MOLECULAR MECHANISM OF GLUCOSE-REGULATED GENE EXPRESSION IN β-CELLS [49].

Lipotoxicity

Free fatty acids (FFAs) acutely enhance insulin secretion by serving as β-cell fuels, whereas prolonged exposure to FFAs (*i.e.,* lipotoxicity) impairs insulin secretion and insulin gene expression, and with time adversely impacts β-cell viability [47, 65]. Hyperlipidemia, in addition to hyperglycemia, has been suggested as a causative force in the relentless deterioration of β-cell function and survival, eventually leading to its demise [66, 67]. Most

patients with T2D are obese and, as a consequence of their insulin resistance, often have hyperlipidemia and elevated FFAs that can compromise β-cell function and viability through intra-islet lipid accumulation (steatosis) [66, 67].

According to the *fuel concept* and the *Randle cycle* hypothesis, the influence of FFAs on insulin secretion is associated with suppression of peripheral glucose uptake, stimulation of hepatic gluconeogenesis, and impeded islet glucose recognition and fatty acid oxidation [68, 69]. Interestingly, the deleterious effects of lipotoxicity on insulin gene expression and β-cell function seem to target PDX-1, as in glucotoxicity, although through different mechanisms [70]. However, the precise nature of the mechanisms by which lipotoxicity exerts its harmful effects on the β-cell remains poorly understood.

Glucolipotoxicity

The theory of glucolipotoxicity (combined glucose and lipid toxicity) has been introduced recently based on the proposal that the alterations in intracellular lipid partitioning underlying the mechanisms of lipotoxicity, causing detrimental effects on β-cell function, are dependent upon hyperglycemia [47, 71]. Considering that physiological levels of glucose and lipids are not toxic, but instead are required for normal β-cell function, going from normoglycemic and normolipidemic conditions to hyperglycemic and hyperlipidemic abnormalities, a combination of these two appears to be conspicuously damaging or toxic to the β-cell. Moreover, lipid metabolism is under tight control of ambient glycemia [72]; therefore, in the presence of physiological glucose concentrations, this theory proposes, elevated FFAs are supposed to be oxidized and should not be harmful to the β-cell. On the other hand, under circumstances where both FFAs and glucose are elevated, high glucose would markedly inhibit fat oxidation [73] and consequently lipid detoxification, and thereby inhibit glucose-induced insulin secretion and insulin gene expression. In fact, according to Poitout and

Robertson, lipotoxicity can be viewed as one mechanism of glucotoxicity [47]. It should be noted, however, that the precise role of elevated fatty acids and glucose in β-cell function is still a matter of some debate and remains to be established.

1.4 Steroid diabetes

In rodents, Hydroxysteroid (11-beta) dehydrogenase 1 (*HSD11B1*) converts inactive 11-dehydrocorticosterone (*DHC*) into active corticosterone, whereas in humans cortisone is converted into cortisol. The mRNA and activity of *HSD11B1* have been shown to be present and up-regulated in pancreatic islets from hyperglycemic mice [74, 75] and the Zucker Diabetic Fatty (ZDF) rat [76], compared to their normoglycemic counterparts. The β-cell may be extra susceptible to glucocorticoid excess, since both iatrogenic Cushing syndrome and steroid-induced diabetes in animal models are associated with loss of glucose-stimulated insulin secretion, and steroid immunosuppressive treatment adversely affects islet transplantation outcome [77]. Dexamethasone causes GLUT-2 degradation, thereby impeding β-cell glucose sensing [78-80]. Glucocorticoids also decrease insulin gene expression [81], increase glucose-6-phosphatase, phosphoenolpyruvate carboxykinase activity and glucose cycling [82, 83], and induce β-cell apoptosis [84], all events occurring in GK rat islets [85]. Since the human insulin gene contains glucocorticoid-sensitive transcriptional elements [86], it may be susceptible to islet overproduction of glucocorticoids. This may also be of therapeutical significance, given the attention currently paid to pharmacological *HSD11B1* inhibitors in clinical trials against type 2 diabetes [87].

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 [82, 83]. Humans with impaired β-cell function (low insulin responders) are predisposed for becoming

overtly diabetic during glucocorticoid therapy [88].

Another gene involved in transducing glucocorticoid effects is serum- and glucocorticoid-inducible kinase 1 (*SGK1*), whose expression is increased by glucocorticoids [89]. *SGK1* appears to be instrumental in directly negatively regulating insulin secretion, since the suppression of glucose-sensitive insulin secretion normally evoked by dexamethasone was lost in islets from *SGK1* knockout mice [89]. In rodent islets, *SGK1* upregulates the activity of voltage-activated K^+ channels (K_v) thereby reducing Ca^{2+} entry and insulin secretion [89]. Since intracellular Ca^{2+} handling appears intrinsically disturbed in GK rat islets [90, 91], and can be deranged by glucocorticoid treatment [79], it seems that also *SGK1* may be of both pathogenetic and therapeutical interest in type 2 diabetes and steroid diabetes.

1.5 Goto-Kakizaki (GK) rats

The Goto-Kakizaki (GK) rat is a non-obese genetic model of T2D that was produced by selective inbreeding (with glucose intolerance as a selection index) of Wistar rats, repeated over many generations [92]. The GK rat is one of the best characterized animal models for genetic susceptibility to T2D, both male and female rats are equally affected with the diabetic trait [93]. The GK rats exhibit mild hyperglycemia, glucose intolerance, impaired glucose-induced insulin secretion, lowered islet insulin content, alteration of tissue insulin sensitivity, histopathological changes in the endocrine pancreas, and β-cell mass depletion [94-99]. While some degree of insulin resistance does exist, this is in all likelihood secondary to hyperglycemia, and the GK rat is clearly a model of β-cell dysfunction [30, 97, 99-103]. According to a number of studies epigenetic, genetic, and environmental programming of the endocrine pancreas seems to contribute to the transmitted β-cell defects in the GK model of T2D [30, 85, 104]. It should also be recognized that variations in diabetes pathogenesis between different colonies of GK rats occur.

Studies on GK rats fed high-fat diet demonstrate deteriorated glucose metabolism, partly due to an insufficient insulin secretion caused by genetic defects and lipotoxicity, whereas in normal Wistar rats high-fat diet induces enhancement of insulin secretion to compensate for the insulin resistance evoked by lipotoxicity [105].

1.6 Pioglitazone

Thiazolidinediones (TZDs), synthetic ligands of peroxisome proliferator–activated receptor γ (*PPARγ*), are antidiabetic agents that improve hyperglycemia and reduce insulin resistance by sensitizing muscle, liver and adipose tissue to insulin [106]. Also, direct beneficial effects on β-cells have been described [107-109], which collectively may contribute in concert to delay the progressive nature of T2D [110-112]. Currently, two TZDs are on the market and approved for the clinical management of T2D, *viz.* rosiglitazone and pioglitazone. Previous studies have suggested that TZDs may protect β-cell function [107, 110], improve insulin secretory capacity, preserve β-cell mass and islet structure [113-115], lower fasting and prandial glycemia as well as FFA levels [106, 116], and protect β-cells from oxidative stress and inflammation [117]. In addition, binding of TZDs to *PPARγ* leads to alteration in the expression of key glucoregulatory genes [118, 119], although the exact mechanism of action has not been completely elucidated. The direct effects of TZDs on β-cell gene expression appear to be both *PPARγ*-dependent and -independent [109]. Importantly, *PPARγ* is abundantly expressed in human islets [120]. The direct activation of *PPARγ* increases expression and activity of proteins involved in β-cell glucose sensing [121, 122], which leads to the stimulation of multiple metabolic pathways that favor the disposal of fatty acids [123] and may thus explain β-cell preservation and protection against lipotoxicity afforded by TZDs.

PPARs are ligand-dependent transcription factors belonging to the nuclear receptor family [124]. In addition to the synthetic ligands (TZDs), PPARs are also activated by a number of natural ligands such as fatty acids and fatty acid-derived molecules. Furthermore, three distinct PPAR isoforms have been identified with distinct tissue distribution and biological activities: *PPARγ*, *PPARδ/β* and *PPARα* [116, 125-127]. Upon heterodimerization with retinoic X receptor (*RXR*), PPARs control transcriptional rates of a large panel of genes implicated in various physiological functions [128, 129]. Moreover, PPARs have been reported to be involved in lipid and lipoprotein metabolism, glucose homeostasis, cellular proliferation and differentiation, apoptosis, and may also play a role in the control of the inflammatory response [130-132] (Fig. 6).

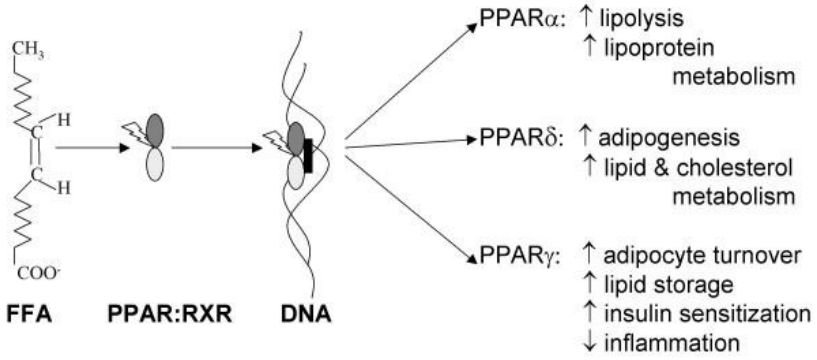

Figure 6. Overview of PPAR activation and effects.

FFA (eg. oleic acid) interact with PPAR, which dimerize with retinoid X receptor (RXR) and translocate to the nucleus where the complex interacts with PPRE to activate gene transcription. The general effects of transcriptional activation of PPARα, PPARδ and PPARγ are shown on the right of the figure.

Reprinted from ref [133]: "Fatehi-Hassanabad Z, Chan CB, Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic beta-cell function, Nutr Metab (Lond), 2005, 5, 2, 1, with kind permission from Copyright © 2005 Fatehi-Hassanabad and Chan; licensee BioMed Central Ltd."

1.7 Exenatide

Exenatide (synthetic Exendin-4), an incretin mimetic and a long-acting analog of glucagon-like peptide 1 (*GLP-1*), shares approximately 50 % sequence identity with mammalian *GLP-1*. Exenatide was approved in the U.S. in 2005 and in Europe in 2007 for the clinical management of T2D. *GLP-1* belongs to the large glucagon superfamily of peptide hormones and promotes β-cell function and survival by interaction with *GLP-1* receptor (*GLP-1R*) [134]. This receptor is abundantly expressed on β-cells, and its occupancy by *GLP-1* activates various pathways and genes required for insulin secretion and β-cell survival [135, 136] (Fig. 7). *GLP-1* is secreted from enteroendocrine L-cells in the small intestine in response to food intake and improves glycemic control through mechanisms quite different from that of insulin. *GLP-1* and its derivatives (*e.g.* exenatide) promote glucose-dependent insulin gene transcription, biosynthesis and secretion (only in the presence of hyperglycemia; hence, functioning as an incretin), further control glycemia via inhibition of glucagon secretion and gastric emptying, reduce appetite and energy intake [137-139], stimulate β-cell lipolysis [140, 141], and promote weight loss in overweight patients [142, 143].

More recent evidence suggests that *GLP-1* receptor agonists (incretins) promote DNA synthesis, stimulate the proliferation of β-cells [144-146] and also the differentiation of human fetal and adult pancreatic precursor cells into mature β-cells, through the expression of pancreatic duodenal homeobox gene-1 (*PDX-1*) [147-150]. Also, these agents may increase β-cell survival, and preserve β-cell mass via inhibition of apoptosis [151].

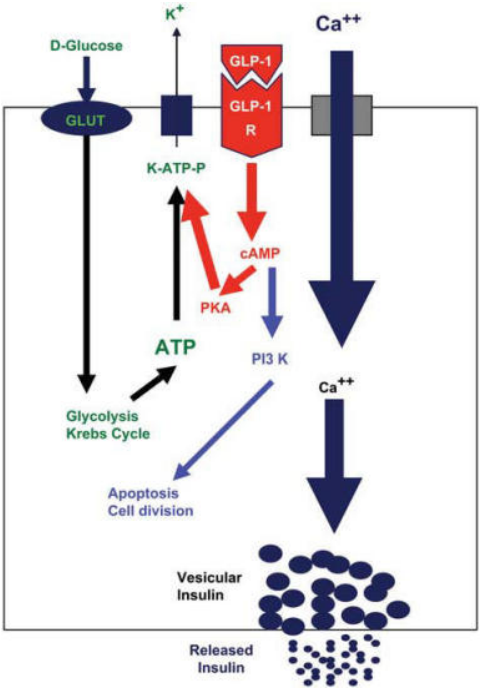

Figure 7. Interplay of glucose and incretins where glucagonlike peptide 1 (GLP-1) is depictedx on the pancreatic b-cell.
The entry and metabolism of glucose generates ATP. Phosphorylation of K-channels by ATP is potentiated by GLP-1 mediated c-AMP protein kinases allow increased calcium entry and subsequent insulin release. cAMP-dependent phopsphoinositol pathways appear to be involved in maintaining b-cell mass by effects on apoptosis and cell division.

