Institutionen för molekylär medicin och kirurgi

Functional and Genetic Studies in Type 2 Diabetes and Obesity

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ABSTRACT

Type 2 diabetes (T2D) and obesity are highly prevalent disorders reflecting a complex interplay of genetics, epigenetics, and environment. They constitute serious health problems and lead to significant morbidity and mortality. The overall aim of this thesis was to shed some light on the molecular mechanisms underlying pathogenesis of T2D and obesity by studies in rat and man.

In **Paper I**, adenylyl cyclase 3 (*Ac3*) mRNA expression levels in pancreatic islets and striatum/hypothalamus regions of brain of diabetic Goto-Kakizaki (GK) rats were higher compared with control Wistar rats, while its expression was intermediate in islets and brain regions of insulin-treated GK rats. This study proposes that increased *Ac3* mRNA expression in these tissues is partially a primary and inherited defect and not solely secondary to hyperglycaemia, and that AC3 may participate in the regulation of glucose homeostasis via insulin secretion and CNS.

In **Paper II**, protein kinase Cα (PKCα) and PKCζ mRNA expressions in pancreatic islets were decreased in GK compared with Wistar rats, with intermediate expressions in the insulin-treated GK group. PKCα and phosphorylated PKCα (p-PKCα) protein expressions in islets were diminished in GK compared with insulin-treated GK and Wistar rats. Islet PKCζ protein expression was reduced in both GK and insulin-treated GK compared with Wistar rats, but p-PKCζ was reduced only in GK compared with insulin-treated GK and Wistar rats. PKCε was lower in islets of GK compared with insulin-treated GK and Wistar rats, at both the mRNA and protein levels. PKCδ and p-PKCδ protein expressions in islets were decreased in GK compared with insulin-treated GK and Wistar rats. In liver, PKCδ and PKCζ mRNA expressions were decreased in both GK and insulin-treated GK compared with Wistar rats. Hepatic PKCζ protein expression was diminished in GK rats with and without insulin treatment compared with Wistar rats. Although PKCδ showed no difference at the protein level, p-PKCδ/PKCδ was reduced in liver of GK compared with Wistar rats. PKCε mRNA expression in liver was down-regulated in insulin-treated GK compared with non-treated GK and Wistar rats. Therefore, this study suggests defects in PKCα and PKCε expressions in pancreatic islets of GK rats secondary to hyperglycaemia. In liver, PKCε mRNA expression in liver could be under control of insulin.

In **Paper III**, mRNA expressions, enzyme activities, and protein levels of succinyl-CoA:3-ketoacid-CoA transferase (SCOT) and ATP citrate lyase (ATPCL) were decreased in pancreatic islets of GK compared with Wistar rats. Two cell lines with the severest knockdown of SCOT enzyme activity and protein, SCOT 1676 and SCOT 1184, showed the severest reduction in secretagogue-stimulated insulin secretion. This study confirms that the mitochondrial pathways involving SCOT, instead of or synergizing with the pathway involving ATPCL, are important potentiators of glucose-stimulated insulin secretion (GSIS).

In **Paper IV**, we studied the association of adrenergic receptor alpha 2A (*ADRA2A*) genetic polymorphisms with obesity and/or T2D in a Swedish cohort. The single nucleotide polymorphism (SNP) rs553668 was associated with T2D in men, but this association disappeared after adjusting for age and body mass index (BMI). Associations were also detected when comparing obese subjects with normal glucose tolerance (NGT) and lean NGT subjects, and in obese but not lean T2D patients. In women, multiple logistic regression regarding SNP rs521674 demonstrated an association with T2D when including age as a covariant. However, correcting for BMI removed this association. When age was included in the model, the increased risk of rs521674 was seen in obese, but not in lean, T2D women. This study provides evidence that *ADRA2A* genetic polymorphisms are mainly associated with obesity and may also relate to T2D in a Swedish population.

In conclusion, this thesis has revealed molecular pathogenetic defects related to T2D and obesity, and may thus create a basis for more precise and improved therapeutic approaches.

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To my wonderful family

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LIST OF PUBLICATIONS

I. **SEED AHMED M.**, KOVOOR, A., NORDMAN, S., ABU SEMAN, N., GU, T., EFENDIC, S., BRISMAR, K., OSTENSON, C. G. & GU, H. F. 2012. Increased expression of adenylyl cyclase 3 in pancreatic islets and central nervous system of diabetic Goto-Kakizaki rats: a possible regulatory role in glucose homeostasis. *Islets,* 4**,** 343-8.

II. **SEED AHMED M.***, PELLETIER, J.*, LEUMANN, H., GU, H. F. & OSTENSON, C. G. Expression of protein kinase C isoforms in pancreatic islets and liver of Goto-Kakizaki rats, a model of type 2 diabetes. *Manuscript*.

III. HASAN, N. M., LONGACRE, M. J., **SEED AHMED, M.**, KENDRICK, M. A., GU, H. F., OSTENSON, C. G., FUKAO, T. & MACDONALD, M. J. 2010. Lower succinyl-CoA:3-ketoacid-CoA transferase (SCOT) and ATP citrate lyase in pancreatic islets of a rat model of type 2 diabetes: knockdown of SCOT inhibits insulin release in rat insulinoma cells. *Archives of biochemistry and biophysics,* 499**,** 62-8.

IV. LANGBERG, E. C.*, **SEED AHMED, M.***, EFENDIC, S., GU, H. F. & OSTENSON, C. G. 2013. Genetic association of adrenergic receptor alpha 2A with obesity and type 2 diabetes. *Obesity (Silver Spring, Md.),* 21**,** 1720-5.

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CONTENTS

LIST OF ABBREVIATIONS

$[Ca^{2+}]_i$	Cytosolic free Ca^{2+}
AC	Adenylyl cyclase
AC3	Adenylyl cyclase 3
ACAA2	Acetyl-CoA acyltransferase 2
ACAT1	Acetyl-CoA acetyltransferase 1
ACh	Acetylcholine
ADP	Adenosine diphosphate
ADRA2A	Adrenergic receptor alpha 2A
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
ATPCL	ATP citrate lyase
BMI	Body mass index
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA
CI	Confidence interval
CNS	Central nervous system
DAG	Diacylglycerol
DNA	Deoxyribonucleic acid
FAM	Carboxyfluorescein
FFA	Free fatty acid
GK	Goto-Kakizaki
GLP1	Glucagon-like peptide 1
GLUT	Glucose transporter
GPCR	G protein-coupled receptor
GSIS	Glucose-stimulated insulin secretion
GWA	Genome-wide association
HOMA-IR	Homeostasis model assessment-insulin resistance
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
INS-1	Insulinoma cell line
IP_3	Inositol triphosphate
K^+_{ATP} channel	ATP-sensitive K^+ channel
KRB	Krebs-Ringer bicarbonate
ME1	Cytosolic malic enzyme
MMS+HB	Monomethylsuccinate-plus-β-hydroxybutyrate
mRNA	Messenger RNA
NGT	Normal glucose tolerance
OR	Odds ratio
PCR	Polymerase chain reaction
PDH	Pyruvate dehydrogenase
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PS	Phosphatidylserine

RGS9	Regulator of G protein signalling 9
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase PCR
SCOT	Succinyl-CoA:3-ketoacid-CoA transferase
SE	Standard error of the mean
shRNA	Short hairpin RNA
siRNA	Short interfering RNA
SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes
TAMRA	Tetramethylrhodamine
TCA cycle	Tricarboxylic acid cycle
WHO	World health organization

1 BACKGROUND

Type 2 diabetes (T2D) and obesity are chronic disorders, which result from complex interactions between genetic, epigenetic, and environmental influences (Drong *et al.*, 2012). Given the increased global prevalence and disastrous public health aftermath of T2D and obesity, substantial research efforts have been devoted to investigate potential causes of the development of these disorders.

1.1 GLUCOSE HOMEOSTASIS

1.1.1 Physiology of plasma glucose regulation

Glucose homeostasis is the essential process for maintaining constant levels of glucose, a principle energy source for the body and the main fuel for the brain and erythrocytes (Siems *et al.*, 2000; Tirone and Brunicardi, 2001). The circulatory system maintains normal cerebral function by providing a constant supply of glucose, the obligate source of energy for the brain under physiological conditions because of the insufficiency of alternative fuels (Unger, 1981; Gerich, 2000). In spite of considerable variations in glucose flux (input or removal), plasma glucose levels are normally maintained within narrow limits that are necessary for the body's well-being (Gerich, 2000). Thus persistent hyperglycaemia has the potential for macro- and micro-vascular complications, as in the case of sub-optimally controlled diabetes mellitus (Forbes and Cooper, 2013), whilst hypoglycaemia results in brain dysfunction and, if severe and prolonged, can lead to permanent brain damage and even death (Cryer and Gerich, 1985).

The plasma glucose concentration depends on the rates of entry of glucose into the circulation and its uptake into the tissues (Gerich, 2000). Glucose entering into the circulation is derived from ingested glucose; from the liver by glycogenolysis, which is the breakdown of the storage form of glucose, glycogen; and from the liver and kidney by gluconeogenesis, the synthesis of new glucose molecules from other precursors (mainly lactate, glycerol, and amino acids) (Gerich, 1993; Stumvoll *et al.*, 1997; Mitrakou, 2011).

Glucose homeostasis is achieved through the harmonized interactions of hormonal and neural networks, the former of which includes the most important glucoregulatory factors insulin, glucagon, and catecholamines, which function in a matter of minutes (Gerich, 2000). Insulin decreases plasma glucose levels by suppressing hepatic and renal glucose production, and promoting glucose uptake and utilization by liver and extrahepatic tissues, primarily skeletal muscle and, to a lesser extent, adipose tissue and cardiac muscle (Gerich, 2000; Pessin and Saltiel, 2000; Khan and Pessin, 2002; Thorens and Mueckler, 2010). Glucagon increases hepatic glucose output, whilst epinephrine promotes both hepatic and renal glucose release (Gerich, 2000). Other important glucoregulatory hormones, including cortisol, growth hormone, thyroid hormone, and angiotensin II, influence glucose levels over a period of hours firstly by stimulating hepatic glucose production (Tirone and Brunicardi, 2001); secondly by altering the sensitivity of the liver, kidney, adipose tissue, and muscle to insulin, glucagon, and catecholamines; and thirdly by affecting the amounts of key enzymes,

glycogen stores, and availability of gluconeogenic precursors (Gerich, 2000; Mitrakou, 2011).

Glucose is a hydrophilic molecule and, therefore, its bidirectional diffusion across the cell membrane is facilitated by glucose transporter (GLUT) proteins. Fourteen GLUTs have been hitherto identified in humans and besides glucose, they transport other substrates including fructose, myoinositol, and urate (Thorens and Mueckler, 2010). One or more GLUT proteins are expressed in nearly every cell type, and GLUTs 1-5, the best characterized isoforms, are either insulin-independent or insulin-dependent; however, GLUT5 is a fructose transporter and its participation in glucose homeostasis is minimal (Mueckler and Thorens, 2013). In summary, GLUT1 is the major glucose transporter in the brain and erythrocytes, and is insulin-independent; GLUT2 is also insulin-independent, and is principally located in pancreatic β-cells and hepatocytes; GLUT3, an insulin-independent transporter, is present in neurons, intestine, testicles, and kidneys; and GLUT4, which is the main insulin-dependent isoform, mediates glucose transport activity in skeletal and cardiac muscle, and in adipose tissue (Tirone and Brunicardi, 2001; Thorens and Mueckler, 2010; Wu and Garvey, 2010; Mueckler and Thorens, 2013).

1.1.2 Control of glucose homeostasis

The islets of Langerhans, which are the endocrine compartment of the pancreas, constitute a major centre for the control of glucose homeostasis via the secretion of two hormones essential in the regulation of plasma glucose, namely insulin and glucagon from the β- and α-cells, respectively. Besides the α- and β-cells, the islets of Langerhans also consist of somatostatin-expressing δ-cells, pancreatic polypeptide-expressing cells, and ghrelin-expressing ε-cells (In't Veld and Marichal, 2010; Jones and Persaud, 2010).

1.1.2.1 Insulin biosynthesis and storage

The biosynthesis of insulin by the β-cells of the pancreatic islets commences by synthesizing preproinsulin on polyribosomes associated with the rough endoplasmic reticulum (Uchizono *et al.*, 2007; Jones and Persaud, 2010). Preproinsulin, guided by the N-terminal signal peptide, is quickly translocated to the lumen of the rough endoplasmic reticulum, where the signal peptide is cleaved by proteolytic enzymes, forming proinsulin (Uchizono *et al.*, 2007). Proinsulin, comprising the A and B chains of insulin joined by the C peptide, is then transported to the Golgi apparatus, where it is packaged into membrane-bound vesicles known as insulin secretory granules or β-granules (Jones and Persaud, 2010). The C peptide chain is enzymatically removed from proinsulin in maturing secretory granules, yielding insulin with its A and B chains correctly aligned. There are at least two populations of insulin secretory granules, the readily releasable pool (1-5% of total granules), which is responsible for the acute first phase of glucose-stimulated insulin secretion (GSIS), and the reserve pool (95-99% of the granules), which is responsible for the sustained second phase of GSIS (Bratanova-Tochkova *et al.*, 2002; Hou *et al.*, 2009). Young newly formed granules are preferentially selected for secretion over older granules, which remain in the storage pool for 3-5 days and are then subject to intracellular degradation (Uchizono *et al.*, 2007). Amongst many nutrients, glucose is the most physiologically relevant regulator

of proinsulin biosynthesis (Taylor *et al.*, 2004; Uchizono *et al.*, 2007). When insulin secretion is triggered, proinsulin biosynthesis is also increased; however, the threshold (2-4 mmol/l glucose) for the stimulation of insulin biosynthesis is lower than that for insulin secretion (4-6 mmol/l glucose) to ensure that insulin stores and, consequently, tight glycaemic control are maintained (Ashcroft *et al.*, 1978; Ashcroft, 1980).

1.1.2.2 Insulin secretion

1.1.2.2.1 Nutrient control of insulin secretion

The secretion of insulin is initiated by the influx of glucose, the most prominent physiological stimulus of insulin secretion, into pancreatic β-cells by facilitated diffusion through specific glucose transporters (GLUT1 and GLUT3 in humans; GLUT2 in rodents) (De Vos *et al.*, 1995; Newsholme *et al.*, 2010; McCulloch *et al.*, 2011; Rorsman and Braun, 2013). Intracellular glucose is first metabolized via glycolysis to pyruvate, which is then metabolized in mitochondria, generating adenosine triphosphate (ATP) that is exported to the cytosol, in conjunction with a concomitant decrease in adenosine diphosphate (ADP) levels (Ashcroft *et al.*, 1994). The increase in the cytoplasmic ATP/ADP ratio leads to closure of ATP-sensitive K^+ (K^+_{ATP}) channels, resulting in depolarization of the β-cell plasma membrane. This in turn culminates in the opening of voltage-dependent Ca^{2+} channels, and an influx of extracellular Ca^{2+}, with subsequent elevation of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) levels. Increased $[Ca^{2+}]_i$ triggers insulin release from the β-cells by the process of exocytosis (Figure 1), which involves fusion of the secretory granule membrane and plasma membrane, together with the opening of a fusion pore connecting the two membranes (Kasai *et al.*, 2010). Pancreatic islets are also characterized by the intrinsic property of a biphasic insulin secretory response to a glucose challenge, in which there is a rapidly rising but transient first phase lasting a few minutes (4-10 min), followed by a sustained second phase of secretion, which continues as long as hyperglycaemia persists (Cerasi, 1975a; Cerasi, 1975b; Nesher and Cerasi, 2002).

In addition to glucose, other nutrients including amino acids and fatty acids play important roles in insulin release, and their cooperation is fundamental in regulating appropriate insulin secretion after a mixed carbohydrate/protein/lipid meal (Nolan and Prentki, 2008; Newsholme *et al.*, 2010). In spite of being poor insulin secretagogues individually, most amino acids require glucose or need to act in certain combinations with each other in order to serve their insulin secretory function (Nolan and Prentki, 2008; Jones and Persaud, 2010); nevertheless, some of them such as leucine, alanine, and arginine can increase insulin release in the absence of glucose (Henquin and Meissner, 1986). Although free fatty acids (FFAs) are essential for efficient GSIS (Stein *et al.*, 1996; Nolan *et al.*, 2006a) and clearly augment it (Corkey *et al.*, 1989; Prentki *et al.*, 1992; Haber *et al.*, 2003; Roduit *et al.*, 2004), long-term exposure to FFAs may have lipotoxic effects on the β-cells (Sako and Grill, 1990; Zhou and Grill, 1994; Haber *et al.*, 2006).

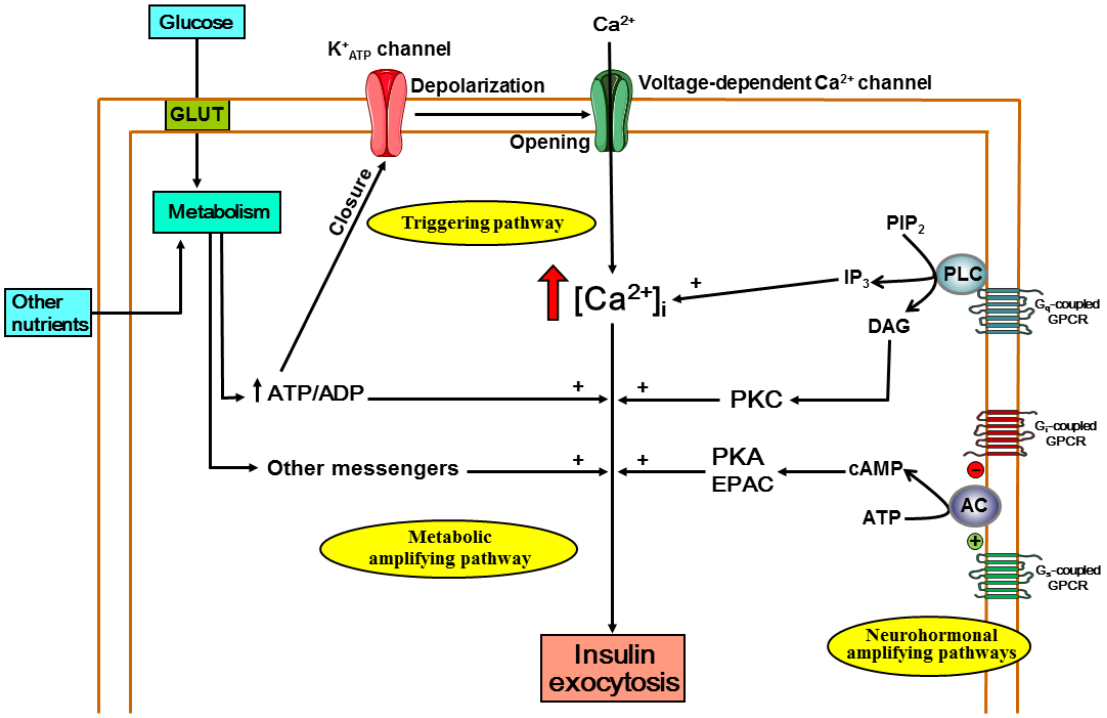

Figure 1. Schematic overview of the triggering and amplifying pathways in the regulation of insulin secretion. *GLUT, glucose transporter (GLUT1 and GLUT3 in humans, GLUT2 in rodents); ATP, adenosine triphosphate; ADP, adenosine diphosphate; K^+_{ATP} channel, ATP-sensitive K^+ channel; $[Ca^{2+}]_i$, cytosolic free Ca^{2+}; GPCR, G protein-coupled receptor; AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; EPAC, exchange protein activated by cAMP; PLC, phospholipase C; PIP_2, phosphatidylinositol bisphosphate; IP_3, inositol triphosphate; DAG, diacylglycerol; ↑, increased; +, stimulation; −, inhibition. The various GPCRs differently influence the signalling pathways in the β-cell according to the activated G protein where G_q activates PLC, while G_s and G_i stimulates and inhibits the formation of cAMP, respectively. (This figure is taken from Henquin (2009), and has been modified with permission of the author.)*

1.1.2.2.2 Non-nutrient regulation of insulin secretion

Nutrient-induced insulin secretion is modulated by other non-nutrient factors including numerous hormones and neurotransmitters, many of which act through the activation of G protein-coupled receptors (GPCRs) in the β-cell membrane (Ahren, 2009). Therefore, effective second-messenger systems operating through a number of intracellular effector systems are required to transduce extracellular neurohormonal signals into an insulin secretory response. In this context, the adenylyl cyclase (AC)/protein kinase A (PKA) and phospholipase C (PLC)/protein kinase C (PKC) pathways form the two most important signalling systems within the β-cell (Zawalich *et al.*, 1997; Furman *et al.*, 2010; Newsholme *et al.*, 2010). Agonists known to stimulate AC (e.g. glucagon, glucagon-like peptide 1 (GLP1), glucose-dependent insulinotropic polypeptide (GIP), pituitary adenylyl cyclase activating polypeptide (PACAP)) potentiate insulin secretion via catalysis of the conversion of ATP to cyclic adenosine monophosphate (cAMP), which activates PKA and exchange proteins activated by cAMP (EPACs) (Straub and Sharp, 1996a; Straub and Sharp, 1996c; Straub and Sharp, 1996b; Straub and Sharp, 2002; Efendic and Portwood, 2004;

Drucker, 2006; Dyachok *et al.*, 2008; Szaszak *et al.*, 2008). Other receptor agonists (e.g. acetylcholine (ACh), cholecystokinin (CCK), arginine vasopressin (AVP)) activate PLC, which promotes the cleavage of phosphatidylinositol bisphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates Ca^{2+} release from the endoplasmic reticulum leading to increased $[Ca^{2+}]_i$, and DAG activates some isoforms of PKC, both of which enhance insulin secretion (Jones and Persaud, 1998; Gilon and Henquin, 2001; Yamazaki *et al.*, 2010). In contrast, some agents (such as islet somatostatin and ghrelin hormones) inhibit insulin secretion through inhibition of AC activity and decreased cAMP production (Sharp, 1996; Jones and Persaud, 2010; Dezaki and Yada, 2012), via activation of K^+_{ATP} channels in the β-cell membrane and a consequent hyperpolarization-induced fall in $[Ca^{2+}]_i$, and by blocking exocytosis at a late step in the stimulus-secretion coupling (Nilsson *et al.*, 1989; Sharp, 1996; Dezaki *et al.*, 2008; Jones and Persaud, 2010).

Whereas the major parasympathetic neurotransmitter, ACh, stimulates insulin secretion, the major sympathetic neurotransmitter, norepinephrine (noradrenaline), as well as circulating catecholamines secreted by the adrenal medulla (mainly epinephrine (adrenaline)) can exert both stimulatory and inhibitory influences on insulin secretion depending on the subtype of adrenergic receptors that are activated (Jones and Persaud, 2010). The stimulatory effects are mediated via β_2-adrenergic receptors through augmentation of cAMP formation by AC (Kuo *et al.*, 1973; Ahren and Lundquist, 1981; Ahrén, 2000; Massarsky *et al.*, 2011), while inhibitory effects are brought about by α_2-adrenergic receptors through activation of K^+_{ATP} channels that results in a reduction of $[Ca^{2+}]_i$, by inhibition of AC-dependent cAMP generation, and via distal attenuation of exocytosis (Hermann and Deckert, 1977; Yamazaki *et al.*, 1982; Ullrich and Wollheim, 1984; Sharp, 1996; Ahrén, 2000; Zhao *et al.*, 2008; Massarsky *et al.*, 2011; Straub and Sharp, 2012).

Adipocyte-derived hormones (adipokines) such as adiponectin, leptin, and resistin are also reported to influence insulin secretion (Jones and Persaud, 2010). Adiponectin induces insulin secretion (Gu *et al.*, 2006; Okamoto *et al.*, 2008) and protects against β-cell apoptosis (Rakatzi *et al.*, 2004); however, although adiponectin receptors are expressed in human and rat pancreatic β-cells (Kharroubi *et al.*, 2003; Gu *et al.*, 2006; Okamoto *et al.*, 2008), the signalling pathways and functional effects induced by their activation in β-cells have not yet been fully clarified. In addition, adiponectin has been shown to enhance insulin sensitivity (Yamauchi *et al.*, 2001), and low plasma adiponectin levels are found to be closely associated with obesity-related illnesses including T2D, atherosclerotic cardiovascular diseases, hypertension, and lipid disorders (Hotta *et al.*, 2000; Maeda *et al.*, 2002; Gil-Campos *et al.*, 2004; Okamoto *et al.*, 2006; Mojiminiyi *et al.*, 2007; Lu *et al.*, 2008; Matsuzawa, 2010). On the other hand, leptin exerts inhibitory effects on insulin secretion (Cases *et al.*, 2001; Marroqui *et al.*, 2012) via activation of β-cell K^+_{ATP} channels accompanied by a marked lowering of $[Ca^{2+}]_i$ (Kieffer *et al.*, 1997; Lupi *et al.*, 1999), by activation of c-Jun N-terminal kinases (Maedler *et al.*, 2008), or through a central effect on the hypothalamus via melanocortin receptors (Muzumdar *et al.*, 2003). Moreover, pancreas-specific knockout of Ob-Rb leptin receptors is associated with enhanced insulin secretion (Morioka *et al.*, 2007). Leptin may also contribute to β-cell mass reduction (Morioka *et al.*, 2007; Maedler *et al.*, 2008). Like leptin, resistin also impairs GSIS in mice (Nakata *et al.*,

2007) and induces apoptosis of rat β-cells (Gao *et al.*, 2009). However, resistin is shown not to be associated with insulin sensitivity, and its function is unknown in humans (Arner, 2005; Utzschneider *et al.*, 2005). Rather, it might have paracrine effects on β-cell function, since it has been expressed in human pancreatic islets (Minn *et al.*, 2003).

1.1.2.2.3 Triggering and amplifying pathways in the regulation of insulin secretion

GSIS is regulated by so-called triggering and amplifying pathways since, in addition to its ability to stimulate insulin secretion via induction of a triggering Ca^{2+} signal (triggering pathway), glucose can also augment the action of triggering Ca^{2+} on exocytosis (metabolic amplifying pathway) (Figure 1) (Henquin, 2000; Westerlund *et al.*, 2001; Henquin, 2011). Furthermore, glucose potentiates Ca^{2+}-triggered insulin secretion induced by non-metabolized agents, like arginine and sulfonylureas, by two mechanisms: augmentation of the triggering Ca^{2+} signal and amplification of the efficacy of this signal on exocytosis (Ishiyama *et al.*, 2006). Another amplifying signal is generated in the neurohormonal amplifying pathway, which mediates the potentiation effects of neurotransmitters (e.g. ACh) and hormones (e.g. GLP1) on nutrient-induced insulin secretion mainly through amplification of Ca^{2+}-induced exocytosis (Figure 1) (Henquin, 2009).

1.1.2.2.4 Role of mitochondrial signalling in modulating insulin secretion

β-cell mitochondria serve as fuel sensors and play a pivotal role in coupling nutrient metabolism to insulin secretion (Maechler *et al.*, 2010). In the mitochondria, glucose-derived pyruvate, which is the terminal product of the glycolytic pathway, is either decarboxylated by pyruvate dehydrogenase (PDH) to produce acetyl-CoA for the glucose oxidation pathway, or carboxylated by pyruvate carboxylase (PC) to oxaloacetate in the anaplerosis (the replenishment of tricarboxylic acid (TCA) cycle intermediates)/cataplerosis (the exit of anaplerotic products from the mitochondria) pathway (Maechler and Wollheim, 2001; Nolan and Prentki, 2008). In the glucose oxidation pathway, acetyl-CoA enters the TCA cycle and is oxidized via mitochondrial oxidative phosphorylation to generate ATP, which is necessary for the elevation of $[Ca^{2+}]_i$ with the successive induction of insulin exocytosis (Maechler *et al.*, 2010). Anaplerotic and cataplerotic (mainly NADPH, malonyl-CoA, and glutamate) products can act as important signals for insulin secretion (Farfari *et al.*, 2000; Maechler and Wollheim, 2001; MacDonald *et al.*, 2005; Hasan *et al.*, 2008; Jensen *et al.*, 2008; Nolan and Prentki, 2008; Jitrapakdee *et al.*, 2010; Maechler *et al.*, 2010).

1.1.2.3 Insulin action

Insulin is exclusively synthesized in, and secreted from, pancreatic β-cells in response to dietary nutrients (mainly glucose) and non-nutrient stimuli (Newsholme *et al.*, 2010). Insulin is an anabolic hormone that regulates whole-body fuel homeostasis via its actions in the liver, skeletal muscle, and adipose tissue, in which it enhances synthesis and reduces degradation of glycogen, lipid, and protein (Wu and Garvey, 2010). The ultimate effect of insulin is to lower plasma glucose levels by decreasing hepatic glucose output and facilitating glucose uptake and utilization by the target tissues (Gerich, 2000). In liver, insulin inhibits glycogenolysis and gluconeogenesis, and

stimulates glycogen synthesis and lipogenesis. Secreted insulin binds to insulin receptors in skeletal muscle and adipose tissue, triggering glucose uptake by these tissues with sequential anabolic effects, which include increased glycogen and protein syntheses in skeletal muscle, and the augmentation of lipogenesis and inhibition of lipolysis in adipose tissue (Nordlie *et al.*, 1999; Pessin and Saltiel, 2000; Radziuk and Pye, 2001; Khan and Pessin, 2002; Stumvoll *et al.*, 2005; Wu and Garvey, 2010).

1.2 TYPE 2 DIABETES

This form of diabetes was previously referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes (Alberti and Zimmet, 1998; American Diabetes Association, 2013).

1.2.1 Definition and description of type 2 diabetes

T2D is a heterogeneous disorder with a complex aetiology caused by the combined effects of genetic inheritance and environmental factors, yet the precise molecular mechanisms underlying the roles of these effects are not unequivocally established (Qi *et al.*, 2008). This disorder is characterized by chronic hyperglycaemia, which is principally due to impaired insulin secretion (insulin deficiency) from the pancreatic β-cells, as well as decreased insulin sensitivity (insulin resistance) in the liver and extrahepatic tissues, mainly skeletal muscle and adipose tissue (Gerich, 1998; Surampudi *et al.*, 2009; Amed *et al.*, 2010; Marchetti *et al.*, 2012). Insulin deficiency in T2D is relative, rather than absolute, in the sense that patients secrete insulin, but not enough to overcome insulin resistance (Alberti, 2010; American Diabetes Association, 2013). Considerable debate exists as to the primacy and relative contribution of insulin deficiency versus insulin resistance in the development of T2D; however, impaired insulin release is the sine qua non for the hyperglycaemia to occur, and the prevailing current view depicts T2D as the result of a genetic β-cell defect that limits compensation for, in most instances, obesity-induced insulin resistance (Polonsky *et al.*, 1996; Gerich, 1998; Kahn, 2001; Ostenson, 2001; Pratley and Weyer, 2001; Gerich, 2003; Alsahli and Gerich, 2010).