1.8 PDX-1

Islet β-cells detect variations in extracellular glucose concentrations and transmit this information to the nucleus to modify the expression of a range of genes, including insulin. *PDX-1* (a.k.a. *Ipf-1*, *Idx-1*, *Iuf-1*, and *Stf-1*) [153] is essential for the maintenance of the expression of glucose-sensitive β-cell specific genes [154], and also for early pancreatic development, β-cell differentiation and in the mature β-cell [155]. Insulin gene transcription is

directly regulated by *PDX-1*, through formation of a complex with transcriptional co-activators on the proximal insulin promoter [156]. *PDX-1*-deficient mice, resulting from a homozygous mutation in the *PDX-1* gene, are born without a pancreas [157] and this has also been observed in a patient with pancreatic agenesis [158]. Germane to this, the heterozygous mutation of *PDX-1* causes mild diabetes in mice [159] and maturity onset diabetes of the young (MODY-4, a monogenic form of T2D) in humans [160].

The *PDX-1* protein is composed of separate functional domains: the highly conserved DNA-binding homeobox domain within the middle that is responsible for DNA binding and glucose-dependent nuclear translocation, the N-terminal region with at least three evolutionarily conserved subdomains that provides the trans-activation potential, and the C-terminal domain [161] (Fig. 8).

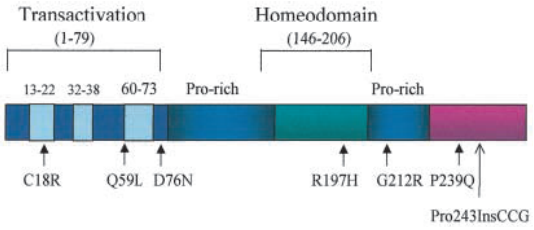

Figure 8. Structure of PDX-1.

Schematic representation of the predicted structure of PDX-1 showing the antennapedia-like homeodomain flanked by two proline rich regions. The NH_2-terminal activation domain comparising three sub-domains is also indicated as are a basic residue-rich nuclear localisation signal (NLS) within helix three of the homeodomain and a pentapeptide motif that interacts with PBX located colse to the homeodomain. Sequences COOH-terminal to the homeodomain (amino acids 144-283) could also have transactivation properties [162].

"With kind permission from Springer Science+Business Media: < Diabetologia, Pancreatic duodenal homeobox-1, PDX-1, a major regulator of beta cell identity and function., 44, 2001, 1203-14, McKinnon CM, Docherty K, figure 1, Diabetologia© Springer-Verlag 2001>." Ref [163].

Glucose-induced activation and translocation of endogenous *PDX-1* from the nuclear periphery to the nucleoplasm [164], triggered possibly by the phosphorylation of *PDX-1* [165] and the activation of phosphoinositide 3-kinase [166], implicates the importance of this element in the activation of insulin gene transcription. Although the mechanisms involved in *PDX-1*-mediated glucose action on insulin gene expression have been extensively addressed, they still remain under investigation.

2 AIMS

- To investigate the long-term *in vitro* effects of palmitate, pioglitazone and/or exenatide on global gene expression in healthy and type 2 diabetic rat and human islets at physiological glucose concentrations (Paper I, II).

- To investigate global alterations in long-term glucose-regulated gene expression in healthy and type 2 diabetic rat islets (Paper III).

- To investigate the molecular mechanisms of transcription factor PDX-1 action in short-term glucose-dependent regulation of insulin gene transcription (Paper IV).

3 MATERIALS AND METHODS

3.1 Drugs (Paper I, II)

Pioglitazone (Actos®) was generously provided by Takeda Pharmaceuticals North America (Deerfield, IL), while exenatide (Byetta®) was graciously donated by Amylin Inc. (San Diego, CA).

3.2 Cell culture

3.2.1 MIN-6 cells (Paper IV)

MIN-6 cells [167] were obtained from Dr J. Miyazaki (Dept. of Nutrition and Physiological Chemistry, Osaka University School of Medicine, Osaka, Japan) and were adopted to culture in Dulbecco's modified Eagle medium (DMEM) containing 10 % fetal calf serum, 100 μg/ml streptomycin and 100 U/ml penicillin, at 5 % CO_2 and 37°C.

3.2.2 Pancreatic islets

3.2.2.1 Rat pancreatic islets (Paper I and III)

Rat pancreatic islets were isolated by collagenase digestion [168] from approximately 3-month-old male diabetic GK and control Wistar rats, purchased from Taconic Europe (barrier EBU 202, Bomholt site, Ry, Denmark). Isolated islets were cultured for 48 hrs at 5 % CO_2 and 37 °C in RPMI 1640 medium, supplemented with 1 % fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 8 mM glucose (paper I) or 3 mM or 20 mM glucose (paper III). All experiments were approved by the local animal ethics committee.

3.2.2.2 Human pancreatic islets (Paper II)

Human diabetic and non-diabetic pancreatic islets were kindly donated by the Uppsala University facility for isolation of human islets from Scandinavian brain-dead donors. The islets were maintained in RPMI 1640 medium, supplemented with 1 % fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 6 mM glucose, at 5 % CO_2 and 37 °C. All experiments were approved by the Karolinska human ethics committee/IRB.

3.3 Online monitoring of GFP by laser scanning confocal microscopy (Paper IV)

Laser scanning confocal microscopy was performed by using a Leica CLSM confocal microscope (Leica Lasertechnik, Heidelberg, Germany) to detect GFP in transfected cells [169]. The following settings were used for the confocal microscope: 100U/1.30 oil Leitz Fluotar objective lens, excitation wavelength 488 nm (krypton/argon laser), excitation filter HQ470/40, dichroic mirror Q495LP, and emission filter HQ525/50.

3.4 Plasmids and transfection (Paper IV)

The plasmid construction details can be found in the respective section of paper IV.

Transfection of MIN-6 cells, grown on 24 mm glass cover slips, was carried out in a 35 mm culture dish by utilizing the lipofectamine technique [170].

3.5 Quantitative RT-PCR (Paper I, II, III)

Total RNA was extracted with the Qiagen RNeasy kit (WVR, Stockholm, Sweden) according to the manufacturer's instructions. The integrity of the extracted RNA was confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Double-stranded cDNA was synthesized with 50 ng of total RNA using the SuperScript Choice system (Invitrogen Inc., Carlsbad, CA).

Quantitative RT-PCR was performed using an ABI Prism 7700 PCR machine (PE Applied Biosystems, Foster City, CA). Selected genes and endogenous reference gene(s) primers and hybridization probes were used (Perkin–Elmer Biosystems, Warrington, UK). These genes were selected based on differential expression grounded on statistical analysis and fold change threshold, a select range of intensities, biological interest and potential roles in diabetes, and primer availability. 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. The relative quantification of genes was determined by using the arithmetic $2^{-\Delta\Delta CT}$ formula according to the Applied Biosystems Instruction Manual of 1997. The expression amount was adjusted to the endogenous reference gene(s) and the values are expressed relative to a reference sample (Paper I, II, and III) (Fig 9).

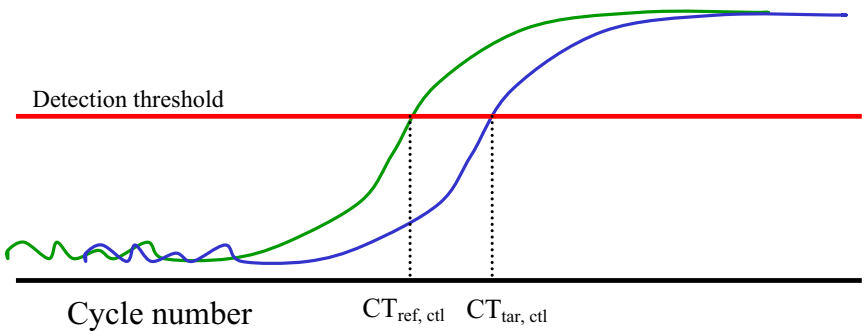

Figure 9. The relative abundance of a target gene to a reference gene in any RNA, e.g. a "control" RNA or a "sample" RNA.

3.6 Insulin content and insulin secretion determination

For short term insulin release experiments, duplicate batches of 10 cultured islets were selected and preincubated at 37 °C for 45 min in Krebs–Ringer bicarbonate buffer (KRB buffer), supplemented with 2 mg/ml BSA, 3 mM glucose and 10 mM HEPES (pH 7.4). The pre-incubation media were discarded and the incubation continued for another 60 min in fresh buffer (3 mM glucose). The medium was collected at the end of incubation and was frozen for the subsequent analysis of insulin concentration. Fresh media, now containing 20 mM glucose, were added to the same islets and incubation continued for another 60 min. At the end of incubation, media were frozen for subsequent analysis of their insulin concentration by ELISA. The islets were then retrieved, ultrasonically disrupted and extracted in acid ethanol for determination of insulin content by ELISA.

3.7 Gene array (Paper I, II, III)

Paper I, II

Sample preparation and processing procedures were performed according to the Affymetrix GeneChip Expression Analysis Manual (www.affymetrix.com) at the microarray core facility, Karolinska Institutet. Briefly, total RNA was extracted with the Qiagen RNeasy kit (WVR, Stockholm, Sweden) according to the manufacturer's instructions. The integrity of the extracted RNA was confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Double-stranded cDNA was synthesized with 50 ng of total RNA using the SuperScript Choice system (Invitrogen Inc., Carlsbad, CA). Synthesis of single-stranded complementary DNA (cDNA) and purification of double-stranded cDNA, synthesis and isolation of biotin-labeled complementary RNA (cRNA), quantification and fragmentation of cRNA, and target hybridization of fragmented cRNA, washing and staining of GeneChip expression probe arrays were all performed according to the manufacturer's instructions (Technical manual of

Affymetrix GeneChip products). Samples were analyzed by the Affymetrix Rat 230 GeneChip containing 31,000 probe sets (Paper I) and by the Affymetrix Human Genome U133 Plus 2.0 Array containing over 47,000 transcripts (Paper II). The level of expression of each gene was revealed by the intensity of hybridization of labeled mRNA. The probe arrays were scanned using the Agilent Gene Array Scanner (Agilent Technologies) and the scanned images were inspected and analyzed using established quality control criteria according to the manufacturer's instructions.

Paper III

Processing of total RNA samples and GeneChip experiments were carried out at Clinical Pharmacogenetics Department, Novartis Pharmaceuticals Corporation (Gaithersburg, MD), essentially as recommended by Affymetrix. 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 sec, 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 7,500 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 of buffer RLT (lysis buffer) with 2-mercaptoethanol, followed by addition of 250 µl 100 % ethanol. The samples were applied to the column and centrifuged for 15 sec at ≥ 8,000 g, with this step repeated with the flow-through. The column was washed twice with 500 µl of buffer RPE (washing buffer) and 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. A starting amount of 10 µg total RNA was used for the synthesis of double stranded cDNA with a commercially available kit (SuperScript™ double-stranded cDNA synthesis kit; Invitrogen Life Technologies, Basel, Switzerland) according to manufacturer's instructions, in the presence of a poly-T primer containing a T7 phage RNA polymerase promoter. The cDNA was then purified on an affinity resin (QIAquick, Qiagen; Basel, Switzerland). The purified cDNA was then transcribed *in vitro* (Bioarray high yield T7 DNA transcription kit; ENZO, Farmingdale, NY) with incorporation of biotinylated ribonucleotides, rUb and rCb. The labeled cRNA was then purified on an affinity resin (RNeasy, Qiagen; Basel, Switzerland), quantified and fragmented by chemical hydrolysis into strands of approximately 50-100 nucleotides. Ten µg of labeled cRNA was hybridized for approximately 16 hours at 45 ºC to an expression probe array. The array was then washed, stained with streptavidin-R-phycoerythrin (SAPE, Molecular Probes; Eugene, OR) and the signal amplified using a biotinylated goat anti-streptavidin antibody (Vector Laboratories; Burlingame, CA) 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; Palo Alto, CA) at an excitation wavelength of 488 nm and emission recorded at 570 nm, resulting in one scanned image (Fig 10).

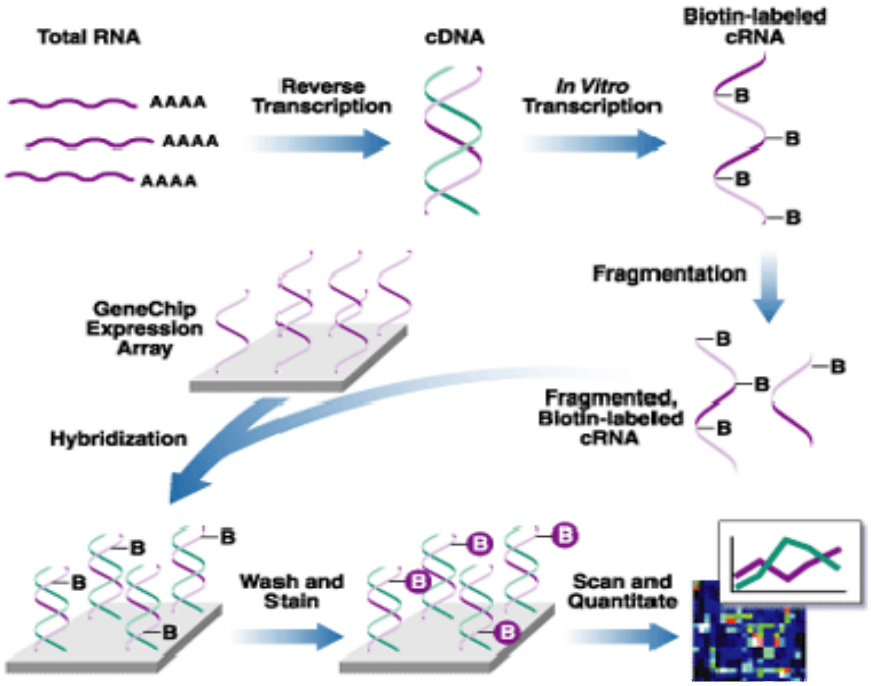

Figure 10. Steps of the expression assay.