The global prevalence of diabetes is rapidly increasing and is regarded as one of the most challenging threats to human health in the 21st century (Guariguata *et al.*, 2011). The prevalence of diabetes worldwide was estimated to be 366 million people in the year 2011, and is expected to reach 552 million by 2030 (Whiting *et al.*, 2011), among whom almost 90-95% are accounted for by T2D (Nolan *et al.*, 2006b; American Diabetes Association, 2013; WHO, 2013a). Diabetes has emerged as a pandemic, with enormous and serious health and socioeconomic burdens in the form of increased morbidity and disability, reduced quality and span of life, altered social roles and family structure, and high economic costs (Ali *et al.*, 2010). Diabetes was internationally estimated to be the fifth leading cause of death (Roglic *et al.*, 2005), and it amply contributes to premature adult mortality (Roglic and Unwin, 2010).

Diabetes evolves through pre-diabetes, also referred to as impaired glucose regulation, which is an intermediate stage of altered glucose metabolism between normal glucose levels and T2D (Alberti and Zimmet, 1998). Pre-diabetes, defined as impaired fasting

glucose (IFG) and/or impaired glucose tolerance (IGT), is associated with an increased risk for the development of diabetes and cardiovascular disease (Bergman, 2010; Buysschaert and Bergman, 2011). About 70% of pre-diabetic subjects have been reported to eventually develop diabetes (Nathan *et al.*, 2007). The term 'intermediate hyperglycaemia' over the term 'pre-diabetes' has been recommended by a joint World Health Organization (WHO) and International Diabetes Federation (IDF) technical advisory group, because the latter term misleadingly implies that everyone with pre-diabetes develops diabetes, and it might also underestimate the significant association with increased cardiovascular risk (WHO/IDF, 2006).

Diagnosis of diabetes and intermediate hyperglycaemic conditions (IFG and IGT) is established by measuring the plasma glucose concentration in a venous sample. T2D diagnostic criteria consist of values of fasting plasma glucose ≥7.0 mmol/l (126 mg/dl) and/or 2-hour post-glucose load ≥11.1 mmol/l (200 mg/dl). Diagnosis of IFG and IGT involves fasting plasma glucose levels of 6.1-6.9 mmol/l (110-125 mg/dl), and 2-hour post-glucose load levels of 7.8-11.0 mmol/l (140-199 mg/dl), respectively (WHO/IDF, 2006; Alberti, 2010; American Diabetes Association, 2013).

T2D is associated with a number of acute and chronic complications. Hyperosmolar hyperglycaemia is a life-threatening metabolic condition, which complicates poorly treated or undiagnosed T2D (Kitabchi *et al.*, 2006; Hansen and Møller, 2010). Chronic hyperglycaemia in T2D leads to devastating long-term vascular consequences, specifically macro-vascular (cerebrovascular disease, coronary heart disease, and peripheral vascular disease) and micro-vascular (retinopathy, nephropathy, and neuropathy) complications (Bailes, 2002; Coccheri, 2007; Kaul *et al.*, 2012; Forbes and Cooper, 2013; WHO, 2013a).

1.2.2 Pathogenesis of type 2 diabetes

T2D is a polygenic disorder, which results from a complex interplay of multiple genes, each of individually small effect, with numerous pre-natal and post-natal environmental factors, the combination of which leads to impairment in insulin secretion and resistance to insulin action (Lander and Schork, 1994; Busch and Hegele, 2001; Hansen, 2003; Wolford and Vozarova de Courten, 2004; Malecki, 2005; Vaxillaire and Froguel, 2010; Kota *et al.*, 2012). In addition, epigenetic modifications are important players in the pathogenesis of T2D (Drong *et al.*, 2012; Dayeh *et al.*, 2013; Simmons, 2013).

1.2.2.1 β-cell defects

Pancreatic β-cells respond to decreases in tissue insulin sensitivity by increasing their function in an effort to maintain glucose homeostasis, up to a point at which they fail and hyperglycaemia becomes manifest (Alsahli and Gerich, 2010). T2D is characterized by progressive deterioration of β-cell function and mass (Wajchenberg, 2010). The β-cell mass is found to be decreased by 35% in T2D patients (Sakuraba *et al.*, 2002), and this reduction in β-cell mass is attributable to increased levels of apoptosis, which are not compensated for by adequate β-cell regeneration (Butler *et al.*, 2003). Defective β-cell function in T2D includes decreased first phase insulin release, dampened insulin pulsatility during the sustained second phase of GSIS, and impaired

insulin biosynthesis (Bergsten *et al.*, 1998; Bergsten, 2000; Wajchenberg, 2007). Nevertheless, neither alone is enough for the development of T2D, and defects in both β-cell mass and function are required for the disorder to become manifest (Kahn *et al.*, 2009).

Several acquired factors, together with a background of a genetic predisposition, can adversely affect β-cell function and survival (Alsahli and Gerich, 2010; Marchetti *et al.*, 2010). Among these factors, chronic exposure to increased concentrations of glucose (hyperglycaemia) and FFAs (hyperlipidaemia) can have detrimental impact, respectively termed glucotoxicity and lipotoxicity, on the β-cells leading to increased apoptosis, decreased GSIS, and various molecular changes (Marchetti *et al.*, 2012). While glucotoxicity occurs independently of hyperlipidaemia, hyperglycaemia is required for the consequences of chronic hyperlipidaemia to ensue (Poitout and Robertson, 2002); hence the term glucolipotoxicity, instead of lipotoxicity, is proposed as being more appropriate to describe the toxic effects of lipids on the β-cells (Prentki *et al.*, 2002; Poitout and Robertson, 2008).

1.2.2.2 Insulin resistance

Insulin resistance can be defined as the inability of insulin to produce its usual biological actions at circulating concentrations that are effective in normal subjects (Yki-Järvinen, 2010); thence, higher than normal levels of plasma insulin are required to maintain normoglycaemia. Insulin resistance is a common feature of several complex disorders comprising obesity, T2D, metabolic syndrome, non-alcoholic fatty liver disease, lipid disorders, hypertension, atherosclerotic cardiovascular disease, and polycystic ovary syndrome (Cohn *et al.*, 2005; Savage *et al.*, 2005; Yki-Järvinen, 2010; Koleva *et al.*, 2013). In the context of glucose homeostasis, insulin resistance leads to impaired inhibition of the production of hepatic glucose and very low density lipoprotein, resulting, respectively, in hyperglycaemia and hypertriglyceridaemia (Yki-Järvinen, 2010). Additionally, insulin resistance is implicated in decreased peripheral uptake of glucose by skeletal muscle and adipose tissue (Petersen and Shulman, 2002). Insulin-resistant states such as obesity are associated with reduced suppression of lipolysis in adipose tissue, which results in increased plasma FFA concentrations and ectopic fat deposition in liver and skeletal muscle, contributing to insulin resistance in these tissues (Perseghin *et al.*, 2003; Donnelly *et al.*, 2005; Frayn *et al.*, 2006). Glucotoxicity and lipotoxicity as the consequences of prolonged hyperglycaemia and hyperlipidaemia, respectively, can directly contribute to the development of insulin resistance and further deteriorate insulin sensitivity in skeletal muscle (Yki-Järvinen, 1992; Ostenson, 2001; Krook *et al.*, 2004).

1.2.3 Risk factors for type 2 diabetes

Several risk factors are known to be associated with increased risk for T2D including genetic as well as behavioural and environmental risk factors (Ma and Tong, 2010).

1.2.3.1 Genetic factors

Ethnic variation, familial aggregation, and concordance between identical twins provides strong evidence for a genetic component in T2D (Barroso, 2005). Ethnic

variability in the prevalence of T2D provides robust support for the contribution of genetic factors, although part of this ethnic variation can be ascribed to the concomitant role of environmental and cultural factors (Abate and Chandalia, 2003; Barroso, 2005; Carulli *et al.*, 2005; Adeyemo and Rotimi, 2010; Chen *et al.*, 2012). Positive family history of T2D is a major independent risk factor that increases the chance for developing T2D in young people by two- to four-fold, depending on the number and degree of relativity of the affected family members, due to both shared genes and shared environment (Grill *et al.*, 1999; Arslanian *et al.*, 2005; Hilding *et al.*, 2006; Ghosh *et al.*, 2010; Heideman *et al.*, 2011). Twin studies have further supported the strong genetic predisposition to T2D, with higher concordance rates detected in monozygotic than in dizygotic twins (Newman *et al.*, 1987; Japan Diabetes Society, 1988; Kaprio *et al.*, 1992; Medici *et al.*, 1999; MacGregor *et al.*, 2000; van Tilburg *et al.*, 2001).

To date, more than 64 common genetic variants with low effect sizes have been identified in genome-wide association (GWA) studies as strongly associated with T2D (Kwak and Park, 2013). However, the added effect of the variants identified so far explains only approximately 10% of the T2D heritability (Manolio *et al.*, 2009; Voight *et al.*, 2010; Kwak and Park, 2013). Most of the identified T2D-related variants are involved in β-cell function rather than in insulin resistance (Florez, 2008; Groop and Lyssenko, 2009; Stolerman and Florez, 2009; Herder and Roden, 2011; Petrie *et al.*, 2011; Wheeler and Barroso, 2011). The strongest associations with T2D are found for gene variants in the loci *TCF7L2* (transcription factor 7-like 2) and *KCNQ1* (K^+ voltage-gated channel, KQT-like subfamily, member 1) (Vaxillaire and Froguel, 2010; Herder and Roden, 2011), both of which are associated with reduced β-cell function (Cauchi and Froguel, 2008; Petrie *et al.*, 2011; Ali, 2013).

1.2.3.2 Early determinants

Maternal and early life environmental factors can have profound effects on health in adulthood through developmental programming. Fetal under-nutrition and/or rapid post-natal growth, as well as fetal over-nutrition, are associated with an increased risk to develop T2D later in life (Berends and Ozanne, 2012; Vignini *et al.*, 2012). Furthermore, birth weight has a U-shaped relationship with lifelong risk of T2D, where both low and high birth weights are linked with an excess risk for T2D (Harder *et al.*, 2007; Whincup *et al.*, 2008).

1.2.3.3 Behavioural and environmental factors

The environmental factors known to impact the development of T2D often relate to lifestyle behaviour, and include physical inactivity, obesity, and tobacco use (Persson *et al.*, 2000; Fulton-Kehoe *et al.*, 2001; Patja *et al.*, 2005; Willi *et al.*, 2007; Venables and Jeukendrup, 2009). Other environmental risk factors comprise high-fat diet, dietary supplements, exposure to organic pollutants, psychological distress, and work-related stress (Agardh *et al.*, 2003; Norberg *et al.*, 2007; Cosgrove *et al.*, 2008; Eriksson *et al.*, 2008; Murea *et al.*, 2012). Obesity, especially if centrally distributed and of long duration, is the most potent environmental driver of T2D (Wild and Byrne, 2006). It is connected to T2D through induction of systemic insulin resistance, which is mainly

mediated by persistently elevated concentrations of FFAs (Boden, 2001; Boden and Shulman, 2002; Kahn *et al.*, 2006; Ye, 2013).

1.3 GOTO-KAKIZAKI RATS

The Goto-Kakizaki (GK) rat is considered as one of the best available rodent strains for the study of inherited T2D, and this explains its extensive use in experimental diabetes research (Ostenson and Efendic, 2007; Portha *et al.*, 2012). The GK rat was developed in Sendai, Japan, by repeated breeding of Wistar rats selected at the upper limit of the normal distribution for glucose tolerance (Goto *et al.*, 1976). Repetition of this selective breeding of glucose-intolerant Wistar rats over numerous generations led to the generation of a non-obese diabetic rat strain, named GK, which develops hyperglycaemia in early life. Thereafter, several GK rat colonies with breeding pairs from Japan were initiated in Paris, France; Dallas, USA; Stockholm, Sweden; Cardiff, UK; Coimbra, Portugal; and Tampa, USA (Portha *et al.*, 2012). Glucose intolerance and impairment of GSIS have been reported as constant features in the different GK rat colonies; however, other properties like β-cell number, insulin content, and islet metabolism seem to differ between some of the various colonies, perhaps reflecting newly introduced genetic modifications and/or differences in the local breeding environments (Portha *et al.*, 2010).

Glucose intolerance in the GK rat results from impaired GSIS due to a β-cell deficit, which is most likely the primary defect leading to T2D in the context of a polygenic background (Abdel-Halim *et al.*, 1993; Ostenson *et al.*, 1993; Abdel-Halim *et al.*, 1994; Hughes *et al.*, 1994; Abdel-Halim *et al.*, 1995; Miralles and Portha, 2001; Ostenson and Efendic, 2007; Portha *et al.*, 2009). Insulin resistance has been demonstrated in liver, some skeletal muscles, and adipose tissue of the GK rat (Bisbis *et al.*, 1993). Insulin action in skeletal muscle of GK rats was fully restored after correction of hyperglycaemia by phlorizin treatment, suggesting that impaired insulin action in the GK rat, at least in skeletal muscle, is secondary to hyperglycaemia (Krook *et al.*, 1997).

1.4 OBESITY

Overweight (defined as a body mass index (BMI) $\geq$ 25 kg/m^2) and obesity (BMI $\geq$ 30 kg/m^2) are conditions of abnormal or excessive fat accumulation, due to the presence of an energy imbalance between calories consumed and calories expended, that may impair health (WHO, 2013b). However, the BMI cut-off points that connect overweight and obesity with the associated health risk must be population-specific, since the relationship between percent body fat and BMI is different amongst different ethnic groups (Deurenberg *et al.*, 1998; Razak *et al.*, 2007). The prevalence of overweight and obesity is dramatically rising worldwide at an alarming rate in both developed and developing countries (WHO, 2000). From 1.46 billion overweight and 502 million obese adults in the year 2008 (Finucane *et al.*, 2011), the global projection for the corresponding numbers by 2030 is estimated to be 2.16 billion and 1.12 billion adults, respectively (Kelly *et al.*, 2008). In addition to reduced life expectancy, increased BMI is a major risk factor for T2D, cardiovascular diseases, musculoskeletal disorders, and some cancers (Haslam and James, 2005).

Common obesity is induced by environmental triggers on a strong yet poorly dissected genetic basis, with the extent of heritability estimated to range from 40% to 70% (Herrera *et al.*, 2011). The most obvious drivers of this observed obesity epidemic are environmental and reside in the food system, including: an increased availability of cheap, palatable, energy-dense foods; improved distribution systems to make food much more accessible and convenient; and more convincing and widespread commercial food promotion (Swinburn *et al.*, 2011). By the same token, low physical activity is a conspicuous determinant of the obesity epidemic (Bray, 2010; Hauner, 2010). Indeed, it is not just confined to 'diet and exercise', and other plausible aetiological factors contributing to the obesity epidemic comprise infections, epigenetic and intrauterine imprinting, increasing maternal age, greater fecundity among people with higher adiposity, assortative mating, decreased sleep, toxic chemicals, drugs, and reduction in variability of ambient temperatures (McAllister *et al.*, 2009).

It is estimated that more than 100 genes, with a small effect for each, contribute to body weight regulation (Hinney *et al.*, 2010). From GWA studies, genetic variation within the *FTO* (fat mass and obesity associated) gene shows the strongest association with obesity, though the functional mechanism is not yet perfectly determined (Korner *et al.*, 2008; Hinney *et al.*, 2010). Like T2D, epigenetic mechanisms greatly contribute to the aetiology and development of obesity (Campion *et al.*, 2009; Youngson and Morris, 2013). The important pathophysiological aspects of obesity are related, firstly, to metastatic (ectopic) fat deposition in pancreatic islets, liver, and muscle; secondly, to fat as a tissue releasing a huge number of active signalling molecules including adiponectin, leptin, and resistin, as well as FFAs; and thirdly, to adipose tissue inflammation with elevated levels of macrophage infiltration and expression of pro-inflammatory cytokines (Roth *et al.*, 2004).

1.5 FUNCTIONAL AND GENETIC STUDIES IN TYPE 2 DIABETES AND OBESITY

The spotlight of this thesis is focused on the molecular aspects of the pathogenesis of T2D and obesity. The investigated targets are described in the same order as in the list of publications.

1.5.1 Adenylyl cyclase 3

ACs are enzymes that catalyze the conversion of ATP to cAMP, which is an important intracellular second messenger potentiating nutrient-induced insulin secretion by the pancreatic β-cells (Seino, 2012). There are at least nine mammalian membrane-bound ACs sharing a common structure of two trans-membrane regions TM1 and TM2, each containing six membrane-spanning helices and two cytoplasmic regions (Halls and Cooper, 2011). Adenylyl cyclase 3 (AC3) has been characterized as one of the Ca^{2+}/calmodulin-regulated isoforms (Cooper, 2003). It has been reported that *Ac3* over-expression, due to the presence of a functional double-point mutation in the promoter region of the *Ac3* gene, is associated with reduced GSIS in GK rats (Abdel-Halim *et al.*, 1998). Additionally, an increased immunoreactivity of AC3 protein has been detected in β- and α-cells of GK rats (Guenifi *et al.*, 2000). Moreover, liver AC activity is

enhanced in the membranes of obese ob/ob mice compared with lean control mice (Begin-Heick, 1994). These findings support a role for AC3 in the pathogenesis of T2D and obesity.

1.5.2 Protein kinase C

PKC is the name given to a family of protein kinase enzymes that are involved in controlling the functions of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on those proteins. PKC isoforms are involved in a wide range of cellular processes, from fundamental activities such as cell growth and differentiation, proliferation, gene expression, signal transduction, secretion and exocytosis, and smooth muscle contraction, to higher functions such as memory (Nishizuka, 1986; Sun and Alkon, 2006). The various PKC isoforms translocate from one subcellular compartment to another when activated by neurotransmitters, hormones, or growth factors, and they differ in structure, expression pattern, subcellular localization, activation, and function (Kanashiro and Khalil, 1998).

According to their enzymatic properties, PKC isoforms have been grouped into smaller subfamilies: conventional PKCs (cPKCs), which comprise α, β1, β2, and γ isoforms, are activated by phosphatidylserine (PS), Ca^{2+}, and diacylglycerol (DAG); novel PKCs (nPKCs), which consist of δ, ε, θ, and η isoforms, are activated by PS and DAG but not Ca^{2+}; atypical PKCs (aPKCs), consisting of ζ and ι\λ isoforms, require neither Ca^{2+} nor DAG for activation; and PKC-related kinases (PRKs), encompassing at least three members (PRKs 1-3), are insensitive to both Ca^{2+} and DAG (Nishizuka, 1988; Mellor and Parker, 1998). In both pancreatic islets and liver, PKC isoforms appear to have important roles in the regulation of glucose homeostasis (Pugazhenthi and Khandelwal, 1995; Biden *et al.*, 2008). In the GK rat pancreas, diminished expression of at least four different PKC isoenzymes, namely PKCα, PKCε, PKCθ, and PKCζ, has been indicated by immunohistochemical staining (Warwar *et al.*, 2006). Recently, PKCδ has been presented to be implicated in insulin sensitivity (Bezy *et al.*, 2011). In addition to its previously suggested role in treatment of insulin resistance, inhibition of PKCε has been proposed to act as a positive regulator of insulin availability (Schmitz-Peiffer and Biden, 2008).

1.5.3 Succinyl-CoA:3-ketoacid-CoA transferase and ATP citrate lyase

The biosynthesis of compounds within mitochondria using carbon derived from insulin secretagogues (anaplerosis) and their export to the cytosol (cataplerosis), such as for the synthesis of short chain acyl-CoAs and for metabolite shuttles, seem to be essential for the stimulation and support of insulin secretion by pancreatic β-cells (MacDonald *et al.*, 2005). In this context, the pathways involving two enzymes, succinyl-CoA:3-ketoacid-CoA transferase (SCOT) and ATP citrate lyase (ATPCL), are intriguing. ATPCL plays a key role in the pyruvate citrate shuttle, as well as in the synthesis of short chain acyl-CoAs and lipids. It catalyzes the cytosolic formation of oxaloacetate from citrate (Jensen *et al.*, 2008). Besides oxaloacetate, ATPCL also forms acetyl-CoA from citrate in the cytosol, and this acetyl-CoA can be converted to three other short chain acyl-CoAs (Figure 2). The pathway that supports GSIS and involves SCOT is one in which the decarboxylation of glucose-derived pyruvate, catalyzed by PDH, forms acetyl-CoA in the mitochondria. This acetyl-CoA is converted to acetoacetyl-CoA by either

acetyl-CoA acetyltransferase 1 (ACAT1) or acetyl-CoA acyltransferase 2 (ACAA2) (MacDonald *et al.*, 2007). SCOT then catalyzes the conversion of acetoacetyl-CoA plus succinate to succinyl-CoA and acetoacetate. The succinyl-CoA can then be converted to succinate by either the GTP or the ATP succinyl-CoA transferase resulting in a regenerative cycle for succinate. The acetoacetate can be exported from the mitochondria to the cytosol, where it can be transformed into the same four short chain acyl-CoAs (Figure 2). Levels of SCOT and ATPCL have been reported to be decreased in the islets of the few humans studied (MacDonald *et al.*, 2009b).

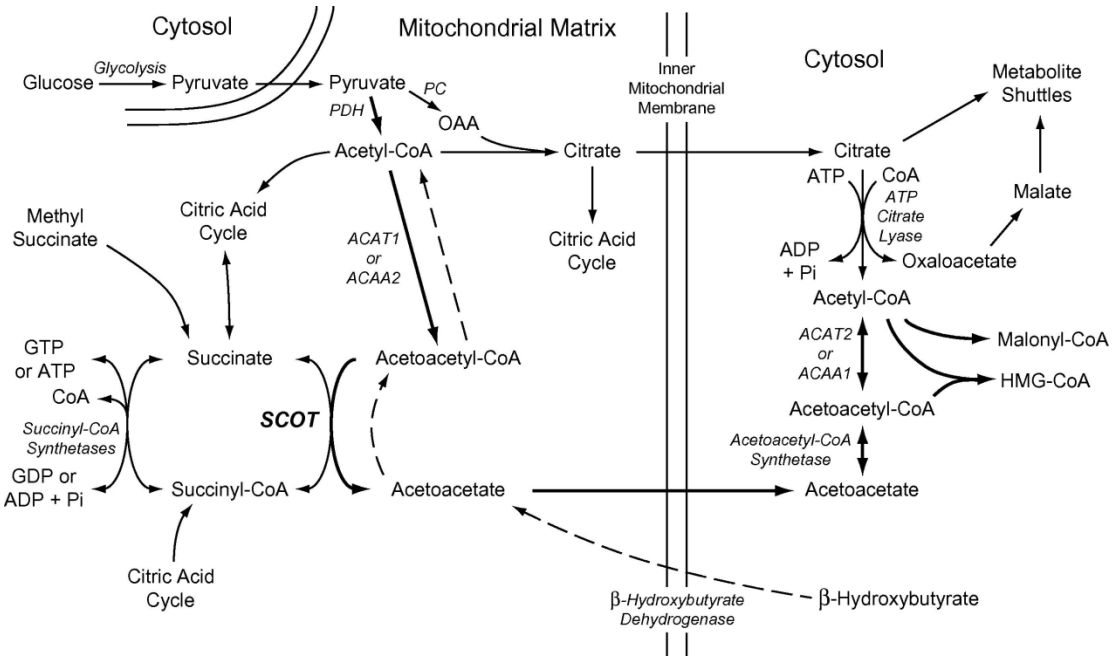

Figure 2. Schematic representation of the roles of SCOT and ATPCL in glucose- or MMS+HB-stimulated insulin secretion. *SCOT, succinyl-CoA:3-ketoacid-CoA transferase; ATPCL, ATP citrate lyase; MMS+HB, monomethylsuccinate-plus-β-hydroxybutyrate. For a detailed description, see the text.*

1.5.4 Adrenergic receptor alpha 2A

Insulin secretion from pancreatic β-cells is inhibited when α2-adrenergic receptors are activated by circulating catecholamines or stimulation of sympathetic pancreatic nerves (Ahrén, 2000). Thus, increased expression of α2-adrenergic receptors in β-cells could contribute to the aetiology of T2D (Devedjian *et al.*, 2000; Wess, 2010). α2A-adrenergic receptor-deficient mice exhibit increased plasma insulin levels, reduced blood glucose levels, and improved glucose tolerance (Savontaus *et al.*, 2008). Recent data indicate that obesity is characterized by sympathetic nervous activation, which, in the long term, may contribute to the development of obesity-related disorders including hypertension, insulin resistance, heart failure, and renal impairment (Lambert *et al.*, 2010). Polymorphisms in the human α2A-adrenergic receptor (*ADRA2A*) gene have been reported to associate with obesity (Ukkola *et al.*, 2001; Lima *et al.*, 2007), fat content and distribution (Oppert *et al.*, 1995; Garenc *et al.*, 2002), elevated glucose levels (Rosmond *et al.*, 2002), and reduced insulin secretion and increased risk of T2D (Rosengren *et al.*, 2010).

2 AIMS AND SIGNIFICANCE

The overall aim of this thesis was to elucidate the molecular pathogenetic defects related to T2D and obesity. Therefore, identification of susceptibility genes and their potential pathomechanisms would pave the way for preventative and therapeutic applications in the future.

Specific aims in the individual papers were:

Paper I – AC3

- To examine AC3 expression in pancreatic islets and in the central nervous system (CNS) of diabetic GK rats, with and without insulin treatment, to evaluate the association of altered AC3 expression with glucose regulation.

Paper II – PKC

- To investigate the expression levels of PKC isoforms (PKCα, PKCδ, PKCε, and PKCζ) in pancreatic islets and liver of GK rats, with and without insulin treatment, for the evaluation of their association with glucose homeostasis.

Paper III – SCOT and ATPCL

- To measure the levels of transcripts that encode SCOT and ATPCL, their protein levels, and also their enzyme activities in pancreatic islets of GK rats.

- To discern the necessity for SCOT in insulin secretion by studying insulin release in four cell lines developed with various levels of knockdown of SCOT protein and enzyme activity.

Paper IV – ADRA2A

- To investigate the association of *ADRA2A* genetic polymorphisms with obesity and/or T2D.

- To study *ADRA2A* mRNA expression in pancreatic islets isolated from T2D patients and non-diabetic control subjects.

3 MATERIALS AND METHODS

This section provides a brief summary of the major methods used in this thesis. For more detailed methodological descriptions, please refer to the individual papers.

3.1 MATERIALS

3.1.1 Animals

All animal experiments were approved by the regional ethics committees.

3.1.1.1 Wistar rats

Normal Wistar rats used as non-diabetic controls (**Papers I**, **II**, and **III**) were purchased from a local commercial breeder (B&K Universal, Sollentuna, Sweden).

3.1.1.2 Goto-Kakizaki rats

The GK rats used in this thesis (**Papers I**, **II**, and **III**) were obtained from our colony at Karolinska University Hospital (Stockholm, Sweden).

3.1.2 Sustained release insulin implants

Half of the GK rats (**Papers I** and **II**) were implanted with sustained release insulin implants containing 26 ± 2 mg/implant of insulin in microrecrystallized palmitic acid (LinShin Inc., Ontario, Canada) for 14 days.

3.1.3 Subjects

A total of 1,177 individuals were included in the study described in **Paper IV**, and all of them were selected from the population-based cohort of Stockholm Diabetes Prevention Programme (SDPP) investigated in both a baseline study and a follow-up study 8–10 years later, as described previously (Eriksson *et al.*, 2008). The individuals are unrelated and of Swedish origin. The subjects were randomly selected healthy controls with normal glucose tolerance (NGT) and BMI of $\leq$ 26 kg/m^2 and without family history of diabetes (n = 580; 394 men and 186 women), obese subjects with NGT and BMI $\geq$ 30 kg/m^2 (n = 198 men, since we did not have access to samples from obese women with NGT), and T2D patients with BMI between 18.4 to 58.6 kg/m^2 (n = 399; 235 men and 164 women). T2D patients were diagnosed according to criteria from WHO (1998), and the standard definition of obesity was used according to the Centre for Disease Control (CDC, 1998) (Alberti and Zimmet, 1998; National Heart, 1998). For T2D patients diagnosed between baseline and follow-up studies, baseline data were used to avoid the influence of lifestyle changes and/or anti-diabetic treatment on phenotypes. All participants gave their informed consent to be included in the study, in accordance with the declaration of Helsinki II, and as approved by the local ethics committee.

3.1.4 Human islets

With support from the Nordic Network for Clinical Islet Transplantation, human pancreatic islets, from both T2D patients and from non-diabetic controls, were isolated from brain-dead, heart-beating, multi-organ donors. The study in **Paper IV** was approved by the Human Research Ethics Committee of Karolinska Institutet.

3.1.5 INS-1 832/13 cell line

The INS-1 832/13 cell line used in the study described in **Paper III** was obtained from Hans Hohmeier and Christopher Newgard. The 832/13 cell line clone was derived from INS-1 cells, which have been stably transfected with a plasmid containing the human proinsulin gene. Such a modification resulted in the generation of cell lines that evidently demonstrate increased and stable responsiveness to both glucose and several of its known potentiators (Hohmeier *et al.*, 2000).

3.2 METHODS

3.2.1 Isolation of rat islets

Isolation of rat islets (**Papers I**, **II**, and **III**) was performed as previously described (Seed Ahmed *et al.*, 2012). Briefly, pancreata were removed after retrograde injection into the pancreatic duct of 24 mg or 9 mg collagenase in 10 ml Hank's solution (SVA, Uppsala, Sweden) in GK or Wistar rats, respectively. After 24 min of incubation at 37°C in a water-bath, the tissue was homogenized and then washed three times in Hank's solution. Enriched islet fractions were prepared on Histopaque gradients by mixing the digested tissue with 1119 and 1077 Histopaque solutions (Sigma-Aldrich, Ayrshire, UK). Following centrifugation of the gradients at 2000 rpm for 20 min, islets were collected from the border of the upper two layers and transferred to Hank's medium. Some of the islets were stored in RNAlater solution (Ambion, Austin, USA) at -20°C for total RNA extraction, and the remaining islets were processed for insulin release measurement.