GeneChip® Microarrays curriculum, Activity #2. (www.Affymetrix.com)

3.8 Microarray data analysis (Paper I, II, III)

Data normalization and statistical analysis were performed by using GeneSpring 7.3 software (Agilent, Santa Clara, CA) or Affymetrix Microarray Analysis Suite 5.0 software (MAS 5.0) (Affymetrix Inc., Santa Clara, CA). The data were normalized by utilizing the 50th percentile (per chip normalization) and the normalization to median (per gene normalization). To determine genes differentially expressed across conditions, Student's *t*-test, the ANOVA test (considering False Discovery Rate, FDR) at *P* values <0.05, and a fold change of >1.5 (Paper

I, II) or >1.1 (Paper III) were performed. Moreover, these genes were assigned to functional groups as described in GeneSpring (Agilent Technologies), Affymetrix Microarray Analysis Suite 5.0 software (MAS 5.0) (Affymetrix Inc., Santa Clara, CA), NetAffx (Affymetrix), and other databases. The differentially regulated transcripts were annotated and then clustered according to functional annotation and average fold change.

3.9 Statistical analysis

Probabilities of chance differences between the experimental groups were analyzed by using Student's *t*-test for paired or unpaired data, where $P<0.05$ was deemed statistically significant. Values are given as the mean ± SEM.

4 RESULTS AND DISCUSSION

Paper I and II

Effects of palmitate, pioglitazone and exenatide on gene expression in pancreatic β-cells

The mechanisms underlying the effects of FFAs on β-cell failure, and β-cell mass reduction, remain controversial. Whether glucose and FFAs alter β-cell gene expression and thereby its function synergistically or separately, or require a genetic predisposition, needs to be addressed. To investigate these issues, we examined the influence of prolonged exposure of FFAs on islet gene expression, and also the impact of pioglitazone and exenatide on this process, at physiological glucose concentrations.

Global gene expression profiling in islets from diabetic GK and normal Wistar rats, and also from diabetic and non-diabetic human donors, revealed changes in the expression of genes involved in epigenetic regulation of cell proliferation and differentiation, metabolism, response to stimulus, transport, and signal transduction.

Among many interesting genes differently regulated in diabetic GK *vs.* normal Wistar rat islets of potential pathogenetic significance were (Paper I):

- X-box binding protein 1 (*Xbp1*) that triggers an endoplasmic reticulum (ER) stress response, which could lead to β-cell death in T2D [171]. Furthermore, recent studies indicate the protective role of exenatide [172] and rosiglitazone [173] against ER stress-associated β-cell death through reduction of genes related to the islet ER stress. In agreement with these studies, *Xbp1* was down-regulated by both pioglitazone and exenatide when incubated with palmitate in our Wistar rat islets. Moreover, *Xbp1* was induced by palmitate in Wistar islets. Thus, pioglitazone and exenatide may afford protection against the apoptotic effect of palmitate by down-regulating *Xbp1* expression in Wistar islets.
- Pyruvate dehydrogenase kinase isoenzymes 2 (*Pdk2*) and 4 (*Pdk4*), which are involved in glucose oxidation and insulin secretion through pyruvate dehydrogenase [174-176]. In the current study *Pdk2* was up-regulated by palmitate in GK islets, while *Pdk2* and *Pdk4* were down-regulated in diabetic GK islets by both pioglitazone and exenatide in the presence of palmitate. Since *Pdk*s exert an inhibitory effect on islet glucose metabolism, through repression of pyruvate dehydrogenase that governs mitochondrial glucose oxidation [174, 175], down-regulation of *Pdk*s by pioglitazone and exenatide could lead to enhanced activity of pyruvate dehydrogenase and thus result in improved glucose oxidation and enhanced insulin secretion.
- Neurogenic differentiation 1 (*Neurod1/BETA2*) plays a role in pancreas development and also in insulin gene transactivation in the β-cell through interaction with PDX-1 [177-179]. Interestingly, targeted disruption of the *Neurod1/BETA2* gene causes severe diabetes in mice and they die 3–5 days after birth. Additionally, *Neurod1/BETA2* null mice exhibit a severe reduction of β-cells and fail to develop

mature islets [180]. Our gene expression profiling revealed a reduction of *Neurod1/BETA2* gene expression by pioglitazone in GK islets in the presence of palmitate, the expression being enhanced when incubated just by palmitate. Our finding with palmitate is in agreement with previous work [181] and this up-regulation of *Neurod1/BETA2* gene expression could be due to the metabolic milieu, *e.g.* exposure to palmitate, the presence of the diabetic condition, or both. Furthermore, pioglitazone seems to normalize the expression level of *Neurod1/BETA2*, thereby potentially restoring insulin gene expression and promoting β-cell survival in our diabetic GK islets. Taking into consideration the importance of *Neurod1/BETA2* in insulin gene expression, and in the morphogenesis of pancreatic islets, it makes *Neurod1/BETA2* a potential therapeutic target in T2D.

- Interleukin-1 (*IL-1*) receptor type I (*Il1r1*), a transmembrane receptor for IL-1 that binds IL-1β (a proinflammatory cytokine), which inhibits β-cell function and promotes β-cell apoptosis [182]. Palmitate induced the expression of *IL1r1* in both GK and Wistar islets, which may eventually invoke β-cell dysfunction and death by augmenting IL-1β toxicity. *IL1r1* up-regulation by palmitate was reduced by pioglitazone and exenatide in our GK islets, whereas in Wistar islets only by pioglitazone. These observations suggest that the protective effect of pioglitazone and exenatide against β-cell destruction and dysfunction may involve reduced islet expression of *IL1r1* and thereby inhibition of *IL-1* binding and its deleterious actions.
- Alluding to the previous point, *IL-1* receptor antagonist (*Il1rn*/*IL-1ra*) is a naturally occurring antagonist of *IL-1* that prevents a biological response to *IL-1* [183]. The expression level of *Il1rn* was increased in our GK islets and reduced in Wistar islets when exposed to palmitate. Considering a previous report where islets from type 2 diabetic patients exhibit a decreased expression of *Il1rn* [184], our results could reflect

the impact of palmitate and/or the diabetic state. Interestingly, both pioglitazone and exenatide were able to normalize *Il1rn* expression in islets exposed to palmitate. Hence, pioglitazone and exenatide may protect islets against the deleterious influence of palmitate through decreasing the expression and the availability of *IL1r1* and at the same time increasing the expression and activity of *Il1rn* to further limit the detrimental impact of *IL-1*.

To confirm the gene regulation observed with microarray analysis, we performed qRT-PCR on samples for selected genes that were differently regulated on the microarrays. The results generated by qRT-PCR showed a highly significant correlation between the levels of gene expression measured by both methods for *Cacna1e* (*$Ca_V2.3$*), *PPAR β/δ*, *Kcnj10* (*Kir1.2*, *Kir4.1*), *HNF4α*, *PDK2*, and *Xbp1* (paper I).

Among many interesting genes of potential pathogenetic significance differently regulated in diabetic *vs.* non-diabetic human islets were (Paper II):

- Transcription factor 7-like 2 (*TCF7L2*/*TCF4*), involved in β-cell proliferation [185] and insulin secretion [186], and identified as the most important T2D susceptibility gene to date [25, 187]. It is known to be up-regulated in human islets from type 2 diabetic donors, and its overexpression in the β-cell reduces insulin secretion [186]. These findings are entirely compatible with our current data, indicating FFA-induced up-regulation of *TCF7L2*/*TCF4* in diabetic islets. Its normalization by pioglitazone and exenatide might furthermore be one way by which these drugs exert beneficial effects in the human β-cell and thus could be part of their antidiabetogenic actions (Fig. 11).

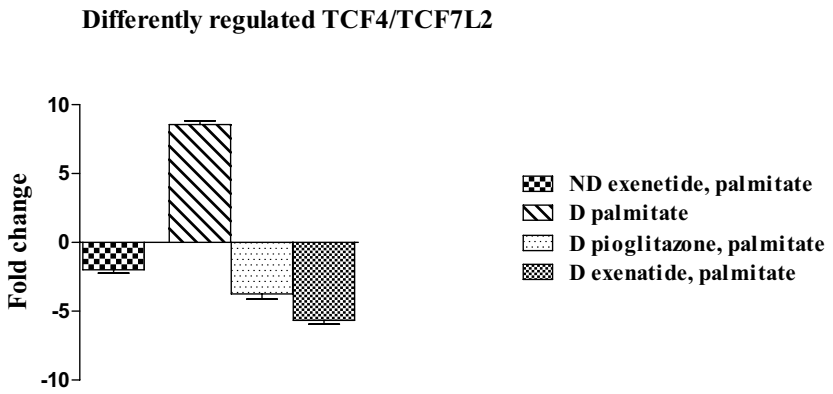

Figure 11. Differently regulated TCF4/TCF7L2 in human islets.
TCF4/TCF7L2 gene expression remained unchanged in the presence of palmitate and palmitate + pioglitazone in non-diabetic islets. D, diabetic islets; ND, non-diabetic islets.

- Regenerating islet-derived 3 alpha (*REG3A*) is a pancreatic secretory protein that seems to mediate β-cell proliferation and differentiation [188]. Both pioglitazone and exenatide induced *REG3A* expression in islets exposed to palmitate. Possibly, pioglitazone and exenatide contribute to maintenance of β-cell mass through induction of *REG3A* when islets become exposed to lipotoxicity, and so signaling through the *REG3A* pathway might be yet another mechanism by which these drugs evoke their salutary influences on the β-cell.
- Growth arrest and DNA-damage-inducible beta (*GADD45B*), involved in β-cell growth arrest, DNA repair, apoptosis [189], and coactivation of RXR or PPAR [190]. Both pioglitazone and exenatide reduced *GADD45B* expression in diabetic islets exposed to palmitate. This may reflect that pioglitazone and exenatide, by normalizing the overexpression of *GADD45B* by palmitate, attempt to restore the balance between apoptosis and proliferation/survival in these human islets.

- Thioredoxin interacting protein (*TXNIP*) that mediates oxidative stress [191], and is negatively influencing β-cell survival and insulin secretion [192-195]. Both pioglitazone and exenatide were able to normalize the overexpression of *TXNIP* induced by palmitate in diabetic islets and it is conceivable that this mechanism might in part explain the positive impact of these drugs in terms of β-cell funtion and insulin secretion in human subjects.
- Aryl hydrocarbon receptor nuclear translocator (*ARNT* [a.k.a. *HIF-1β*]) expression has previously been reported to be significantly decreased in islets from type 2 patients, *ARNT* gene silencing in isolated β-cells was found to grossly impair glucose-stimulated insulin secretion, and *ARNT* knock-out animals showed severe glucose intolerance [196]. Interestingly and consistent with these previous findings, *ARNT* down-regulation by palmitate was normalized by both pioglitazone and exenatide in our diabetic islets (Fig. 12). This may indicate *ARNT* as one important molecular target for pioglitazone and exenatide in human islets, by which these drugs maintain optimal insulin secretion and β-cell function in diabetic patients.

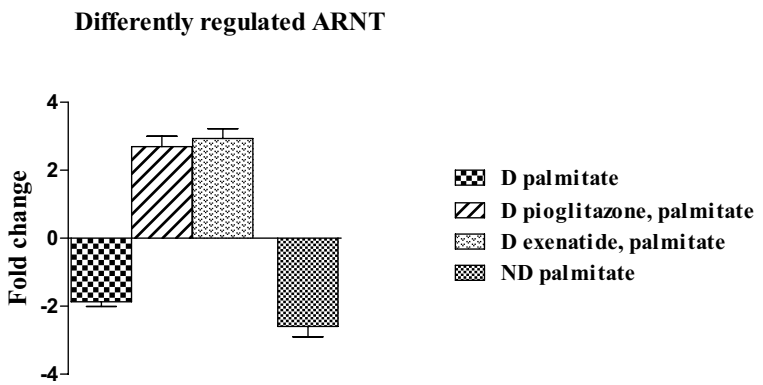

Figure 12. Differently regulated ARNT in human islets.
ARNT gene expression remained unchanged in the presence of palmitate and pioglitazone + exenatide in non-diabetic islets. D, diabetic islets; ND, non-diabetic islets.

- Acetyl-Coenzyme A carboxylase alpha (*ACACA*) is essential in nutrient-induced insulin secretion [197, 198]. Both pioglitazone and exenatide up-regulated *ACACA* gene expression suppressed by palmitate in diabetic islets (Fig. 13). This indicates the normalizing effect by these drugs on palmitate-induced imbalance between fatty acid breakdown and synthesis by increasing lipogenesis, thereby probably positively impacting long term β-cell function and survival by ameliorating lipotoxicity and restoring normal glucose sensitivity in these human islets.