3.2.2 Insulin release from isolated rat islets

Some of the isolated islets (**Papers I** and **II**), in batches of three islets each, were incubated for 60 min at 37°C in a slowly shaking water-bath, in 300 µl Krebs-Ringer bicarbonate (KRB) buffer solution with 3.3 or 16.7 mmol/l glucose. After incubation, 200 µl was aspirated to new tubes, which were kept at -20°C until insulin release was measured using radioimmunoassay (RIA) (Herbert *et al.*, 1965).

3.2.3 RNA extraction and real-time RT-PCR

In rats (**Papers I**, **II**, and **III**) and human islets (**Paper IV**), total cellular RNA was extracted using RNeasy mini kits, following the manufacturer's protocol for tissues (Qiagen, Hilden, Germany). To minimize the risk of RNA degradation, samples were kept on ice when not performing the extraction, and all working surfaces and tools were cleaned with RNase Away solution (Sigma, Buchs, Switzerland) before use. Reverse-transcription for cDNA from mRNA samples was performed using QuantiTect reverse transcription kits (Qiagen).

Real-time RT-PCR (reverse transcriptase-polymerase chain reaction) was performed with TaqMan gene expression assays specific to the target genes, with an ABI 7300 real-time PCR system (Applied Biosystems, Foster City, USA). All genes were normalized to 18S, β-actin, or GAPDH. The probes for all of the TaqMan gene expression assays employed were labeled with carboxyfluorescein (FAM) as a reporter dye, and tetramethylrhodamine (TAMRA) as a quencher dye. Amplifications were performed using the 5′-nuclease TaqMan method with a two-step PCR protocol (95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min) in an ABI 7300 real-time PCR system (Applied Biosystems). Experiments were replicated at least twice. Gene expression data were analyzed using the relative quantification method based upon the standard curve.

3.2.4 Western blot

Western blotting is a robust and widely exploited technique used to identify and quantify proteins from various types of samples, with the help of specific antibodies. It involves the sequential steps of gel electrophoresis to separate the proteins in the sample, transfer to a membrane, blocking, and detection with specific antibodies targeting the proteins of interest (Renart *et al.*, 1979; Towbin *et al.*, 1979).

The proteins were extracted in the samples (**Papers II** and **III**) using lysis buffers containing protease inhibitors. The protein concentrations were measured with the Bradford assay using bovine serum albumin (BSA) as a standard. Proteins in the lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to nitrocellulose or polyvinylidene fluoride (PVDF) membranes. After blocking with 5% fat-free milk, membranes were probed for the target proteins using suitably diluted primary antibodies. Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were used for detection. Proteins were visualized using an enhanced chemiluminescence procedure. Quantification was carried out using Luminescent Image Analyzer (Image Reader LAS-100 Pro v1.0, Fujifilm) and ImageJ software (v1.47b, National Institute of Health).

3.2.5 Insulin release from stable cell lines

Insulin release from stable cell lines derived from INS-1 832/13 cells and expressing a short hairpin RNA (shRNA) targeting *Scot* mRNA was measured (**Paper III**) as previously described (MacDonald *et al.*, 2007). One day before an insulin release experiment was to be performed, the medium was replaced with fresh medium modified to contain 5 mmol/l glucose. Two hours before the experiment, the medium was replaced with KRB buffer containing 3 mmol/l glucose and 0.5% BSA. Cells were washed once with this solution, and insulin release was studied in 1 ml of this same solution containing secretagogues or non-secretagogues as controls. After one hour at 37°C, samples of incubation solution were collected, centrifuged to sediment any cells floating in the incubation solution, and an aliquot of the supernatant fraction was removed and saved for insulin measurements by RIA. The plates were then washed once with Krebs-Ringer solution containing no added protein. Water was added to the plates and the mixture containing the cells was removed and saved for estimation of total protein by the Bradford method, using a dye reagent from Bio-Rad.

3.2.6 Enzyme activities

Enzyme activities were measured (**Paper III**) in supernatant fractions of whole-cell homogenates or mitochondria from pancreatic islets or cell lines prepared as previously described (MacDonald *et al.*, 2007). Briefly, cells and islets were homogenized in KMSH solution, and then fractionated by differential centrifugation as previously described (MacDonald *et al.*, 2007; MacDonald *et al.*, 2009a). SCOT was measured at 30°C in Tris-chloride buffer containing sodium acetoacetate, succinyl-CoA, and $MgCl_2$. The rate of acetoacetyl-CoA formation was followed by measuring the increase in absorbance at 310 nm. After the background rate was obtained, the reaction was started with the addition of acetoacetate. The background rate was subtracted from the total rate to give the rate attributable to the enzyme. ATPCL was measured in the presence of citrate, coenzyme-A, ATP, $MgCl_2$, NADH, dithiothreitol, and malate dehydrogenase in Tris-chloride buffer, at 37°C. The formation of oxaloacetate was measured by the oxidation of NADH. After the background rate was measured in the presence of enzyme source, the enzyme reaction was started with the addition of citrate. Cytosolic malic enzyme (ME1) was measured spectrophotometrically by monitoring NADPH formation at 340 nm in Tris-chloride buffer containing malate, NADP, $MgCl_2$, and DTT, at 37°C, as previously described (MacDonald *et al.*, 2009a).

3.2.7 SCOT knockdown by siRNA

In vivo expression of short interfering RNAs (siRNAs) was accomplished (**Paper III**) using hygromycin selectable DNA vectors that express shRNA. Four regions of the *Scot* gene (*Scot* 68, *Scot* 713, *Scot* 1676, and *Scot* 1184) were targeted with shRNA in INS-1 832/13 cells resulting in cell lines with varying degrees of knockdown of SCOT protein. Pools of cells stably transfected with each plasmid vector targeting one *Scot* mRNA target were used for measurements of SCOT and control enzymes, and to study insulin release.

3.2.8 Insulin content measurement

The insulin content of the cell lines was measured (**Paper III**) by RIA in acid-ethanol extracts of the cells. In brief, one hour after adding acid-ethanol to each well containing cells, the cell extract was removed and vortexed vigorously and frozen until use, when a portion of it was diluted in potassium phosphate buffer containing 0.5% BSA before insulin was measured.

3.2.9 Genotyping

Genotyping was performed (**Paper IV**) using TaqMan allelic discrimination (ABI 7300, Applied Biosystems). This is a probe technology which is dependent upon the 5′-nuclease activity of the enzyme Taq DNA polymerase (Holland *et al.*, 1991) and two single-stranded fluorogenic probes, one for each allele. The fluorescent probe is a dual-labeled oligonucleotide with a 5′-reporter dye and a 3′-quencher dye. Tetrachlorofluorescein (TET) is covalently linked to the 5′ end of the probe for the detection of allele 1 and FAM is covalently linked to the 5′ end of the probe for the detection of allele 2. Both reporters are quenched by TAMRA, which is attached at the 3′ end of each probe. When the probe is intact, the fluorescence of the reporter is quenched due to its proximity to the quencher. The TaqMan probe hybridizes to a

target sequence of DNA within the PCR product, which is determined by forward and reverse primers that hybridize during PCR to specific sequences within the target DNA. During the extension step of the amplification reaction, the 5′-nuclease activity of the Taq DNA polymerase cleaves off the reporter. Therefore, the reporter is separated from the quencher and the resulting fluorescence signal is proportional to the amount of amplified product in the sample (Figure 3) (Lyamichev *et al.*, 1993; Applied Biosystems, 2010).

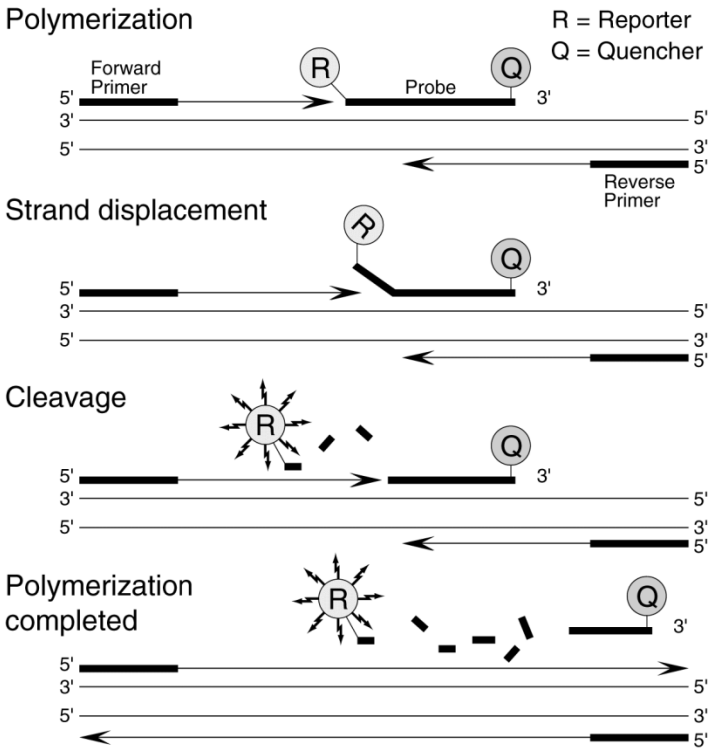

Figure 3. TaqMan allelic discrimination. *The 5′-nuclease activity of Taq DNA polymerase during the extension phase of PCR is depicted. (This picture is taken from TaqMan Allelic Discrimination, Demonstration Kit Protocol, no. 4303267E released by Applied Biosystems (2010).)*

3.2.10 Statistical analysis

In **Papers I**, **II**, and **III**, data were presented as means ± standard error of the mean (SE) for normally distributed variables, or as geometric means (95% confidence interval (CI)) for non-normally distributed variables. To normalize the skewed distribution, natural logarithmic transformation was applied before analysis when needed. The comparisons of continuous variables between independent groups were performed by unpaired Student's t-test, or by one-way ANOVA (analysis of variance) followed by Tukey or Newman-Keuls post-hoc tests. P-values of 0.05 or less ($p \leq 0.05$) were considered significant. Data were analyzed using PASW version 18, IBM SPSS Statistics version 20 (IBM SPSS, Chicago, IL, USA), and GraphPad Prism version 6.0 (GraphPad Software Inc., La Jolla, CA, USA).

In **Paper IV**, data were presented as means (95% CI) or means ± SE for normally distributed variables, or as geometric means (95% CI) for non-normally distributed variables, which have been logarithmically transformed before analysis.

Hardy-Weinberg equilibrium was tested by χ^2-test (Weir, 1996; Mayo, 2008). Allele distributions were compared between cases and controls, and odds ratios (ORs) and 95% CIs were calculated to test for associations. Multiple logistic regression analysis, with results expressed as ORs (95% CI), was performed to study genotype distribution differences between cases and controls after adjustments for potential confounders. Comparisons were carried out in men and women separately. The homeostasis model assessment was used to assess insulin resistance (HOMA-IR). Based on the fasting glucose and insulin levels, HOMA-IR was calculated according to the following equation: fasting plasma glucose (mmol/l) × fasting plasma insulin (mU/l)/22.5 (Matthews *et al.*, 1985). The unpaired Student's t-test was used to compare the differences between two independent groups. $P \leq 0.05$ was considered significant. All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA) and PASW version 18 (IBM SPSS, Chicago, IL, USA) software.

4 RESULTS AND DISCUSSION

4.1 INCREASED EXPRESSION OF ADENYLYL CYCLASE 3 IN PANCREATIC ISLETS AND CENTRAL NERVOUS SYSTEM OF DIABETIC GOTO-KAKIZAKI RATS: A POSSIBLE REGULATORY ROLE IN GLUCOSE HOMEOSTASIS (PAPER I)

In this study, we investigated *Ac3* mRNA expression levels in pancreatic islets and regions of striatum/hypothalamus of diabetic GK rats with and without insulin treatment. In addition, in order to ascertain whether AC3 co-localizes and interacts with regulator of G protein signalling 9 (RGS9) in brain, we examined *Ac3* expression in striatum and hypothalamus dissected from *Rgs9* knockout (*Rgs9*$^{-/-}$) and wild-type (*Rgs9*$^{+/+}$) mice, because it has been shown that RGS9 is enriched in the striatum region of brain (Chen *et al.*, 2000; Waugh *et al.*, 2011).

We have found that *Ac3* mRNA expression levels in pancreatic islets and regions of the striatum/hypothalamus of brains from GK, insulin-treated GK, and control Wistar rats were different (Figure 4A and B). *Ac3* mRNA expression was increased in islets of GK compared with Wistar rats (p = 0.016, Figure 4A), while its expression was intermediate in insulin-treated GK rat islets. This *Ac3* expression pattern was also observed in the striatum/hypothalamus from the three groups of rats, i.e. *Ac3* mRNA expression levels in the striatum/hypothalamus of GK rats were increased compared with Wistar rats (p = 0.021, Figure 4B). Furthermore, similar levels of expression of *Ac3* were observed in the striatum and hypothalamus of *Rgs9* knockout mice compared with wild-type mice.

A **B**

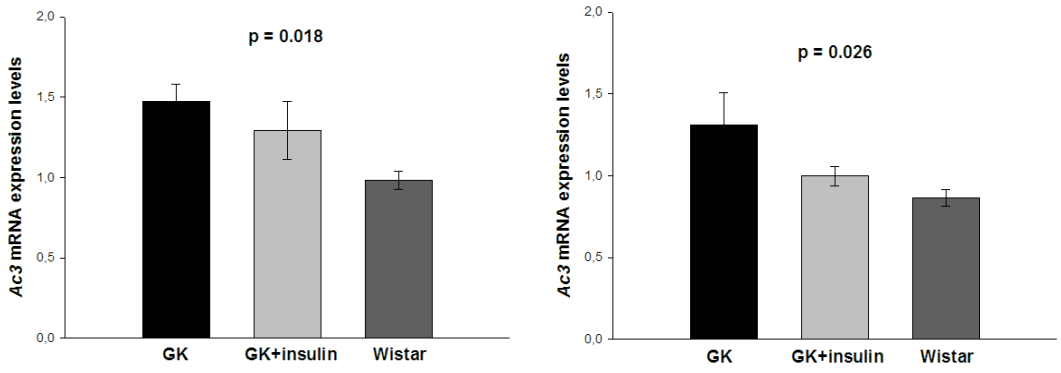

Figure 4. Ac3 mRNA expression levels in pancreatic islets (A) and striatum/hypothalamus regions of brain (B). *Ac3 mRNA expression levels in both islets and striatum/hypothalamus of GK rats were increased compared with Wistar rats (p = 0.016 and p = 0.021, respectively). Data are means ± SE. P-value from one-way ANOVA is given in the figure. Pairwise comparisons were performed by Tukey post-hoc test.*

We applied insulin treatment to normalize blood glucose levels in GK rats for two weeks. Comparison analyses between Wistar, GK rats with and without insulin treatment provided us with the opportunity to investigate whether over-expression of *Ac3* is a primary defect, or secondary to the diabetic state (hyperglycaemia). Data in the present study indicate that *Ac3* is over-expressed in pancreatic islets of GK compared with Wistar rats, a finding consistent with a previous observation from our laboratory in which increased *Ac3* expression was suggested to account for impaired GSIS (Abdel-Halim *et al.*, 1998). In addition, we have shown that the *Ac3* gene is over-expressed in the striatum/hypothalamus of GK rats compared with Wistar rats. Interestingly, the *Ac3* expression patterns in islets and striatum/hypothalamus are similar. Thus, *Ac3* expression levels in both islets and striatum/hypothalamus of insulin-treated GK rats were intermediate between the expression levels in GK and Wistar rats. This shows that normalization of plasma glucose levels in GK rats with insulin treatment tended to decrease the augmented levels of *Ac3* mRNA expression in this strain. Since *Ac3* is expressed in striatum and hypothalamus tissues from *Rgs9* knockout mice, it seems unlikely that the *Ac3* and *Rgs9* genes interact. Our attempts to analyze AC3 expression at the protein level with Western blotting technique failed, mainly due to the high homologies of AC3 with other AC isoforms, and because of the lack of specific high-affinity antibodies (Hanoune and Defer, 2001). Moreover, several reports demonstrate that neuronal signalling, consisting of both afferent and efferent autonomic nerves, plays important roles in inter-organ metabolic communication and systemic homeostasis (Yamada *et al.*, 2008; Katagiri *et al.*, 2009). Based upon our observations, *Ac3* is over-expressed not only in islets but also, in a similar pattern, in striatum/hypothalamus of GK rats, suggesting the existence of a functional link between CNS and pancreatic islets via AC3 regulation.

It has been demonstrated that *AC3* genetic polymorphisms are associated with obesity in Swedish men with and without T2D (Nordman *et al.*, 2008). This association was replicated in an adult Chinese population (Wang *et al.*, 2010). Furthermore, *Ac3* knockout mice exhibit obesity as they age, mainly due to decreased locomotor activity, hyperphagia, and leptin insensitivity (Wang *et al.*, 2009). Thus, AC3 appears to be of genetic and biological relevance in the regulation of body weight and glucose homeostasis (Gu, 2010). In the current study, body weights of GK rats with and without insulin treatment were lower compared with Wistar rats. Taken together with the aforementioned studies, AC3 is proposed to play an important role in body weight regulation, which could be mediated by CNS.

In conclusion, this study provides evidence that *Ac3* mRNA expression is increased in pancreatic islets and striatum/hypothalamus of GK rats, at least partly due to the diabetic state. AC3 may participate in the regulation of glucose homeostasis via insulin secretion and CNS, without obvious interaction with RGS9. In addition, it may have a primary role in regulating body weight through CNS.

4.2 EXPRESSION OF PROTEIN KINASE C ISOFORMS IN PANCREATIC ISLETS AND LIVER OF GOTO-KAKIZAKI RATS, A MODEL OF TYPE 2 DIABETES (PAPER II)

In this study, we examined the expression levels of PKC isoforms, namely PKCα, PKCδ, PKCε, and PKCζ, in pancreatic islets and liver of GK rats with and without insulin treatment.

We have shown that PKCα and PKCζ mRNA expression levels were under-expressed in pancreatic islets of GK compared with Wistar rats ($p < 0.05$ and $p < 0.01$, respectively), while their expressions in insulin-treated GK rats were intermediate between those in GK and Wistar rats (Figure 5A and D). PKCα and phosphorylated PKCα (p-PKCα) protein expressions were decreased in islets of GK compared with insulin-treated GK ($p < 0.001$ and $p < 0.05$, respectively) and Wistar rats ($p < 0.05$) (Figure 6A and E). Furthermore, PKCα protein expression was enhanced in islets of insulin-treated GK compared with Wistar rats ($p < 0.01$). However, the ratio of p-PKCα to PKCα (p-PKCα/PKCα) in islets did not show any difference between the three groups of rats. Whereas PKCζ protein expression in islets was diminished in both GK and insulin-treated GK compared with Wistar rats ($p < 0.05$), p-PKCζ was decreased only in GK compared with insulin-treated GK ($p < 0.01$) and Wistar rats ($p < 0.05$) (Figure 6D and H). With regard to PKCε mRNA expression in islets, it was down-regulated in GK compared with insulin-treated GK ($p < 0.05$) and Wistar rats ($p < 0.01$) (Figure 5C). At the protein level, the expressions of PKCε and p-PKCε were reduced in GK compared with insulin-treated GK ($p < 0.05$) and Wistar rats ($p < 0.05$) (Figure 6C and G). In spite of being unchanged at the mRNA level, PKCδ protein expression was lower in islets of GK compared with insulin-treated GK ($p < 0.01$) and Wistar rats ($p < 0.01$) (Figure 6B). Moreover, islet p-PKCδ protein expression was decreased in GK rats, to the extent that it was non-quantifiable (Figure 6F).

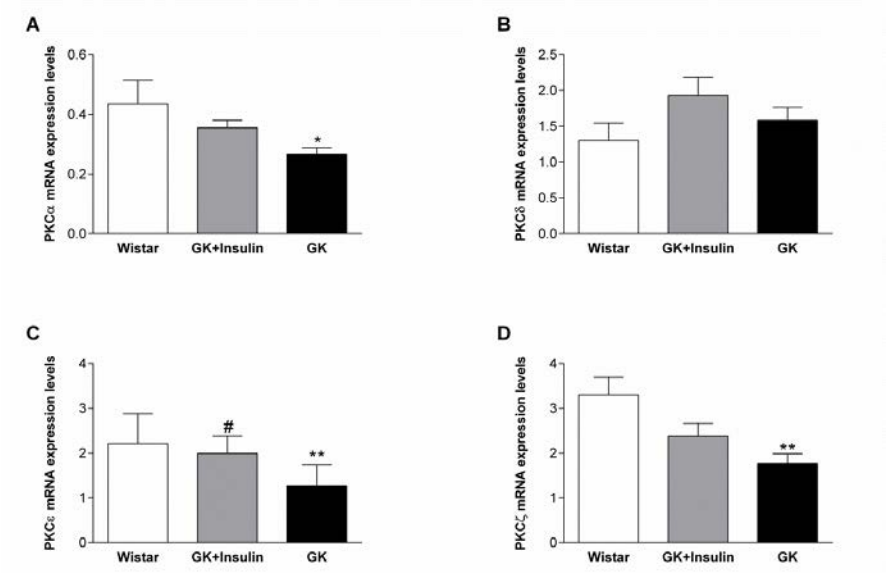

Figure 5. mRNA expression levels of PKCα (A), PKCδ (B), PKCε (C), and PKCζ (D) in pancreatic islets of Wistar, insulin-treated GK, and non-treated GK rats. *Data are means ± SE for all PKC isoforms, except for PKCε, for which data have been shown as geometric means (95% Cl). *p < 0.05, **p < 0.01 vs. Wistar rats; #p < 0.05 vs. GK rats.*

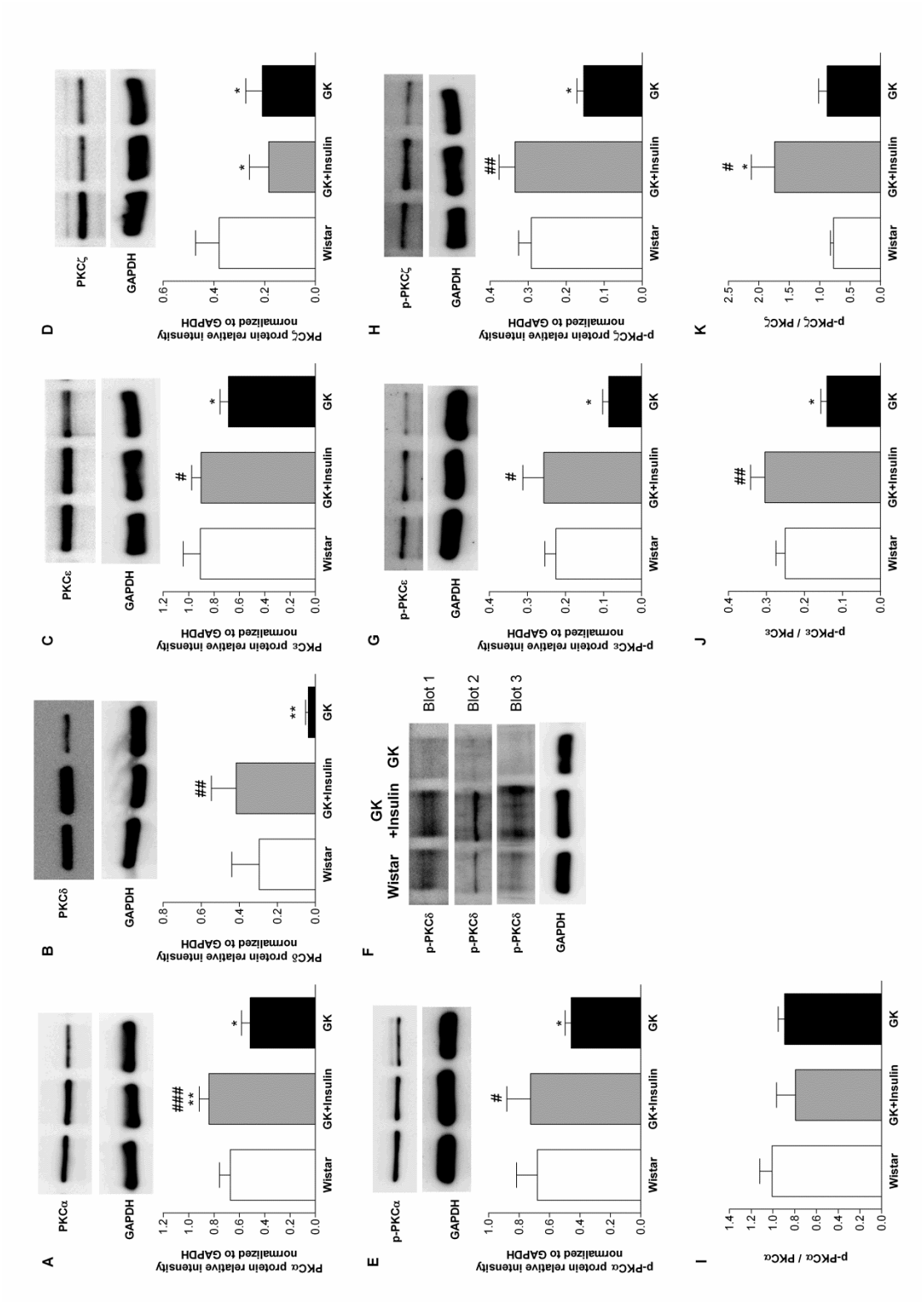

Figure 6. Representative immunoblots and densitometric analyses of PKCα (A), PKCδ (B), PKCε (C), PKCζ (D), p-PKCα (E), p-PKCε (G), and p-PKCζ (H) protein expressions in pancreatic islets of Wistar, insulin-treated GK, and non-treated GK rats. *The relative expression of p-PKCδ (F) was too low for densitometric analysis, thus p-PKCδ expression is presented with three different representative Western blots. Calculation of the ratio of phosphorylated protein to total protein allowed for assessing the sensitivity of proteins in islets from the three groups of rats (I-K). Data are means ± SE. *p < 0.05, **p < 0.01 vs. Wistar rats; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. GK rats.*

In liver, PKCδ and PKCζ mRNA expressions were decreased in both GK ($p < 0.001$) and insulin-treated GK rats ($p < 0.01$ and $p < 0.001$, respectively) compared with Wistar rats (Figure 7B and D). Hepatic PKCζ protein expression was diminished in both GK rats with and without insulin treatment compared with Wistar rats ($p < 0.05$) (Figure 8D). Although PKCε mRNA expression was down-regulated in liver of insulin-treated GK compared with non-treated GK ($p < 0.01$) and Wistar rats ($p < 0.05$) (Figure 7C), it displayed no difference in expression at the protein level. Also, PKCα in liver exhibited no difference between the three groups of rats either at the mRNA or protein levels.

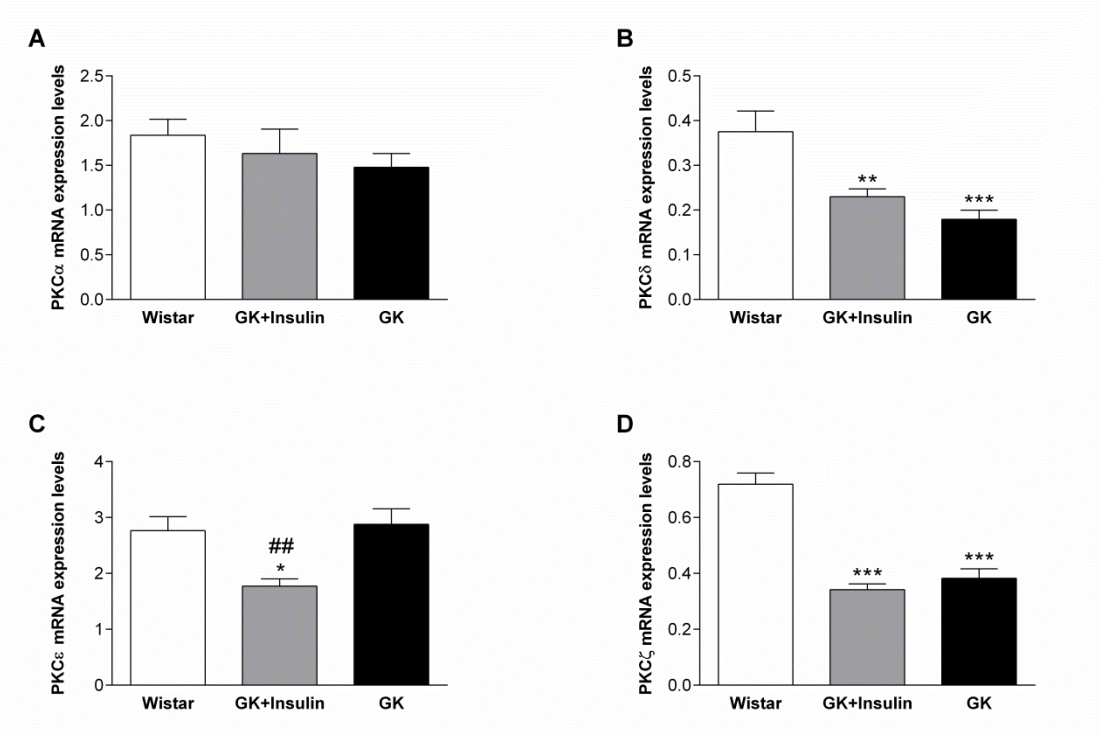

Figure 7. mRNA expression levels of PKCα (A), PKCδ (B), PKCε (C), and PKCζ (D) in livers of Wistar, insulin-treated GK, and non-treated GK rats. *Data are means ± SE. *p < 0.05, **p < 0.01, ***p < 0.001 vs. Wistar rats; ##p < 0.01 vs. GK rats.*