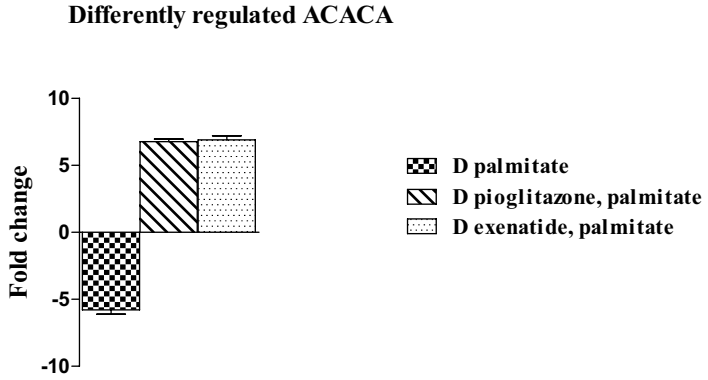

Figure 13. Differently regulated ACACA in human islets.
ACACA gene expression remained unchanged in the presence of palmitate and palmitate + pioglitazone/exenatide in non-diabetic islets. D, diabetic islets.

To confirm the gene regulation observed with microarray analysis, we performed qRT-PCR on samples for selected genes that were differently regulated on the microarrays. The results generated by qRT-PCR showed a highly significant correlation between the levels of gene expression measured by both methods for *GADD45B*, *GAPDH*, *PPY*, *REG3A*, *TXNIP*, *CEL*, *ARNT*, *ISL1*, *TCF7L2*/*TCF4*, and *DAD1*(paper II).

Epigenetic regulation of gene expression turned out to be the largest transcript group in this study and most influenced by palmitate, pioglitazone and/or exenatide, consisting of genes involved in cell proliferation and differentiation, metabolism, response to stimulus, transport, and signal transduction pathways.

Environmental influences, such as consumption of high-caloric foods, sedentary life style and obesity, and consequently, chronically elevated levels of glucose and fatty acids, collectively create an environment that may contribute to insulin resistance and β-cell dysfunction [199-202]. Taking into account that population gene pools shift quite slowly, whereas exposure to adverse environments is increasing and occurs rapidly, the increased diabetes prevalence may suggest that transgenerational epigenetic responses controlled by environmental factors might be significantly contributing to the development of T2D [203]. Epigenetic modifications of the genome through changes in gene expression allow an organism to respond to the environmental factors, which seem to be involved in β-cell failure in T2D [204]. Given that fatty acids are not merely passive energy-providing fuels but are also metabolic regulators, controlling the activity or abundance of key transcription factors, it may seem likely that FFAs influence the epigenetic regulation of gene expression involved in T2D.

The expression of genes in apoptotic pathways was not noticeably influenced by palmitate in our study, possibly due to the absence of chronically elevated level of glucose (*i.e.,* concomitant glucotoxicity). Previous studies have suggested that only under conditions of elevated glucose levels, prolonged exposure to pathological levels of fatty acids results in accumulation of triglycerides in β-cells and impairment of insulin secretion, insulin gene expression, and cell viability [65, 105, 205-208]. Thus, these findings could reflect that lipotoxicity may require concomitant hyperglycemia (*i.e.*, glucolipotoxicity) to exert harmful effects on β-cell gene expression and function, unless abnormalities in fatty acid metabolism exist due to a certain genetical background (Fig. 14). A genetic predisposition seems to be

required for the inhibitory effect of chronically elevated plasma FFAs on β-cell gene expression and function [105]. Also, lipotoxicity appears more prominent in individuals with a genetic predisposition to obesity and T2D [209-214], who manifest abnormalities in lipolysis and lipid oxidation [133, 215, 216].

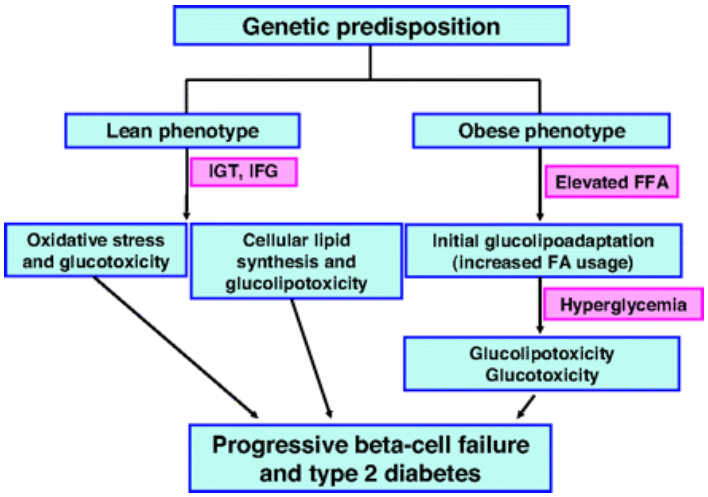

Figure 14. Contribution of glucotoxicity and glucolipotoxicity to the development of type 2 diabetes.

In this hypothesis, lean and obese individuals who develop type 2 diabetes primarily have polygenic defects that predispose them to the disease. Because of genetic abnormalities the lean phenotype develops impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG), which exposes the β-cell to chronic hyperglycemia. Hyperglycemia generates ROS which cause oxidative stress and worsened β-cell dysfunction. Alternatively but not exclusively, abnormally high blood glucose concentrations cause increased cellular lipid synthesis and glucolipotoxicity, which also causes deterioration in β-cell function. The obese phenotype has intrinsically elevated blood free fatty acid (FFA) levels which causes the β-cell to switch to preferential fatty-acid metabolism (glucolipoadaptation). Over time, however, the β-cell can no longer adapt and glucolipoadaptation evolves toward glucolipotoxicity. This in turn leads to hyperglycemia, which eventually overwhelms the β-cell and leads to its frank dysfunction. FA, Fatty acid.

Regarding the outcomes from these studies, pioglitazone and exenatide may exert potential salutary effects on preservation and augmentation of β-cell mass against the detrimental impact by lipotoxicity through regulation of genes controlling proliferation, growth, differentiation, thus maintaining the balance between β-cell apoptosis and proliferation. Considering that these two drugs target different components of the epigenetic machinery, our findings suggest that they can be of complimentary utility in restoring normal gene activity and may protect the human β-cell from lipotoxic insult by mechanisms that may also have significance in the clinical management of T2D in therapeutical efforts aiming at conferring β-cell protection. Finally, since the epigenetic misregulations involved in the etiology of disease seem to be reversible, they can be utilized for clinical diagnostics and also as therapeutic targets in preserving or restoring β-cell function in the treatment of T2D.

Paper III

Effects of glucose on gene expression in normal and diabetic rat β-cells

Glucotoxicity, *i.e.*, chronic hyperglycemia, causes adverse structural and functional changes in cellular components of insulin production and may contribute to the progressive impairment of insulin secretion characteristic of β-cells in T2D [218, 219]. Furthermore, glucotoxicity may evoke impairments in insulin gene expression and secretion, and also in β-cell glucose-regulated gene expression. The molecular mechanisms by which chronic glucose toxicity induces these β-cell lesions remain, however, poorly understood and need to be addressed in great detail.

The current study (III) was performed to identify the molecular mechanisms involved in long-term glucose regulation of normal and diabetic β-cell gene expression, at basal and supra-physiological glucose concentrations *in vitro*. Gene expression analyses in islets from diabetic GK and non-diabetic Wistar rats revealed changes in glucose-regulated gene expression involved in glucose metabolism, signaling, transport, incretin action, glucocorticoid handling,

apoptosis, transcription, proliferation and immune response that clearly distinguish diabetic animals from controls. These findings indicate that after long-term exposure to high glucose, the expression of many islet genes is altered and this may result in an altered β-cell phenotype. In this investigation we used a gene array with a larger number of transcripts compared with other studies, which illustrates a broader picture of the complex dynamics of β-cell adaptation to changes in glycemia.

Among many interesting genes differently regulated in this study were:

- ATP-binding cassette, sub-family C, member 8 (*ABCC8/SUR1*) and its potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11/KIR6.2*) were up-regulated by high glucose in GK rat islets, which may contribute to the attenuation of glucose-sensitive insulin secretion characteristic of GK rat islets.
- Hydroxysteroid (11-beta) dehydrogenase 1 (*HSD11B1*) [74, 76] and serum/glucocorticoid regulated kinase 1 (*SGK1*) [89], that are key elements in steroid metabolism, important effectors of β-cell glucocorticoid handling, and known to influence glucose-sensitive insulin secretion, were abnormally expressed in diabetic GK islets. The upregulation of these two genes in diabetic islets may enhance not only local islet synthesis of glucocorticoids (through *HSD11B1*), in effect creating an intracellular Cushing syndrome, but also their downstream effects (through effector targets such as *SGK1*) that can all adversely impact β-cell function and viability (see Section 1.4 above).
- The potassium voltage-gated channel, Shab-related subfamily, member 1 (delayed rectifier potassium channel, *Kv2.1*) (*KCNB1*) is the dominant K_v in rodent and human islets, contributing to some 85 % of their steady-state outward current, and thereby negatively regulates Ca^{2+} dynamics and insulin secretion [220]. The up-regulation of *KCNB1 (Kv2.1*) by high glucose in GK islets is thus 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 [221], it is possible that *KCNB1 (Kv2.1*) overexpression is an intrinsic β-cell defect in the GK rat that contributes to its loss of glucose sensitivity.

- The *CD36* gene that was up-regulated by high glucose in GK islets, might be involved in β-cell dysfunction, given that *CD36* functions as a transporter for oxidized LDL-cholesterol, suppresses insulin gene transcription and promotes β-cell death [222, 223]. *CD36* facilitates the major fraction of long-chain FFA uptake into β-cells, in which it was found to specifically mediate the inhibitory effects of FFA on insulin secretion [224]. This tantalizing connection, *i.e.,* the upregulation of FFA uptake by high glucose in diabetic islets, could thus provide an inextricable link between glucotoxicity and lipotoxicity. Although GK rats are not hyperlipidemic [225], the increased *CD36* expression may promote functional suppression and possibly lipoapoptosis as part of glucolipotoxicity [71, 102]. In accordance with such a scenario, previous reports indicate that endogenous FFA catabolism is greater in GK islets than in Wistar islets [226], and that high-fat feeding impedes glucose-sensitive insulin secretion in islets from GK rats while not affecting Wistar islets [105].
- Expression of the gastric inhibitory polypeptide receptor (glucose-dependent insulinotropic polypeptide receptor) (*GIPR*) was lower in GK low glucose compared to GK high glucose islets. This suggests a tonic reduction of incretin signaling in GK islets, potentially a major contributor to the islet defect in this diabetes model.
- Several important effectors of β-cell fuel sensing, protein phosphorylation and exocytosis, such as phosphoenolpyruvate carboxykinase (*PEPCK/PCK*), protein kinase, AMP-activated, beta 1 non-catalytic subunit/ beta 2 non-catalytic subunit (*PRKAB1, PRKAB2*), protein kinase C (*PRKC*) isoforms, and phospholipase C, beta 1

(*PLCB1*) were also dysregulated in GK islets (see paper III for details), which might contribute to β-cell failure and the diabetic phenotype in this animal model.

- Several potentially critical factors controlling cell proliferation and apoptosis were also aberrantly expressed in GK islets: Cyclin D1 (*CCND1*), insulin-like growth factor binding protein 3 (*IGFBP3*), and Apolipoprotein E (*APOE*), yet other defects that could be involved in β-cell dysfunction and appearance of diabetes in these animals (see paper III for details).

In order to validate the qualitative changes in gene expression revealed by the microarray analyses, the expression of selected genes was also confirmed by realtime qRT-PCR. The same expression pattern as that identified by the microarray analyses was found by realtime qRT-PCR for *HSD11B1*, *SGK1*, *IGFBP3*, and *APOE*.

Using pancreatic islets isolated from non-obese normal and T2D animal models provides a unique opportunity to identify and study primarily β-cell lesions, *i.e.,* the β-cell genes associated with impaired glucose-induced insulin secretion, avoiding potential confounding influence of significant obesity-associated insulin resistance. Most studies of this kind in the past have been performed in transformed β-cell lines that may be far from optimal for determining gene expression patterns associated with diabetes in native β-cells. Insulinoma cell lines are notoriously poor substitutes for studies of normal β-cell function, basically because they are tumors whose gene expression and growth pattern are grossly deranged.
Genetic alterations in expression profiles of β-cells result in phenotypic changes; therefore, identification of the genes that encode differentially expressed transcripts may lead to the identification of underlying genetic differences accounting for diabetes. The identification of the glucose-responsive β-cell genes involved in this animal model may provide clues to the

pathogenesis of human T2D, and also how the abnormal β-cell function, regenerative potential and viability can be restored to normal by selective drugs and may thus represent targets for pharmacological intervention against β-cell failure.