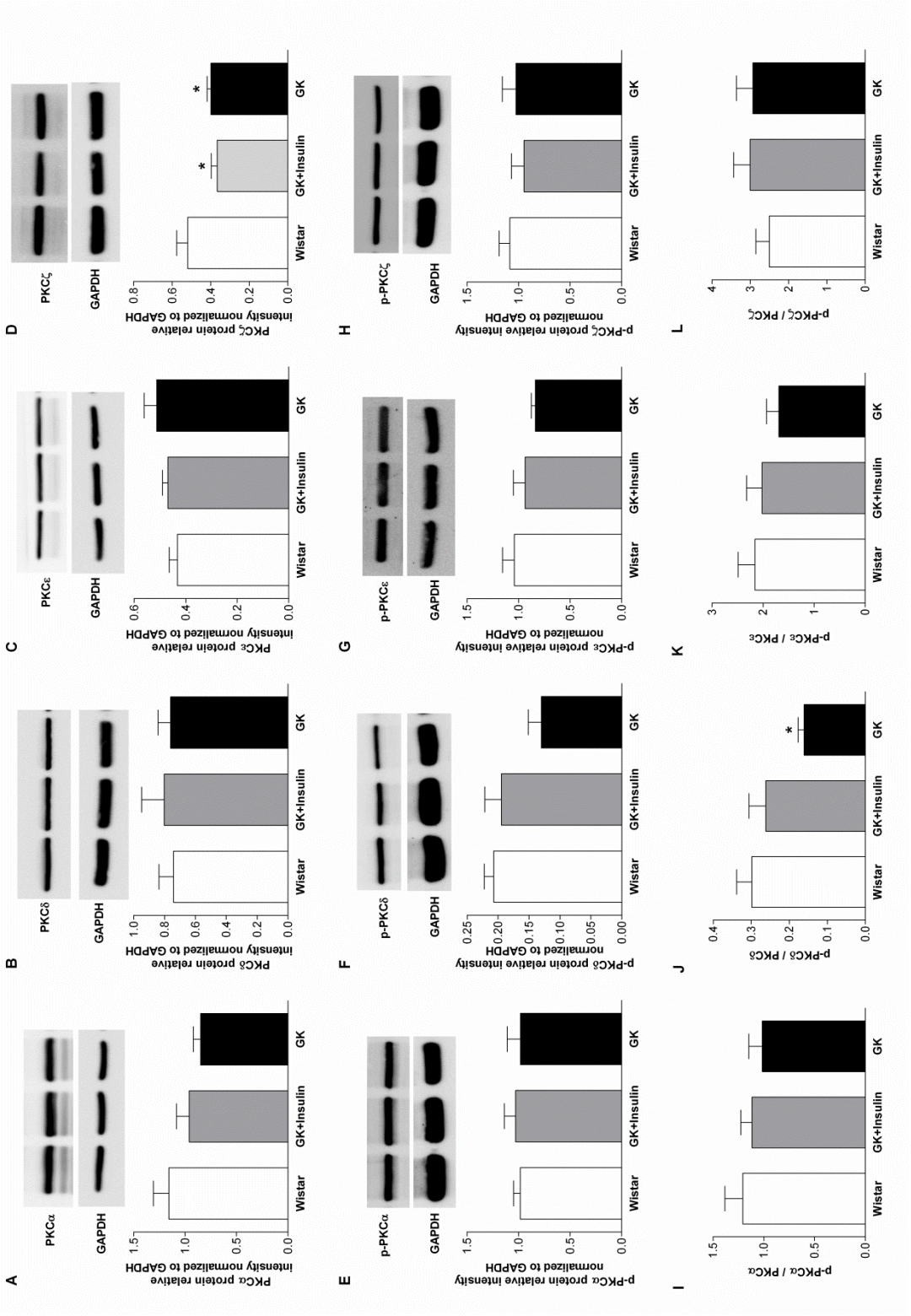

Figure 8. Representative immunoblots and densitometric analyses of PKCα (A), PKCδ (B), PKCε (C), PKCζ (D), p-PKCα (E), p-PKCδ (F), p-PKCε (G), and p-PKCζ (H) protein expressions in livers of Wistar, insulin-treated GK, and non-treated GK rats. *Calculation of the ratio of phosphorylated protein to total protein allowed for assessing the sensitivity of proteins in livers from the three groups of rats (I-L). Data are means ± SE. *p < 0.05 vs. Wistar rats.*

In the GK rat, diminished expression of four different PKC isoenzymes, namely PKCα, PKCε, PKCθ, and PKCζ, has been demonstrated in pancreatic islets by immunohistochemical staining (Warwar *et al.*, 2006). PKCα is proposed to play a role in glucose-induced insulin granule recruitment for exocytosis and insulin secretion (Ganesan *et al.*, 1990; Calle *et al.*, 1992; Ganesan *et al.*, 1992; Yedovitzky *et al.*, 1997; Yaney *et al.*, 2002; Zhang *et al.*, 2004; Warwar *et al.*, 2006), and its expression is shown to be modulated by hyperglycaemia (Nesher *et al.*, 2001; Warwar *et al.*, 2006). This is congruent with our finding in the present study in which islet PKCα was decreased in GK compared with Wistar rats, with an intermediate expression in insulin-treated GK rats suggesting a partial contribution of hyperglycaemia to the decreased expression of PKCα in GK rat islets. Notably, GSIS was enhanced in islets from insulin-treated versus non-treated GK rats, suggesting at least a partial restoration of insulin exocytosis.

PKCδ is reported to have a non-redundant role in GSIS from pancreatic β-cells (Uchida *et al.*, 2007). Inconsistently, the overexpression of kinase-negative PKCδ in pancreatic β-cells protects mice from high-fat diet-induced glucose intolerance and β-cell dysfunction (Hennige *et al.*, 2010). These seemingly conflicting findings could be explained by the difference between the whole-body knockout in contrast with the β-cell-specific inhibition of PKCδ performed in these two studies, respectively. Additionally, the latter study examined the effect of PKCδ in glucose homeostasis after high-fat feeding. Moreover, Frangioudakis *et al.* have found that deletion of PKCδ protects against high-fat diet-induced glucose intolerance and improves glucose tolerance in chow-fed mice (Frangioudakis *et al.*, 2009). Data from the present study showed that islet PKCδ mRNA expression was not affected in non-treated GK rats. Notwithstanding this, the protein expressions of PKCδ and p-PKCδ were reduced in GK rat islets, but the insulin-treated GK group exhibited increased protein expression levels, which were comparable to those of Wistar rats. In liver, PKCδ is suggested to regulate hepatic insulin sensitivity and hepatosteatosis in mice and men (Bezy *et al.*, 2011). Global or liver-specific inactivation of PKCδ results in improved glucose tolerance and insulin sensitivity. Also, liver-specific over-expression of PKCδ leads to hepatic insulin resistance. Furthermore, PKCδ expression is enhanced in livers of obese and T2D obese subjects (Bezy *et al.*, 2011). In the current study, hepatic mRNA expression of PKCδ was under-expressed in the GK rat, which is a non-obese diabetic model. Even though hepatic PKCδ was not affected in GK rats at the protein level, the p-PKCδ/PKCδ was decreased in GK compared with Wistar rats ($p < 0.05$) (Figure 8J); and this is consistent with the fact that insulin resistance is not the main, but a contributory, pathophysiological feature in the diabetic state in GK rats (Bisbis *et al.*, 1993; Ostenson and Efendic, 2007).

In many cell types, PKCε has been identified to be implicated in the regulation of survival pathways via activation of Akt, which is also known as protein kinase B (Matsumoto *et al.*, 2001). Also, PKCε protects islets and improves their survival during the phase of isolation, contributing to preserved islet cell mass (Kvezereli *et al.*, 2008). Incidentally, deletion of PKCε, in a milieu of lipid oversupply, improves glucose stimulated insulin secretion and prevents glucose intolerance in mice (Schmitz-Peiffer *et al.*, 2007). In the present study, islet PKCε was reduced in GK rats at the mRNA and protein levels. In addition, islet p-PKCε/PKCε was reduced in GK compared with

insulin-treated GK ($p < 0.01$) and Wistar rats ($p < 0.05$) (Figure 6J), which could be due to very low expression of p-PKCε (Figure 6G). In liver, PKCε mRNA, but not protein, was decreased in insulin-treated GK rats, suggesting the involvement of insulin in the control of PKCε expression, at least at the mRNA level.

PKCζ, through mTOR activation, is essential for growth factor-induced pancreatic β-cell proliferation with a concomitant improvement in β-cell function (Buteau *et al.*, 2001; Vasavada *et al.*, 2007; Velazquez-Garcia *et al.*, 2011). Of note, GK rats at fetal stage from the Paris colony display a reduction in pancreatic β-cell mass, which is maintained in the adult animal and apparently predates the onset of diabetes (hyperglycaemia) at about three or four weeks of age (Movassat *et al.*, 1997). In contrast, in the Stockholm colony, β-cell density and the relative volume of islet endocrine cells were alike in two- to three-month-old GK and control Wistar rats (Ostenson and Efendic, 2007). Additionally, GK pups from the Stockhom colony were already hyperglycaemic at the first week of age (Abdel-Halim *et al.*, 1994). In the present study, although PKCζ protein expression, in comparison with Wistar rats, was reduced in islets of GK and insulin-treated GK rats, the latter group demonstrated considerable enhancement in the levels of phosphorylated PKCζ, and the p-PKCζ/PKCζ was increased in insulin-treated GK compared with both non-treated GK and Wistar rats ($p < 0.05$) (Figure 6K). In hepatocytes, PKCζ mediates the phosphatidylinositol 3-kinase (PI3K) effect on insulin internalization in a Rab5-dependent manner (Fiory *et al.*, 2004). Given that defective insulin internalization and, consequently, hyperinsulinaemia may also cause secondary insulin resistance in animal models (Poy *et al.*, 2002), PKCζ activation could be important for improving insulin sensitivity. Though hepatic PKCζ protein expression was diminished in both GK rats with and without insulin treatment compared with Wistar rats in the current study, the p-PKCζ and p-PKCζ/PKCζ were not different amongst the three groups of rats, and that could be explained by the fact that the GK rat is not a severely insulin-resistant model. Collectively, enhancing PKCζ activity may be of value as a therapeutic strategy for the treatment of diabetes.

In conclusion, data from the pancreatic islets of GK rats in this study suggest that defects in PKCα and PKCε expressions are secondary to hyperglycaemia, since the expression pattern was restored after insulin treatment. Furthermore, insulin treatment increased islet p-PKCζ expression. In liver, PKCε mRNA expression in liver could be under control of insulin. Moreover, the capacity of hepatic PKCδ to be phosphorylated is diminished in GK rats.

4.3 LOWER SUCCINYL-COA:3-KETOACID-COA TRANSFERASE (SCOT) AND ATP CITRATE LYASE IN PANCREATIC ISLETS OF A RAT MODEL OF TYPE 2 DIABETES: KNOCKDOWN OF SCOT INHIBITS INSULIN RELEASE IN RAT INSULINOMA CELLS (PAPER III)

In this study, we measured the levels of transcripts that encode SCOT and ATPCL, their protein levels, and their enzyme activities in pancreatic islets of GK rats. Further, we studied whether insulin secretion was decreased in proportion to SCOT knockdown in four cell lines with varying degrees of knockdown of SCOT protein.

We have found that SCOT and ATPCL mRNA expressions were decreased ($p < 0.001$) and their enzyme activities were lower ($p < 0.01$ and $p < 0.001$, respectively) in pancreatic islets of GK compared with Wistar rats. In addition, SCOT and ATPCL protein levels were diminished in islets of GK compared with Wistar rats.

Four regions in the *Scot* gene were targeted with shRNA in INS-1 832/13 cells resulting in cell lines with varying degrees of knockdown of SCOT protein and enzyme activity in whole-cell homogenates and mitochondria compared with the parent INS-1 832/13 cell line and the CHS cell line that contains a non-targeting insert. The two cell lines, SCOT 1676 and SCOT 1184, with about 75% knockdown of whole-cell SCOT protein and SCOT enzyme activity, as well as mitochondrial SCOT activity, showed 70-80% reductions in glucose- or monomethylsuccinate-plus-β-hydroxybutyrate (MMS+HB)-stimulated insulin release compared with the control cell lines (Figure 9). Less inhibition of insulin release was observed with the two cell lines, SCOT 713 and SCOT 68, in which the extent of SCOT knockdown was less. The cell line with the least knockdown of SCOT activity and protein, SCOT 713, showed only about 25% decreases in glucose- and MMS+HB-stimulated insulin release. The SCOT 68 cell line showed about a 50% decrease in SCOT levels, and a 50% decrease in GSIS, but no decrease in MMS+HB-stimulated insulin release (Figure 9). To make sure that the decreases in secretagogue-stimulated insulin release in SCOT-targeted cell lines were not due to an off-target effect, the activities of ME1 and ATPCL were measured as control enzymes in these cell lines; these were not different from those of the control cell line. The insulin content of the cell lines with severely decreased secretagogue-stimulated insulin release, SCOT 1676 and SCOT 1184, was lower than that of the control CHS cell line. In contrast, the other two cell lines with moderately decreased insulin release, SCOT 713 and SCOT 68, showed no differences in insulin content from that of the CHS cell line. However, since only 2-5% of the total insulin content of the cell lines was secreted during the 1-hour period of stimulation, it is improbable that these reductions in insulin content were big enough to be the reason for the diminished insulin release. The lower insulin content might indicate that the SCOT reaction in some way affects insulin biosynthesis.

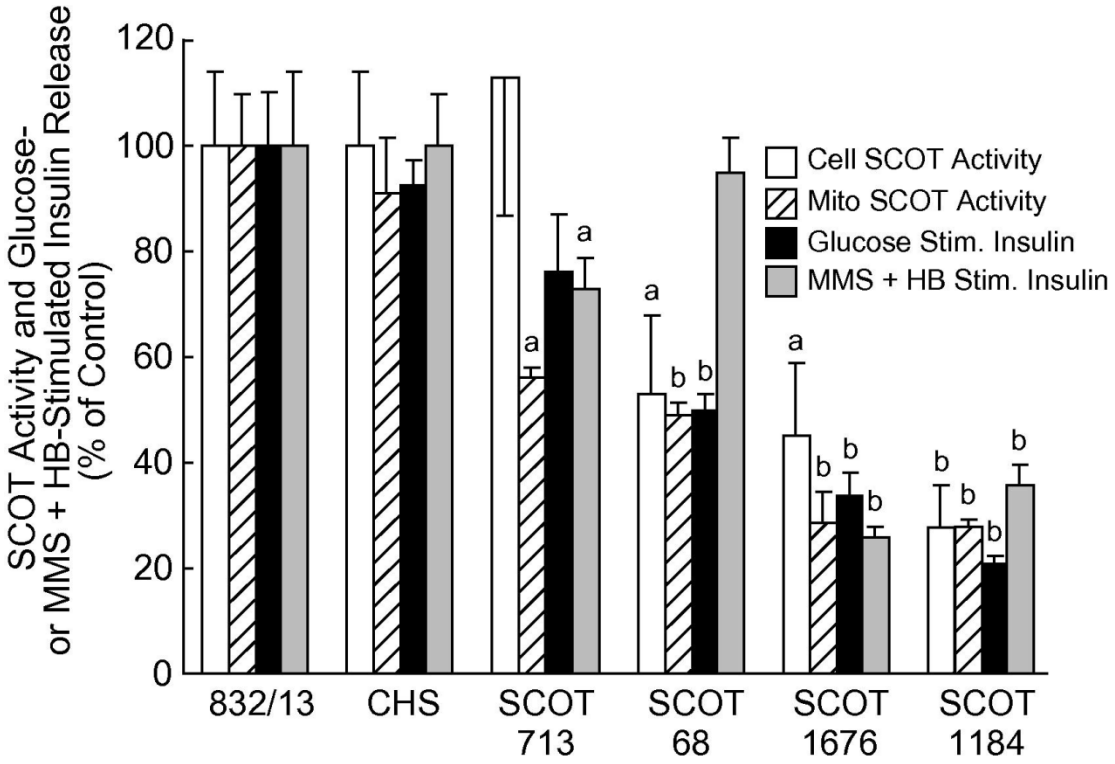

Figure 9. Decreased glucose- or MMS+HB-stimulated insulin release is proportional to knockdown of SCOT enzyme activity in cell lines derived from INS-1 832/13 cells. *Data are means ± SE. [a]p < 0.05, [b]p < 0.001 vs. the INS-1 832/13 control or the CHS control cell line transfected with a non-targeting shRNA insert.*

The findings in the present study, which demonstrate decreased mRNA expressions, protein levels, and enzyme activities of SCOT and ATPCL in pancreatic islets of GK rats, have confirmed the results obtained from a small number of isolated human pancreatic islets in a previous study (MacDonald *et al.*, 2009b). The under-expression of SCOT and ATPCL at the mRNA level in pancreatic islets of GK rats in this study is most likely a primary contribution to the impaired insulin secretion and abnormal islet function seen in GK islets, since insulin treatment of GK rats did not increase expression of the mRNA transcripts that encode SCOT and ATPCL (unpublished data).