Paper IV

Identification of amino acid sequences responsible for the nuclear translocation of transcription factor PDX-1 in β-cells

PDX-1 regulates, in concert with other transcription factors, the expression of the insulin gene and a number of other genes involved in maintaining β-cell identity and function. Moreover, short-term activation of insulin gene transcription in response to changes in glycemia seems to be mediated through nuclear translocation of the PDX-1 [164, 227]. The molecular basis and the mechanism of PDX-1 nuclear translocation remain poorly understood and need to be addressed. Both phosphorylation/dephosphorylation and the presence of a nuclear localization signal (NLS), very often formed by a stretch of the positively charged amino acids arginine and lysine, have been shown to be involved in nuclear translocation of transcription factors [228]. Phosphorylation of transcription factors is one of the mechanisms to unmask a hidden NLS and thereby allow for binding of the cargo to its receptor. This mechanism seems also to be involved in the nuclear translocation of PDX-1, since it has been reported that translocated PDX-1 is phosphorylated [227]. The NLS sequence is usually recognized and targeted by a receptor, karyopherin or importin, in the cytoplasm and later translocates into the nucleus through the nuclear pore complex by the GTPase Ran [229, 230].

In order to identify amino acid residues in PDX-1 that are responsible for its nuclear translocation, site-directed mutagenesis of putative phosphorylation sites and positively charged amino acid residues in putative NLS motifs of GFP-tagged PDX-1 was performed. The glucose-stimulated nuclear translocation of each mutant was studied by using laser scanning confocal microscopy.

Analysis of the mouse PDX-1 amino acid sequence did not reveal a classical NLS that fulfills all the named requirements. Our investigation revealed that not a specific phosphorylation site, but rather the presence of the NLS motif RRMKWKK, is necessary and, in conjunction with the integrity of the `helix 3' domain of the PDX-1 homeodomain, sufficient for nuclear translocation of PDX-1 in insulin-producing MIN-6 cells. The position of the NLS sequence, *i.e.,* being part of the homeodomain, is in agreement with a previous observation [162].

Considering the reported glucose-stimulated nuclear translocation of PDX-1, and a 15 kDa shift in molecular weight after translocation by Macfarlane *et al.* [227], we were unable to observe any glucose-dependent cytoplasmic-nuclear cycling of PDX-1. This could be due to the fact that the localization of freshly translated cytoplasmic PDX-1 molecules would have been missed in our detection system, because the formation of the GFP fluorophore (tagged to PDX-1) takes 30-60 min after translation. Therefore, the observed glucose-dependency in [227] may reflect the combination of the glucose-dependency of PDX-1 biosynthesis and its energy-dependent nuclear translocation rather than a glucose-dependent cytoplasmic-nuclear cycling of PDX-1.

5 CONCLUSIONS

- At physiological glucose concentrations, the TZD PPARγ agonist pioglitazone and/or the incretin analog and GLP-1 receptor agonist exenatide modulate changes evoked by the fatty acid palmitate (as a model of lipotoxicity) in normal and diabetic rodent and human β-cell gene expression. These include genes involved in epigenetic regulation of gene expression, cell proliferation and differentiation, metabolism, response to stimulus, transport, and signal transduction (Paper I, II).

- Lipotoxicity may require hyperglycemia (*e.g.*, glucolipotoxicity) to exert harmful effects on β-cell gene expression and function, unless abnormalities in fatty acid metabolism exist due to a genetic predisposition to obesity and T2D (Paper I, II).

- Pioglitazone and exenatide appear to target different components of the β-cell lipid-responsive genes and seem to restore normal gene expression in human and rat islets, which may protect the β-cell from lipotoxic insult known to occur in T2D (Paper I, II).

- The glucose-responsive β-cell genes involved in glucose metabolism, signaling, ion transport, incretin action, glucocorticoid handling, apoptosis, transcription, proliferation and immune response clearly distinguish diabetic animals from non-diabetic controls (Paper III).

- Long-term exposure to high glucose alters the expression of many islet genes, which may result in an altered β-cell phenotype (Paper III).

- The NLS motif RRMKWKK is necessary and, in conjunction with the integrity of the `helix 3' domain of the PDX-1 homeodomain, is sufficient for the nuclear import of PDX-1 in MIN-6 β-cells (Paper IV).

6 ACKNOWLEDGMENTS

This work was carried out at the Department of Clinical Science and Education, Karolinska Institutet, Södersjukhuset.
I would like to express my sincere gratitude to all of you who have supported me during these years and made this thesis possible. Especially, I would like to thank:

Professor **Åke Sjöholm**, my great boss and chief supervisor, for never ending enthusiasm, strong leadership, encouragement and support, for providing an excellent research environment, for immense knowledge in diabetes, for great scientific advice and guidance, and also for giving me scientific freedom and believing in me.

Dr. **Qimin Zhang**, my co-supervisor, for many valuable discussions, comments and advice.

Docent **Ingo Leibiger**, for sharing your knowledge in molecular biology and teaching me the basics of diabetes and scientific thinking.

Dr. **Tilo Moede**, my co-author, for teaching me everything about confocal microscopy.

Dr. **Zhen Huang**, my co-author, for helping me with the islet isolation, and also for all other non-scientific discussions about fashion and hand bags.

Lotta Engström and **Mia Landström**, for your kindness, and for helping me with animals.

Professor **Sari Ponzer**, the previous chairman of the Department of Clinical Science and Education, and Professor **Göran Elinder**, the current chairman of the Department, for giving me the opportunity to do my Ph.D. work at this department and providing such an excellent research environment.

All administrative staff at KI/SÖS, especially **Anita Stålsäter-Pettersson**, **Lina Rejnö**, **Anne Edgren**, **Anne Kaskela**, **Monika Dahlberg**, **Viveca Holmberg**, **Christer von Essen**, **Matts Jonsson**, **Hans Pettersson** and **Stefan Rosfors** for being extremely kind and helpful during all of these years.

All the former Ph.D. students in the Rolf Luft Center, for creating a pleasant working environment, for the wonderful social activities and all the fun we had. I never forget you.

Lotta Larson, Christina Häll, and Monica Nordlund for being so lovely and kind to me, for our lunches, coffee breaks and talks.

My colleagues: **Tony, Annika, Özlem, Nina, Henrik**, **Lotta, Jeannette,** and **Mohamed** for chats and support. Especially **Nina**, for ordering all the things that I needed for my work. **Jeannette**, for keeping the Research Center in order and fixing everything. **Özlem**, for helping me with the Western blot.

All of my dear friends (not necessarily in order):

Sodabeh, for your friendship, for being always my friend (like a sister), for believing in me and being there for me especially when my mother passed away, and for all the great memories.
Mina, for your friendship, and for all the wonderful memories.
Arezoo, for your friendship, and our lovely childhood memories together.

Morvarid, my dear friend, for your friendship, for our time in Uppsala and the wonderful memories.
Nasrin, for your friendship, for being there for me in the past, and for all the lovely memories.
Julia and **Belia**, my dear friends, for your friendship, for your support and encouragement, for your advice, for believing in me, for our talks and wonderful memories.
Sagarika and **Behrous D.**, for your friendship, for all talks and advice, and the great time before moving to the U.S.
Amanda J., for your friendship and support.
Jessica, for your friendship, for your support and understanding, for all talks and advice about microarrays.
Roshan, Camelia, and **Dipu,** my dear friends, for your friendship, for being there for me, for all talks and great memories.
Mio caro amico **Carletto**, per la tua amicizia, per capirmi di tutto che ho passato sempre ascoltando quando ho avuto dei problemi, per i buoni consigli, per il appoggio e incoraggiamento e per ogni bellissimo ricordo che noi abbiamo passato assieme.

All my dear **uncles**, **aunts**, my **cousins**, and **second-cousins**. Especially, my **youngest aunt** and her daughters; **Mina, Mitra**, and **Mojgan** for allways being there for me, for your love, for your support and encouragement. My cousin, **Beigom**, for your love and support. My **uncle in NY**, for your never ending support and encouragement.

My amazing, loving **family**; my **father**, and my **brothers**. I can not put my feelings and my appreciation into words. I would not be here without your love.

My amazing, gracious, loving, beautiful **mother**; I am who I am because of you and your love. You gave me wings to fly but strength and values to stay steady.

7 REFERENCES

1. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414** (6865): 782-7.

2. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes* 1995; **44** (11): 1249-58.

3. Kahn SE, Haffner SM, Heise MA, Herman WH, Holman RR, Jones NP, Kravitz BG, Lachin JM, O'Neill MC, Zinman B, Viberti G. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med* 2006; **355** (23): 2427-43.

4. Efanova IB, Zaitsev SV, Zhivotovsky B, Kohler M, Efendic S, Orrenius S, Berggren PO. Glucose and tolbutamide induce apoptosis in pancreatic beta-cells. A process dependent on intracellular Ca2+ concentration. *J Biol Chem* 1998; **273** (50): 33501-7.

5. Maedler K, Carr RD, Bosco D, Zuellig RA, Berney T, Donath MY. Sulfonylurea induced beta-cell apoptosis in cultured human islets. *J Clin Endocrinol Metab* 2005; **90** (1): 501-6.

6. Accili D. Lilly lecture 2003: the struggle for mastery in insulin action: from triumvirate to republic. *Diabetes* 2004; **53** (7): 1633-42.

7. Dedoussis GV, Kaliora AC, Panagiotakos DB. Genes, diet and type 2 diabetes mellitus: a review. *Rev Diabet Stud* 2007; **4** (1): 13-24.

8. Sjoholm A, Nystrom T. Endothelial inflammation in insulin resistance. *Lancet* 2005; **365** (9459): 610-2.

9. Sjoholm A, Nystrom T. Inflammation and the etiology of type 2 diabetes. *Diabetes Metab Res Rev* 2006; **22** (1): 4-10.

10. Diamond J. The double puzzle of diabetes. *Nature* 2003; **423** (6940): 599-602.

11. Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes* 2000; **49** (12): 2201-7.

12. Barnett AH, Eff C, Leslie RD, Pyke DA. Diabetes in identical twins. A study of 200 pairs. *Diabetologia* 1981; **20** (2): 87-93.

13. Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. *Diabetologia* 1987; **30** (10): 763-8.

14. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H. Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. *Diabetologia* 1999; **42** (2): 139-45.

15. Medici F, Hawa M, Ianari A, Pyke DA, Leslie RD. Concordance rate for type II diabetes mellitus in monozygotic twins: actuarial analysis. *Diabetologia* 1999; **42** (2): 146-50.

16. Vaag A, Henriksen JE, Madsbad S, Holm N, Beck-Nielsen H. Insulin secretion, insulin action, and hepatic glucose production in identical twins discordant for non-insulin-dependent diabetes mellitus. *J Clin Invest* 1995; **95** (2): 690-8.

17. Elbein SC, Hasstedt SJ, Wegner K, Kahn SE. Heritability of pancreatic beta-cell function among nondiabetic members of Caucasian familial type 2 diabetic kindreds. *J Clin Endocrinol Metab* 1999; **84** (4): 1398-403.

18. Elbein SC, Wegner K, Kahn SE. Reduced beta-cell compensation to the insulin resistance associated with obesity in members of caucasian familial type 2 diabetic kindreds. *Diabetes Care* 2000; **23** (2): 221-7.

19. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* 1998; **19** (4): 491-503.

20. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; **26** (1): 76-80.

21. Silander K, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. *Diabetes* 2004; **53** (4): 1141-9.

22. Gloyn AL, Weedon MN, Owen KR, Turner MJ, Knight BA, Hitman G, Walker M, Levy JC, Sampson M, Halford S, McCarthy MI, Hattersley AT, Frayling TM. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 2003; **52** (2): 568-72.

23. Tsuchiya T, Schwarz PE, Bosque-Plata LD, Geoffrey Hayes M, Dina C, Froguel P, Wayne Towers G, Fischer S, Temelkova-Kurktschiev T, Rietzsch H, Graessler J, Vcelak J, Palyzova D, Selisko T, Bendlova B, Schulze J, Julius U, Hanefeld M, Weedon MN, Evans JC, Frayling TM, Hattersley AT, Orho-Melander M, Groop L, Malecki MT, Hansen T, Pedersen O, Fingerlin TE, Boehnke M, Hanis CL, Cox NJ, Bell GI. Association of the calpain-10 gene with type 2 diabetes in Europeans: results of pooled and meta-analyses. *Mol Genet Metab* 2006; **89** (1-2): 174-84.

24. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. *Nat Genet* 2005; **37** (8): 863-7.

25. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38** (3): 320-3.

26. Florez JC. Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: Where are the insulin resistance genes? *Diabetologia* 2008; **51** (7): 1100-10.

27. Hales CN, Barker DJ. Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* 1992; **35** (7): 595-601.

28. Aerts L, Van Assche FA. Animal evidence for the transgenerational development of diabetes mellitus. *Int J Biochem Cell Biol* 2006; **38** (5-6): 894-903.

29. Junien C, Nathanielsz P. Report on the IASO Stock Conference 2006: early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type II diabetes. *Obes Rev* 2007; **8** (6): 487-502.

30. Gill-Randall R, Adams D, Ollerton RL, Lewis M, Alcolado JC. Type 2 diabetes mellitus--genes or intrauterine environment? An embryo transfer paradigm in rats. *Diabetologia* 2004; **47** (8): 1354-9.

31. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001; **60** 5-20.

32. Baetens D, Malaisse-Lagae F, Perrelet A, Orci L. Endocrine pancreas: three-dimensional reconstruction shows two types of islets of langerhans. *Science* 1979; **206** (4424): 1323-5.

33. Brissova M, Fowler MJ, Nicholson WE, Chu A, Hirshberg B, Harlan DM, Powers AC. Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy. *J Histochem Cytochem* 2005; **53** (9): 1087-97.

34. Bennett BD, Jetton TL, Ying G, Magnuson MA, Piston DW. Quantitative subcellular imaging of glucose metabolism within intact pancreatic islets. *J Biol Chem* 1996; **271** (7): 3647-51.

35. Kulkarni RN. The islet beta-cell. *Int J Biochem Cell Biol* 2004; **36** (3): 365-71.

36. Bonner-Weir S. Perspective: Postnatal pancreatic beta cell growth. *Endocrinology* 2000; **141** (6): 1926-9.

37. Bonner-Weir S. Islet growth and development in the adult. *J Mol Endocrinol* 2000; **24** (3): 297-302.

38. Leahy JL. Natural history of beta-cell dysfunction in NIDDM. *Diabetes Care* 1990; **13** (9): 992-1010.

39. Weyer C, Bogardus C, Mott DM, Pratley RE. The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. *J Clin Invest* 1999; **104** (6): 787-94.

40. Bergman RN. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 1989; **38** (12): 1512-27.

41. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444** (7121): 840-6.

42. Rhodes CJ. Type 2 diabetes-a matter of beta-cell life and death? *Science* 2005; **307** (5708): 380-4.

43. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* 2003; **52** (1): 102-10.

44. Donath MY, Halban PA. Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia* 2004; **47** (3): 581-9.

45. Lingohr MK, Buettner R, Rhodes CJ. Pancreatic beta-cell growth and survival--a role in obesity-linked type 2 diabetes? *Trends Mol Med* 2002; **8** (8): 375-84.

46. Unger RH, Zhou YT. Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 2001; **50 Suppl 1** S118-21.

47. Poitout V, Robertson RP. Minireview: Secondary beta-cell failure in type 2 diabetes--a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 2002; **143** (2): 339-42.

48. de Koning EJ, Bonner-Weir S, Rabelink TJ. Preservation of beta-cell function by targeting beta-cell mass. *Trends Pharmacol Sci* 2008; **29** (4): 218-27.

49. Schuit F, Flamez D, De Vos A, Pipeleers D. Glucose-regulated gene expression maintaining the glucose-responsive state of beta-cells. *Diabetes* 2002; **51 Suppl 3** S326-32.

50. Sander M, German MS. The beta cell transcription factors and development of the pancreas. *J Mol Med* 1997; **75** (5): 327-40.

51. Webb GC, Akbar MS, Zhao C, Steiner DF. Expression profiling of pancreatic beta cells: glucose regulation of secretory and metabolic pathway genes. *Proc Natl Acad Sci U S A* 2000; **97** (11): 5773-8.

52. Cras-Meneur C, Inoue H, Zhou Y, Ohsugi M, Bernal-Mizrachi E, Pape D, Clifton SW, Permutt MA. An expression profile of human pancreatic islet mRNAs by Serial Analysis of Gene Expression (SAGE). *Diabetologia* 2004; **47** (2): 284-99.

53. Marselli L, Thorne J, Ahn YB, Omer A, Sgroi DC, Libermann T, Otu HH, Sharma A, Bonner-Weir S, Weir GC. Gene expression of purified beta-cell tissue obtained from human pancreas with laser capture microdissection. *J Clin Endocrinol Metab* 2008; **93** (3): 1046-53.

54. Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, Brady JN, Harlan DM. Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. *Endocrinology* 2002; **143** (9): 3695-8.

55. Hui H, Wang C, Li H, Bulotta A, D'Amico E, Khoury N, Nguyen E, Di Mario U, Chen IY, Perfetti R. Gene expression profiling of cultured human islet preparations. *Diabetes Technol Ther* 2004; **6** (4): 481-92.

56. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes* 2003; **52** (3): 581-7.

57. Gleason CE, Gonzalez M, Harmon JS, Robertson RP. Determinants of glucose toxicity and its reversibility in the pancreatic islet beta-cell line, HIT-T15. *Am J Physiol Endocrinol Metab* 2000; **279** (5): E997-1002.

58. Moran A, Zhang HJ, Olson LK, Harmon JS, Poitout V, Robertson RP. Differentiation of glucose toxicity from beta cell exhaustion during the evolution of defective insulin gene expression in the pancreatic islet cell line, HIT-T15. *J Clin Invest* 1997; **99** (3): 534-9.

59. Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S, Polonsky KS. Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. *Diabetes* 1998; **47** (3): 358-64.

60. Donath MY, Gross DJ, Cerasi E, Kaiser N. Hyperglycemia-induced beta-cell apoptosis in pancreatic islets of Psammomys obesus during development of diabetes. *Diabetes* 1999; **48** (4): 738-44.

61. Tajiri Y, Moller C, Grill V. Long-term effects of aminoguanidine on insulin release and biosynthesis: evidence that the formation of advanced glycosylation end products inhibits B cell function. *Endocrinology* 1997; **138** (1): 273-80.

62. Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes* 1999; **48** (4): 927-32.

63. Tanaka Y, Gleason CE, Tran PO, Harmon JS, Robertson RP. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci U S A* 1999; **96** (19): 10857-62.

64. Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, Fujitani Y, Kamada T, Kawamori R, Yamasaki Y. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. *J Clin Invest* 1997; **99** (1): 144-50.

65. McGarry JD, Dobbins RL. Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 1999; **42** (2): 128-38.

66. Unger RH. Reinventing type 2 diabetes: pathogenesis, treatment, and prevention. *Jama* 2008; **299** (10): 1185-7.

67. Grill V, Bjorklund A. Impact of metabolic abnormalities for beta cell function: Clinical significance and underlying mechanisms. *Mol Cell Endocrinol* 2008;

68. Zhou YP, Grill VE. Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 1994; **93** (2): 870-6.

69. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 1998; **14** (4): 263-83.

70. Poitout V, Hagman D, Stein R, Artner I, Robertson RP, Harmon JS. Regulation of the insulin gene by glucose and fatty acids. *J Nutr* 2006; **136** (4): 873-6.

71. Prentki M, Joly E, El-Assaad W, Roduit R. Malonyl-CoA signaling, lipid partitioning, and glucolipotoxicity: role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes* 2002; **51 Suppl 3** S405-13.

72. Prentki M, Corkey BE. Are the beta-cell signaling molecules malonyl-CoA and cystolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes* 1996; **45** (3): 273-83.

73. Lehtihet M, Welsh N, Berggren PO, Cook GA, Sjoholm A. Glibenclamide inhibits islet carnitine palmitoyltransferase 1 activity, leading to PKC-dependent insulin exocytosis. *Am J Physiol Endocrinol Metab* 2003; **285** (2): E438-46.

74. Davani B, Khan A, Hult M, Martensson E, Okret S, Efendic S, Jornvall H, Oppermann UC. Type 1 11beta -hydroxysteroid dehydrogenase mediates glucocorticoid activation and insulin release in pancreatic islets. *J Biol Chem* 2000; **275** (45): 34841-4.

75. Ortsater H, Alberts P, Warpman U, Engblom LO, Abrahmsen L, Bergsten P. Regulation of 11beta-hydroxysteroid dehydrogenase type 1 and glucose-stimulated insulin secretion in pancreatic islets of Langerhans. *Diabetes Metab Res Rev* 2005; **21** (4): 359-66.

76. Duplomb L, Lee Y, Wang MY, Park BH, Takaishi K, Agarwal AK, Unger RH. Increased expression and activity of 11beta-HSD-1 in diabetic islets and prevention with troglitazone. *Biochem Biophys Res Commun* 2004; **313** (3): 594-9.

77. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343** (4): 230-8.

78. Gremlich S, Roduit R, Thorens B. Dexamethasone induces posttranslational degradation of GLUT2 and inhibition of insulin secretion in isolated pancreatic beta cells. Comparison with the effects of fatty acids. *J Biol Chem* 1997; **272** (6): 3216-22.

79. Lambillotte C, Gilon P, Henquin JC. Direct glucocorticoid inhibition of insulin secretion. An in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 1997; **99** (3): 414-23.

80. Ohneda M, Johnson JH, Inman LR, Unger RH. GLUT-2 function in glucose-unresponsive beta cells of dexamethasone-induced diabetes in rats. *J Clin Invest* 1993; **92** (4): 1950-6.

81. Philippe J, Giordano E, Gjinovci A, Meda P. Cyclic adenosine monophosphate prevents the glucocorticoid-mediated inhibition of insulin gene expression in rodent islet cells. *J Clin Invest* 1992; **90** (6): 2228-33.

82. Davani B, Portwood N, Bryzgalova G, Reimer MK, Heiden T, Ostenson CG, Okret S, Ahren B, Efendic S, Khan A. Aged transgenic mice with increased glucocorticoid sensitivity in pancreatic beta-cells develop diabetes. *Diabetes* 2004; **53 Suppl 1** S51-9.

83. Ling ZC, Khan A, Delauny F, Davani B, Ostenson CG, Gustafsson JA, Okret S, Landau BR, Efendic S. Increased glucocorticoid sensitivity in islet beta-cells: effects on glucose 6-phosphatase, glucose cycling and insulin release. *Diabetologia* 1998; **41** (6): 634-9.

84. Weinhaus AJ, Bhagroo NV, Brelje TC, Sorenson RL. Dexamethasone counteracts the effect of prolactin on islet function: implications for islet regulation in late pregnancy. *Endocrinology* 2000; **141** (4): 1384-93.

85. Portha B. Programmed disorders of beta-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. *Diabetes Metab Res Rev* 2005; **21** (6): 495-504.

86. Fernandez-Mejia C, Medina-Martinez O, Martinez-Perez L, Goodman PA. The human insulin gene contains multiple transcriptional elements that respond to glucocorticoids. *Pancreas* 1999; **18** (4): 336-41.

87. Seckl JR, Walker BR. Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 2001; **142** (4): 1371-6.

88. Wajngot A, Giacca A, Grill V, Vranic M, Efendic S. The diabetogenic effects of glucocorticoids are more pronounced in low- than in high-insulin responders. *Proc Natl Acad Sci U S A* 1992; **89** (13): 6035-9.

89. Ullrich S, Berchtold S, Ranta F, Seebohm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F. Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. *Diabetes* 2005; **54** (4): 1090-9.

90. Kato S, Ishida H, Tsuura Y, Tsuji K, Nishimura M, Horie M, Taminato T, Ikehara S, Odaka H, Ikeda I, Okada Y, Seino Y. Alterations in basal and glucose-stimulated voltage-dependent Ca2+ channel activities in pancreatic beta cells of non-insulin-dependent diabetes mellitus GK rats. *J Clin Invest* 1996; **97** (11): 2417-25.

91. Varadi A, Molnar E, Ostenson CG, Ashcroft SJ. Isoforms of endoplasmic reticulum Ca(2+)-ATPase are differentially expressed in normal and diabetic islets of Langerhans. *Biochem J* 1996; **319 (Pt 2)** 521-7.

92. Goto Y, Kakizaki M, Masaki N. Production of spontaneous diabetic rats by repetition of selective breeding. *Tohoku J Exp Med* 1976; **119** (1): 85-90.

93. Abdel-Halim SM, Guenifi A, Luthman H, Grill V, Efendic S, Ostenson CG. Impact of diabetic inheritance on glucose tolerance and insulin secretion in spontaneously diabetic GK-Wistar rats. *Diabetes* 1994; **43** (2): 281-8.

94. Portha B, Serradas P, Bailbe D, Suzuki K, Goto Y, Giroix MH. Beta-cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. *Diabetes* 1991; **40** (4): 486-91.

95. Ostenson CG, Khan A, Abdel-Halim SM, Guenifi A, Suzuki K, Goto Y, Efendic S. Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. *Diabetologia* 1993; **36** (1): 3-8.

96. Abdel-Halim SM, Ostenson CG, Andersson A, Jansson L, Efendic S. A defective stimulus-secretion coupling rather than glucotoxicity mediates the impaired insulin secretion in the mildly diabetic F1 hybrids of GK-Wistar rats. *Diabetes* 1995; **44** (11): 1280-4.

97. Guenifi A, Abdel-Halim SM, Hoog A, Falkmer S, Ostenson CG. Preserved beta-cell density in the endocrine pancreas of young, spontaneously diabetic Goto-Kakizaki (GK) rats. *Pancreas* 1995; **10** (2): 148-53.

98. Movassat J, Saulnier C, Portha B. Beta-cell mass depletion precedes the onset of hyperglycaemia in the GK rat, a genetic model of non-insulin-dependent diabetes mellitus. *Diabete Metab* 1995; **21** (5): 365-70.

99. Movassat J, Saulnier C, Serradas P, Portha B. Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. *Diabetologia* 1997; **40** (8): 916-25.

100. Suzuki N, Aizawa T, Asanuma N, Sato Y, Komatsu M, Hidaka H, Itoh N, Yamauchi K, Hashizume K. An early insulin intervention accelerates pancreatic beta-cell dysfunction in young Goto-Kakizaki rats, a model of naturally occurring noninsulin-dependent diabetes. *Endocrinology* 1997; **138** (3): 1106-10.

101. Picarel-Blanchot F, Berthelier C, Bailbe D, Portha B. Impaired insulin secretion and excessive hepatic glucose production are both early events in the diabetic GK rat. *Am J Physiol* 1996; **271** (4 Pt 1): E755-62.

102. Koyama M, Wada R, Sakuraba H, Mizukami H, Yagihashi S. Accelerated loss of islet beta cells in sucrose-fed Goto-Kakizaki rats, a genetic model of non-insulin-dependent diabetes mellitus. *Am J Pathol* 1998; **153** (2): 537-45.

103. Kimura K, Toyota T, Kakizaki M, Kudo M, Takebe K, Goto Y. Impaired insulin secretion in the spontaneous diabetes rats. *Tohoku J Exp Med* 1982; **137** (4): 453-9.

104. Calderari S, Gangnerau MN, Meile MJ, Portha B, Serradas P. Is defective pancreatic beta-cell mass environmentally programmed in Goto-Kakizaki rat model of type 2 diabetes?: insights from crossbreeding studies during suckling period. *Pancreas* 2006; **33** (4): 412-7.

105. Briaud I, Kelpe CL, Johnson LM, Tran PO, Poitout V. Differential effects of hyperlipidemia on insulin secretion in islets of langerhans from hyperglycemic versus normoglycemic rats. *Diabetes* 2002; **51** (3): 662-8.

106. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004; **351** (11): 1106-18.

107. Zeender E, Maedler K, Bosco D, Berney T, Donath MY, Halban PA. Pioglitazone and sodium salicylate protect human beta-cells against apoptosis and impaired function induced by glucose and interleukin-1beta. *J Clin Endocrinol Metab* 2004; **89** (10): 5059-66.

108. Zhang F, Sjoholm K, Zhang Q. Pioglitazone acutely influences glucose-sensitive insulin secretion in normal and diabetic human islets. *Biochem Biophys Res Commun* 2006; **351** (3): 750-5.

109. Richardson H, Campbell SC, Smith SA, Macfarlane WM. Effects of rosiglitazone and metformin on pancreatic beta cell gene expression. *Diabetologia* 2006; **49** (4): 685-96.

110. Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, Ochoa C, Tan S, Berkowitz K, Hodis HN, Azen SP. Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. *Diabetes* 2002; **51** (9): 2796-803.

111. Knowler WC, Hamman RF, Edelstein SL, Barrett-Connor E, Ehrmann DA, Walker EA, Fowler SE, Nathan DM, Kahn SE. Prevention of type 2 diabetes with troglitazone in the Diabetes Prevention Program. *Diabetes* 2005; **54** (4): 1150-6.

112. Gerstein HC, Yusuf S, Bosch J, Pogue J, Sheridan P, Dinccag N, Hanefeld M, Hoogwerf B, Laakso M, Mohan V, Shaw J, Zinman B, Holman RR. Effect of rosiglitazone on the frequency of diabetes in patients with impaired glucose tolerance or impaired fasting glucose: a randomised controlled trial. *Lancet* 2006; **368** (9541): 1096-105.

113. Finegood DT, McArthur MD, Kojwang D, Thomas MJ, Topp BG, Leonard T, Buckingham RE. Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. *Diabetes* 2001; **50** (5): 1021-9.

114. Diani AR, Sawada G, Wyse B, Murray FT, Khan M. Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. *Am J Physiol Endocrinol Metab* 2004; **286** (1): E116-22.

115. Kawasaki F, Matsuda M, Kanda Y, Inoue H, Kaku K. Structural and functional analysis of pancreatic islets preserved by pioglitazone in db/db mice. *Am J Physiol Endocrinol Metab* 2005; **288** (3): E510-8.

116. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. *Nat Med* 2004; **10** (4): 355-61.

117. Ishida H, Takizawa M, Ozawa S, Nakamichi Y, Yamaguchi S, Katsuta H, Tanaka T, Maruyama M, Katahira H, Yoshimoto K, Itagaki E, Nagamatsu S. Pioglitazone improves insulin secretory capacity and prevents the loss of beta-cell mass in obese diabetic db/db mice: Possible protection of beta cells from oxidative stress. *Metabolism* 2004; **53** (4): 488-94.

118. Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 1996; **45** (12): 1661-9.

119. Hofmann C, Lorenz K, Williams D, Palazuk BJ, Colca JR. Insulin sensitization in diabetic rat liver by an antihyperglycemic agent. *Metabolism* 1995; **44** (3): 384-9.

120. Dubois M, Pattou F, Kerr-Conte J, Gmyr V, Vandewalle B, Desreumaux P, Auwerx J, Schoonjans K, Lefebvre J. Expression of peroxisome proliferator-activated receptor gamma (PPARgamma) in normal human pancreatic islet cells. *Diabetologia* 2000; **43** (9): 1165-9.

121. Kim HI, Kim JW, Kim SH, Cha JY, Kim KS, Ahn YH. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* 2000; **49** (9): 1517-24.

122. Kim HI, Cha JY, Kim SY, Kim JW, Roh KJ, Seong JK, Lee NT, Choi KY, Kim KS, Ahn YH. Peroxisomal proliferator-activated receptor-gamma upregulates glucokinase gene expression in beta-cells. *Diabetes* 2002; **51** (3): 676-85.

123. Parton LE, Diraison F, Neill SE, Ghosh SK, Rubino MA, Bisi JE, Briscoe CP, Rutter GA. Impact of PPARgamma overexpression and activation on pancreatic islet gene expression profile analyzed with oligonucleotide microarrays. *Am J Physiol Endocrinol Metab* 2004; **287** (3): E390-404.

124. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell* 1995; **83** (6): 835-9.

125. Braissant O, Foufelle F, Scotto C, Dauca M, Wahli W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996; **137** (1): 354-66.

126. Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 1997; **46** (8): 1319-27.

127. Granneman J, Skoff R, Yang X. Member of the peroxisome proliferator-activated receptor family of transcription factors is differentially expressed by oligodendrocytes. *J Neurosci Res* 1998; **51** (5): 563-73.

128. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta* 1996; **1302** (2): 93-109.

129. Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. *Embo J* 1992; **11** (2): 433-9.

130. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; **20** (5): 649-88.

131. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. *Nature* 1996; **384** (6604): 39-43.

132. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflamm Res* 2000; **49** (10): 497-505.

133. Fatehi-Hassanabad Z, Chan CB. Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic beta-cell function. *Nutr Metab (Lond)* 2005; **2** (1): 1.

134. Drucker DJ. The biology of incretin hormones. *Cell Metab* 2006; **3** (3): 153-65.

135. Chepurny OG, Hussain MA, Holz GG. Exendin-4 as a stimulator of rat insulin I gene promoter activity via bZIP/CRE interactions sensitive to serine/threonine protein kinase inhibitor Ro 31-8220. *Endocrinology* 2002; **143** (6): 2303-13.

136. Jhala US, Canettieri G, Screaton RA, Kulkarni RN, Krajewski S, Reed J, Walker J, Lin X, White M, Montminy M. cAMP promotes pancreatic beta-cell survival via CREB-mediated induction of IRS2. *Genes Dev* 2003; **17** (13): 1575-80.

137. Toft-Nielsen MB, Madsbad S, Holst JJ. Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 1999; **22** (7): 1137-43.

138. Willms B, Werner J, Holst JJ, Orskov C, Creutzfeldt W, Nauck MA. Gastric emptying, glucose responses, and insulin secretion after a liquid test meal: effects of exogenous glucagon-like peptide-1 (GLP-1)-(7-36) amide in type 2 (noninsulin-dependent) diabetic patients. *J Clin Endocrinol Metab* 1996; **81** (1): 327-32.

139. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1993; **36** (8): 741-4.

140. Sorhede Winzell M, Ahren B. Glucagon-like peptide-1 and islet lipolysis. *Horm Metab Res* 2004; **36** (11-12): 795-803.

141. Yaney GC, Civelek VN, Richard AM, Dillon JS, Deeney JT, Hamilton JA, Korchak HM, Tornheim K, Corkey BE, Boyd AE, 3rd. Glucagon-like peptide 1 stimulates lipolysis in clonal pancreatic beta-cells (HIT). *Diabetes* 2001; **50** (1): 56-62.

142. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368** (9548): 1696-705.

143. Zander M, Madsbad S, Madsen JL, Holst JJ. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002; **359** (9309): 824-30.

144. Egan JM, Bulotta A, Hui H, Perfetti R. GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells. *Diabetes Metab Res Rev* 2003; **19** (2): 115-23.

145. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 1999; **48** (12): 2270-6.

146. List JF, Habener JF. Glucagon-like peptide 1 agonists and the development and growth of pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 2004; **286** (6): E875-81.

147. Wang X, Cahill CM, Pineyro MA, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 regulates the beta cell transcription factor, PDX-1, in insulinoma cells. *Endocrinology* 1999; **140** (10): 4904-7.

148. Movassat J, Beattie GM, Lopez AD, Hayek A. Exendin 4 up-regulates expression of PDX 1 and hastens differentiation and maturation of human fetal pancreatic cells. *J Clin Endocrinol Metab* 2002; **87** (10): 4775-81.

149. Zhou J, Pineyro MA, Wang X, Doyle ME, Egan JM. Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. *J Cell Physiol* 2002; **192** (3): 304-14.

150. Stoffers DA, Kieffer TJ, Hussain MA, Drucker DJ, Bonner-Weir S, Habener JF, Egan JM. Insulinotropic glucagon-like peptide 1 agonists stimulate expression of homeodomain protein IDX-1 and increase islet size in mouse pancreas. *Diabetes* 2000; **49** (5): 741-8.

151. Li Y, Hansotia T, Yusta B, Ris F, Halban PA, Drucker DJ. Glucagon-like peptide-1 receptor signaling modulates beta cell apoptosis. *J Biol Chem* 2003; **278** (1): 471-8.

152. Ranganath LR. The entero-insular axis: implications for human metabolism. *Clin Chem Lab Med* 2008; **46** (1): 43-56.

153. Ohlsson H, Karlsson K, Edlund T. IPF1, a homeodomain-containing transactivator of the insulin gene. *Embo J* 1993; **12** (11): 4251-9.

154. Sharma A, Stein R. Glucose-induced transcription of the insulin gene is mediated by factors required for beta-cell-type-specific expression. *Mol Cell Biol* 1994; **14** (2): 871-9.

155. Cerf ME, Muller CJ, Du Toit DF, Louw J, Wolfe-Coote SA. Transcription factors, pancreatic development, and beta-cell maintenance. *Biochem Biophys Res Commun* 2005; **326** (4): 699-702.

156. Iype T, Francis J, Garmey JC, Schisler JC, Nesher R, Weir GC, Becker TC, Newgard CB, Griffen SC, Mirmira RG. Mechanism of insulin gene regulation by the pancreatic transcription factor Pdx-1: application of pre-mRNA analysis and chromatin immunoprecipitation to assess formation of functional transcriptional complexes. *J Biol Chem* 2005; **280** (17): 16798-807.

157. Jonsson J, Carlsson L, Edlund T, Edlund H. Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* 1994; **371** (6498): 606-9.

158. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF. Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. *Nat Genet* 1997; **15** (1): 106-10.

159. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev* 1998; **12** (12): 1763-8.

160. Stoffers DA, Ferrer J, Clarke WL, Habener JF. Early-onset type-II diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 1997; **17** (2): 138-9.

161. Stoffers DA, Thomas MK, Habener JF. Homeodomain Protein IDX-1 A Master Regulator of Pancreas Development and Insulin Gene Expression. *Trends Endocrinol Metab* 1997; **8** (4): 145-51.

162. Lu M, Miller C, Habener JF. Functional regions of the homeodomain protein IDX-1 required for transactivation of the rat somatostatin gene. *Endocrinology* 1996; **137** (7): 2959-67.

163. McKinnon CM, Docherty K. Pancreatic duodenal homeobox-1, PDX-1, a major regulator of beta cell identity and function. *Diabetologia* 2001; **44** (10): 1203-14.

164. Rafiq I, Kennedy HJ, Rutter GA. Glucose-dependent translocation of insulin promoter factor-1 (IPF-1) between the nuclear periphery and the nucleoplasm of single MIN6 beta-cells. *J Biol Chem* 1998; **273** (36): 23241-7.

165. Elrick LJ, Docherty K. Phosphorylation-dependent nucleocytoplasmic shuttling of pancreatic duodenal homeobox-1. *Diabetes* 2001; **50** (10): 2244-52.

166. Rafiq I, da Silva Xavier G, Hooper S, Rutter GA. Glucose-stimulated preproinsulin gene expression and nuclear trans-location of pancreatic duodenum homeobox-1 require activation of phosphatidylinositol 3-kinase but not p38 MAPK/SAPK2. *J Biol Chem* 2000; **275** (21): 15977-84.

167. Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K. Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. *Endocrinology* 1990; **127** (1): 126-32.

168. Lacy PE, Kostianovsky M. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes* 1967; **16** (1): 35-9.

169. Leibiger B, Moede T, Schwarz T, Brown GR, Kohler M, Leibiger IB, Berggren PO. Short-term regulation of insulin gene transcription by glucose. *Proc Natl Acad Sci U S A* 1998; **95** (16): 9307-12.

170. Leibiger B, Leibiger IB. Functional analysis of DNA-elements involved in transcriptional control of the human glucose transporter 2 (GLUT 2) gene in the insulin-producing cell line beta TC-3. *Diabetologia* 1995; **38** (1): 112-5.

171. Karaskov E, Scott C, Zhang L, Teodoro T, Ravazzola M, Volchuk A. Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis. *Endocrinology* 2006; **147** (7): 3398-407.

172. Tsunekawa S, Yamamoto N, Tsukamoto K, Itoh Y, Kaneko Y, Kimura T, Ariyoshi Y, Miura Y, Oiso Y, Niki I. Protection of pancreatic beta-cells by exendin-4 may involve the reduction of endoplasmic reticulum stress; in vivo and in vitro studies. *J Endocrinol* 2007; **193** (1): 65-74.

173. Loffler M, Bilban M, Reimers M, Waldhausl W, Stulnig TM. Blood glucose-lowering nuclear receptor agonists only partially normalize hepatic gene expression in db/db mice. *J Pharmacol Exp Ther* 2006; **316** (2): 797-804.

174. Randle PJ, Priestman DA, Mistry S, Halsall A. Mechanisms modifying glucose oxidation in diabetes mellitus. *Diabetologia* 1994; **37 Suppl 2** S155-61.

175. Randle PJ, Priestman DA, Mistry SC, Halsall A. Glucose fatty acid interactions and the regulation of glucose disposal. *J Cell Biochem* 1994; **55 Suppl** 1-11.

176. Xu J, Han J, Epstein PN, Liu YQ. Regulation of PDK mRNA by high fatty acid and glucose in pancreatic islets. *Biochem Biophys Res Commun* 2006; **344** (3): 827-33.

177. Naya FJ, Stellrecht CM, Tsai MJ. Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. *Genes Dev* 1995; **9** (8): 1009-19.

178. Glick E, Leshkowitz D, Walker MD. Transcription factor BETA2 acts cooperatively with E2A and PDX1 to activate the insulin gene promoter. *J Biol Chem* 2000; **275** (3): 2199-204.

179. Ohneda K, Mirmira RG, Wang J, Johnson JD, German MS. The homeodomain of PDX-1 mediates multiple protein-protein interactions in the formation of a transcriptional activation complex on the insulin promoter. *Mol Cell Biol* 2000; **20** (3): 900-11.

180. Naya FJ, Huang HP, Qiu Y, Mutoh H, DeMayo FJ, Leiter AB, Tsai MJ. Diabetes, defective pancreatic morphogenesis, and abnormal enteroendocrine differentiation in BETA2/neuroD-deficient mice. *Genes Dev* 1997; **11** (18): 2323-34.

181. Lupi R, Bugliani M, Del Guerra S, Del Prato S, Marchetti P, Boggi U, Filipponi F, Mosca F. Transcription factors of beta-cell differentiation and maturation in isolated human islets: effects of high glucose, high free fatty acids and type 2 diabetes. *Nutr Metab Cardiovasc Dis* 2006; **16** (6): e7-8.

182. Bendtzen K, Mandrup-Poulsen T, Nerup J, Nielsen JH, Dinarello CA, Svenson M. Cytotoxicity of human pI 7 interleukin-1 for pancreatic islets of Langerhans. *Science* 1986; **232** (4757): 1545-7.

183. Arend WP, Guthridge CJ. Biological role of interleukin 1 receptor antagonist isoforms. *Ann Rheum Dis* 2000; **59 Suppl 1** i60-4.

184. Maedler K, Sergeev P, Ehses JA, Mathe Z, Bosco D, Berney T, Dayer JM, Reinecke M, Halban PA, Donath MY. Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. *Proc Natl Acad Sci U S A* 2004; **101** (21): 8138-43.

185. Liu Z, Habener JF. Glucagon-like peptide-1 activation of TCF7L2-dependent Wnt signaling enhances pancreatic beta cell proliferation. *J Biol Chem* 2008; **283** (13): 8723-35.

186. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjogren M, Ling C, Eriksson KF, Lethagen AL, Mancarella R, Berglund G, Tuomi T, Nilsson P, Del Prato S, Groop L. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. *J Clin Invest* 2007; **117** (8): 2155-63.

187. Smith U. TCF7L2 and type 2 diabetes--we WNT to know. *Diabetologia* 2007; **50** (1): 5-7.

188. Suzuki Y, Yonekura H, Watanabe T, Unno M, Moriizumi S, Miyashita H, Okamoto H. Structure and expression of a novel rat RegIII gene. *Gene* 1994; **144** (2): 315-6.

189. Eizirik DL, Bjorklund A, Cagliero E. Genotoxic agents increase expression of growth arrest and DNA damage--inducible genes gadd 153 and gadd 45 in rat pancreatic islets. *Diabetes* 1993; **42** (5): 738-45.

190. Yi YW, Kim D, Jung N, Hong SS, Lee HS, Bae I. Gadd45 family proteins are coactivators of nuclear hormone receptors. *Biochem Biophys Res Commun* 2000; **272** (1): 193-8.

191. Junn E, Han SH, Im JY, Yang Y, Cho EW, Um HD, Kim DK, Lee KW, Han PL, Rhee SG, Choi I. Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. *J Immunol* 2000; **164** (12): 6287-95.

192. Corbett JA. Thioredoxin-interacting protein is killing my beta-cells! *Diabetes* 2008; **57** (4): 797-8.

193. Chen J, Saxena G, Mungrue IN, Lusis AJ, Shalev A. Thioredoxin-interacting protein: a critical link between glucose toxicity and beta-cell apoptosis. *Diabetes* 2008; **57** (4): 938-44.

194. Minn AH, Hafele C, Shalev A. Thioredoxin-interacting protein is stimulated by glucose through a carbohydrate response element and induces beta-cell apoptosis. *Endocrinology* 2005; **146** (5): 2397-405.

195. Minn AH, Pise-Masison CA, Radonovich M, Brady JN, Wang P, Kendziorski C, Shalev A. Gene expression profiling in INS-1 cells overexpressing thioredoxin-interacting protein. *Biochem Biophys Res Commun* 2005; **336** (3): 770-8.

196. Gunton JE, Kulkarni RN, Yim S, Okada T, Hawthorne WJ, Tseng YH, Roberson RS, Ricordi C, O'Connell PJ, Gonzalez FJ, Kahn CR. Loss of ARNT/HIF1beta mediates altered gene expression and pancreatic-islet dysfunction in human type 2 diabetes. *Cell* 2005; **122** (3): 337-49.

197. Zhang S, Kim KH. Acetyl-CoA carboxylase is essential for nutrient-induced insulin secretion. *Biochem Biophys Res Commun* 1996; **229** (3): 701-5.

198. Zhang S, Kim KH. Essential role of acetyl-CoA carboxylase in the glucose-induced insulin secretion in a pancreatic beta-cell line. *Cell Signal* 1998; **10** (1): 35-42.

199. Lebovitz HE. Type 2 diabetes: an overview. *Clin Chem* 1999; **45** (8 Pt 2): 1339-45.

200. Riddle MC. Tactics for type II diabetes. *Endocrinol Metab Clin North Am* 1997; **26** (3): 659-77.

201. Groop LC, Widen E, Ferrannini E. Insulin resistance and insulin deficiency in the pathogenesis of type 2 (non-insulin-dependent) diabetes mellitus: errors of metabolism or of methods? *Diabetologia* 1993; **36** (12): 1326-31.

202. Boden G. Fatty acids and insulin resistance. *Diabetes Care* 1996; **19** (4): 394-5.

203. Lindsay RS, Bennett PH. Type 2 diabetes, the thrifty phenotype - an overview. *Br Med Bull* 2001; **60** 21-32.

204. Simmons RA. Developmental origins of beta-cell failure in type 2 diabetes: the role of epigenetic mechanisms. *Pediatr Res* 2007; **61** (5 Pt 2): 64R-67R.

205. Jacqueminet S, Briaud I, Rouault C, Reach G, Poitout V. Inhibition of insulin gene expression by long-term exposure of pancreatic beta cells to palmitate is dependent on the presence of a stimulatory glucose concentration. *Metabolism* 2000; **49** (4): 532-6.

206. Gremlich S, Bonny C, Waeber G, Thorens B. Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin, and somatostatin levels. *J Biol Chem* 1997; **272** (48): 30261-9.

207. Ritz-Laser B, Meda P, Constant I, Klages N, Charollais A, Morales A, Magnan C, Ktorza A, Philippe J. Glucose-induced preproinsulin gene expression is inhibited by the free fatty acid palmitate. *Endocrinology* 1999; **140** (9): 4005-14.

208. Briaud I, Harmon JS, Kelpe CL, Segu VB, Poitout V. Lipotoxicity of the pancreatic beta-cell is associated with glucose-dependent esterification of fatty acids into neutral lipids. *Diabetes* 2001; **50** (2): 315-21.

209. Gulli G, Ferrannini E, Stern M, Haffner S, DeFronzo RA. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. *Diabetes* 1992; **41** (12): 1575-86.

210. Lillioja S, Mott DM, Spraul M, Ferraro R, Foley JE, Ravussin E, Knowler WC, Bennett PH, Bogardus C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin-dependent diabetes mellitus. Prospective studies of Pima Indians. *N Engl J Med* 1993; **329** (27): 1988-92.

211. Vauhkonen I, Niskanen L, Vanninen E, Kainulainen S, Uusitupa M, Laakso M. Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited. Metabolic studies on offspring of diabetic probands. *J Clin Invest* 1998; **101** (1): 86-96.

212. Lillioja S, Bogardus C, Mott DM, Kennedy AL, Knowler WC, Howard BV. Relationship between insulin-mediated glucose disposal and lipid metabolism in man. *J Clin Invest* 1985; **75** (4): 1106-15.

213. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Del Prato S, DeFronzo RA. Obesity and insulin resistance in humans: a dose-response study. *Metabolism* 1990; **39** (5): 452-9.

214. Paolisso G, Tagliamonte MR, Rizzo MR, Gualdiero P, Saccomanno F, Gambardella A, Giugliano D, D'Onofrio F, Howard BV. Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type II diabetes. *Diabetologia* 1998; **41** (10): 1127-32.

215. Felber JP, Ferrannini E, Golay A, Meyer HU, Theibaud D, Curchod B, Maeder E, Jequier E, DeFronzo RA. Role of lipid oxidation in pathogenesis of insulin resistance of obesity and type II diabetes. *Diabetes* 1987; **36** (11): 1341-50.

216. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 2000; **49** (5): 677-83.

217. Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr Rev* 2008; **29** (3): 351-66.

218. Kruszynska YT, Olefsky JM. Cellular and molecular mechanisms of non-insulin dependent diabetes mellitus. *J Investig Med* 1996; **44** (8): 413-28.

219. Polonsky KS, Sturis J, Bell GI. Seminars in Medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus - a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 1996; **334** (12): 777-83.

220. MacDonald PE, Wheeler MB. Voltage-dependent K(+) channels in pancreatic beta cells: role, regulation and potential as therapeutic targets. *Diabetologia* 2003; **46** (8): 1046-62.

221. Hughes SJ, Faehling M, Thorneley CW, Proks P, Ashcroft FM, Smith PA. Electrophysiological and metabolic characterization of single beta-cells and islets from diabetic GK rats. *Diabetes* 1998; **47** (1): 73-81.

222. Cnop M, Hannaert JC, Grupping AY, Pipeleers DG. Low density lipoprotein can cause death of islet beta-cells by its cellular uptake and oxidative modification. *Endocrinology* 2002; **143** (9): 3449-53.

223. Okajima F, Kurihara M, Ono C, Nakajima Y, Tanimura K, Sugihara H, Tatsuguchi A, Nakagawa K, Miyazawa T, Oikawa S. Oxidized but not acetylated low-density lipoprotein reduces preproinsulin mRNA expression and secretion of insulin from HIT-T15 cells. *Biochim Biophys Acta* 2005; **1687** (1-3): 173-80.

224. Noushmehr H, D'Amico E, Farilla L, Hui H, Wawrowsky KA, Mlynarski W, Doria A, Abumrad NA, Perfetti R. Fatty acid translocase (FAT/CD36) is localized on insulin-containing granules in human pancreatic beta-cells and mediates fatty acid effects on insulin secretion. *Diabetes* 2005; **54** (2): 472-81.

225. Zhou YP, Ostenson CG, Ling ZC, Grill V. Deficiency of pyruvate dehydrogenase activity in pancreatic islets of diabetic GK rats. *Endocrinology* 1995; **136** (8): 3546-51.

226. Sener A, Malaisse-Lagae F, Ostenson CG, Malaisse WJ. Metabolism of endogenous nutrients in islets of Goto-Kakizaki (GK) rats. *Biochem J* 1993; **296 (Pt 2)** 329-34.

227. Macfarlane WM, McKinnon CM, Felton-Edkins ZA, Cragg H, James RF, Docherty K. Glucose stimulates translocation of the homeodomain transcription factor PDX1 from the cytoplasm to the nucleus in pancreatic beta-cells. *J Biol Chem* 1999; **274** (2): 1011-6.

228. Boulikas T. Putative nuclear localization signals (NLS) in protein transcription factors. *J Cell Biochem* 1994; **55** (1): 32-58.

229. Moroianu J. Molecular mechanisms of nuclear protein transport. *Crit Rev Eukaryot Gene Expr* 1997; **7** (1-2): 61-72.

230. Mattaj IW, Englmeier L. Nucleocytoplasmic transport: the soluble phase. *Annu Rev Biochem* 1998; **67** 265-306.

8 ARTICLES I-IV