Two previous studies, in which siRNA or shRNA were used to knockdown ATPCL in pancreatic islets or cell lines, showed that GSIS is not inhibited (Joseph *et al.*, 2007; MacDonald *et al.*, 2007), while one study describing siRNA knockdown of the enzyme in INS-1 832/13 cells showed that insulin release is inhibited (Guay *et al.*, 2007). In the current study, knockdown of SCOT with shRNA lowered GSIS in the INS-1 832/13 cell line. The pathway involving SCOT is redundant with the pathways involving ATPCL with respect to the supply of carbon precursors of short chain acyl-CoAs to the cytosol (Figure 2), and this may be why knockdown of ATPCL does not usually inhibit insulin release. The reason why knockdown of SCOT alone inhibits insulin release is unclear at present, but might be because SCOT is located inside the mitochondria and may be necessary for mitochondrial homeostasis, in addition to its role in the supply of acetoacetate for export to the cytosol for formation of short chain acyl-CoAs. The two cell lines with the severest knockdown of SCOT enzyme activity and protein, SCOT 1676 and SCOT 1184, showed the most prominent reductions in insulin secretion

stimulated by glucose or MMS+HB. The two cell lines with lesser levels of knockdown of SCOT protein and SCOT enzyme activity, SCOT 713 and SCOT 68, showed a lowering of glucose- and/or MMS+HB-stimulated insulin release, which was of less magnitude and of greater variability.

When MMS+HB are applied to the cells to stimulate insulin secretion (Figure 2), the pathway that involves SCOT begins with the conversion of β-hydroxybutyrate to acetoacetate by β-hydroxybutyrate dehydrogenase. Acetoacetate can be exported to the cytosol to directly form short chain acyl-CoAs as described above. Exogenously derived acetoacetate can also react with succinyl-CoA from the TCA cycle, to generate acetoacetyl-CoA and succinate in a reaction catalyzed by SCOT. The added succinate derived from the exogenously added methyl succinate, via a mass action effect, can also increase succinyl-CoA to feed the SCOT reaction and in addition, the succinate can replenish other four-carbon intermediates of the TCA cycle. Either ACAT1 or ACAA2 can split the resulting acetoacetyl-CoA into two acetyl-CoA molecules, which can condense with oxaloacetate to form citrate for metabolism in the TCA cycle, in order to generate energy for the stimulation of insulin release; alternatively, citrate is exported to the cytosol to be further processed by ATPCL as described above.

In conclusion, this study verifies that the mitochondrial pathways involving SCOT, instead of or in concert with the pathway involving ATPCL, are important potentiators of GSIS.

4.4 GENETIC ASSOCIATION OF ADRENERGIC RECEPTOR ALPHA 2A WITH OBESITY AND TYPE 2 DIABETES (PAPER IV)

In this study, we investigated the association of *ADRA2A* genetic polymorphisms with obesity and/or T2D in a Swedish cohort. In addition, we studied *ADRA2A* mRNA expression in isolated human pancreatic islets.

The single nucleotide polymorphism (SNP) rs553668 in the *ADRA2A* gene revealed an association between T2D patients and lean NGT subjects in men (OR = 1.47; 95% CI = 1.08-2.01; p = 0.015; Table 1), but this association was lost after adjusting for age and BMI in the multiple logistic regression model. Carriers with the A/A genotype had an increased risk of disease. However, male T2D patients had BMIs ranging from 18.4-45.6 kg/m^2. To determine whether this polymorphism is associated with T2D or obesity, we analyzed obese NGT and lean NGT subjects, and found an association for the A allele with obesity (OR = 1.49; 95% CI = 1.07-2.07; p = 0.017). We also compared lean T2D patients versus lean NGT subjects, but no association was found. However, when comparing obese T2D male patients with lean NGT male subjects, a difference was observed (OR = 1.62; 95% CI = 1.06-2.49; p = 0.026). Using multiple logistic regression analysis for SNP rs553668, and including age in the model, we compared obese NGT and lean NGT groups, and detected an association (OR = 2.82; 95% CI = 1.03-7.74; p = 0.044). There was no association when comparing lean T2D and lean NGT, but when looking at obese T2D and lean NGT subjects, we found an association (OR = 4.17; 95% CI = 1.02-17.00; p = 0.046). For a summary of the comparisons for SNP rs553668 in men, see Table 1.

Table 1. SNP rs553668: genotype distribution and association in men

Comparisons	Genotype distribution			MAF	Allele frequency difference			Multiple logistic regression						
	G/G n (%)	G/A n (%)	A/A n (%)		OR	95% CI	P	G/G[a]	G/A			A/A		
								OR	OR	95% CI	P	OR	95% CI	P
T2D vs. Lean NGT	158/295 (35/65)	65/89 (42/58)	10/7 (59/41)	0.18/0.13	1.47	1.08-2.01	0.015	1.00	1.26	0.82-1.92	0.295[b]	3.09	0.99-9.59	0.051[b]
T2D vs. Lean NGT								1.00	0.96	0.47-1.96	0.913[c]	2.29	0.34-15.49	0.394[c]
Obese NGT vs. Lean NGT	134/295 (31/69)	55/89 (38/62)	9/7 (56/44)	0.18/0.13	1.49	1.07-2.07	0.017	1.00	1.35	0.91-2.01	0.132[b]	2.82	1.03-7.74	0.044[b]
Lean T2D vs. Lean NGT	40/295 (12/88)	11/89 (11/89)	2/7 (22/78)	0.14/0.13	1.09	0.61-1.95	0.780	1.00	0.97	0.46-2.06	0.934[b]	1.76	0.25-12.58	0.572[b]
Obese T2D vs. Lean NGT	56/295 (16/84)	26/89 (23/77)	4/7 (36/64)	0.20/0.13	1.62	1.06-2.49	0.026	1.00	1.35	0.75-2.43	0.319[b]	4.17	1.02-17.00	0.046[b]
Obese T2D vs. Obese NGT	56/134 (29/71)	26/55 (32/68)	4/9 (31/69)	0.20/0.18	1.09	0.69-1.72	0.709	1.00	0.83	0.44-1.57	0.558[b]	1.10	0.30-4.07	0.889[b]
Lean+Obese T2D vs. Lean+Obese NGT	96/429 (18/82)	37/144 (20/80)	6/16 (27/73)	0.18/0.15	1.22	0.86-1.73	0.265	1.00	1.02	0.64-1.63	0.943[b]	1.69	0.58-4.95	0.339[b]

MAF, minor allele frequency; OR, odds ratio; T2D, type 2 diabetes; NGT, normal glucose tolerance. Genotype data on SNP rs553668 are missing for two subjects with T2D and three lean subjects with NGT. [a]Reference group; [b]Adjusted for age; [c]Adjusted for age and body mass index (BMI).

Multiple logistic regression analysis was carried out for SNP rs521674 in women. When including age in the model, comparison of T2D patients with lean NGT subjects showed that A/A genotype carriers had an increased OR compared with T/T genotype carriers (OR = 7.61; 95% CI = 1.70-34.17; p = 0.008; Table 2). Correction for BMI removed this association. The T2D group in women includes subjects with BMIs ranging from 19.7-58.6 kg/m^2. Comparing lean T2D and lean NGT groups did not show any association, although we detected a risk in obese T2D versus lean NGT (OR = 10.89; 95% CI = 1.11-107.10; p = 0.041). We also found an association between lean and obese T2D versus lean NGT (OR = 5.10; 95% CI = 1.13-22.98; p = 0.034). With regard to A/T genotype carriers compared with T/T genotype carriers at SNP rs521674 in women, multiple logistic regression analysis including age in the model revealed an increase in the OR, when comparing T2D patients with lean NGT subjects (OR = 6.35; 95% CI = 1.39-29.10; p = 0.017). Adding BMI into the model removed this association. There was no association when comparing lean T2D and lean NGT, but when looking at obese T2D and lean NGT subjects, we found an association (OR = 10.50; 95% CI = 1.06-104.21; p = 0.045). Moreover, we detected an association between lean and obese T2D versus lean NGT (OR = 4.91; 95% CI = 1.07-22.50; p = 0.040). For a summary of the comparisons for SNP rs521674 in women, see Table 2.

Table 2. SNP rs521674: genotype distribution and association in women

Comparisons	Genotype distribution			MAF	Allele frequency difference			Multiple logistic regression						
	A/A n (%)	A/T n (%)	T/T n (%)		OR	95% CI	P	T/T[a]	A/T			A/A		
								OR	OR	95% CI	P	OR	95% CI	P
T2D vs. Lean NGT	103/106 (49/51)	54/60 (47/53)	3/14 (18/82)	0.19/0.24	1.40	0.97-2.03	0.072	1.00	6.35	1.39-29.10	0.017[b]	7.61	1.70-34.17	0.008[b]
T2D vs. Lean NGT								1.00	3.89	0.58-20.19	0.163[c]	2.66	0.43-16.61	0.296[c]
Lean T2D vs. Lean NGT	22/106 (17/83)	12/60 (17/83)	2/14 (13/87)	0.22/0.24	1.13	0.62-2.07	0.687	1.00	2.28	0.35-14.77	0.389[b]	2.23	0.36-13.83	0.391[b]
Obese T2D vs. Lean NGT	46/106 (30/70)	32/60 (35/65)	1/14 (7/93)	0.22/0.24	1.18	0.75-1.85	0.470	1.00	10.50	1.06-104.21	0.045[b]	10.89	1.11-107.10	0.041[b]
Lean+Obese T2D vs. Lean NGT	68/106 (39/61)	44/60 (42/58)	3/14 (18/82)	0.22/0.24	1.17	0.79-1.73	0.449	1.00	4.91	1.07-22.50	0.040[b]	5.10	1.13-22.98	0.034[b]

MAF, minor allele frequency; OR, odds ratio; T2D, type 2 diabetes; NGT, normal glucose tolerance. Genotype data on SNP rs521674 are missing for four subjects with T2D and six lean subjects with NGT. [a]Reference group; [b]Adjusted for age; [c]Adjusted for age and body mass index (BMI).

Although *ADRA2A* mRNA expression was detectable in isolated human pancreatic islets, no differences were found between the diabetic and control groups.

In the present study, comparison analyses were carried out in men and women separately because gender seems to be an important player when analyzing the genetics of obesity and its related disorders. Differences demonstrated in other genes among men and women include receptor protein tyrosine phosphatase sigma (*RPTPσ*) (Langberg *et al.*, 2007), angiotensin-converting enzyme (*ACE*) (O'Donnell *et al.*, 1998), low-density lipoprotein-related protein-associated protein 1 (*LRPAP1*), thrombospondin I (*THBS1*), acetyl-Coenzyme A acetyltransferase 2 (*ACAT2*), integrin beta 3 (*ITGB3*), coagulation factor II (*F2*), P-selectin (*SELP*), prolylcarboxypeptidase (*PRCP*) (McCarthy *et al.*, 2003), and neuropeptide Y (*NPY*) (Nordman *et al.*, 2005).

SNP rs553668 is a tagSNP and is located in the 3′ region of the *ADRA2A* gene. Genetic variations in the 3′ region can be involved in regulating message stability. Alteration in this region of the *ADRA2A* gene can result in altered expression levels, due to the increased stability of the *ADRA2A* mRNA (Michel *et al.*, 1999). SNP rs553668 has been reported to be associated with the risk for obesity (Ukkola *et al.*, 2001; Lima *et al.*, 2007), hypertension in blacks (Lockette *et al.*, 1995; Li *et al.*, 2006), cardiovascular diseases (Flordellis *et al.*, 2004), platelet aggregation (Freeman *et al.*, 1995), childhood attention deficit hyperactivity disorder (ADHD) (Park *et al.*, 2005), and endurance in athletes of Caucasian origin (Wolfarth *et al.*, 2000). Having completed the current study, it was reported that rs553668 is associated with reduced insulin secretion and increased risk of T2D in a population from Finland and southern Sweden (Rosengren *et al.*, 2010). This association was verified at the functional level in human pancreatic islets. Risk allele carriers showed over-expression of *ADRA2A* in islets, decreased insulin secretion, and reduced number of docked insulin granules *in vitro*. These effects were corrected by ADRA2A-antagonism (Rosengren *et al.*, 2010). Although *ADRA2A* mRNA expression was detectable in the isolated human pancreatic islets used in the present study, no difference in expression was detected between the diabetic and control groups. These divergent results may be explained by the fact that T2D is a complex disease with many different entities and the involvement of multiple genes, as well as epigenetic and environmental factors. Also, in the study by Rosengren *et al.*, the individuals were not categorized into diabetic and control groups, as has been done in this study.

SNP rs521674 is situated in the 5′ region of the *ADRA2A* gene. This polymorphism has previously been described and used as a genetic marker because of its location (Belfer *et al.*, 2005; Kurnik *et al.*, 2006). Genetic variants found in the 5′ flanking region could be involved in transcription binding sites in the promoter region and may therefore influence gene expression. Responsivity to α_2-adrenergic stimulation differs noticeably between individuals. This could be due to receptor regulatory processes (Brodde and Bock, 1984; Michel *et al.*, 1991) or due to hereditary inter-individual variability in α_2-adrenergic receptor responsiveness (Luthra *et al.*, 1991). Our results in the current study suggest that SNP rs521674 might be associated with obesity and possibly also to T2D. This association was seen in obese and not lean T2D when age was included in the multiple logistic regression model. We did not have access to samples from obese women with NGT, and therefore could not compare this group with the lean NGT

female group. We found an association between the combined group of lean and obese T2D patients and lean NGT subjects after adjusting for age, and were not able to exclude a link to T2D.

In conclusion, our data in this study support the notion that *ADRA2A* plays an important role in the pathogenesis of obesity, and might also modify the progression to T2D. Data indicate that SNP rs553668 is mainly associated with obesity in men. For women, SNP rs521674 might be associated with obesity and possibly also with T2D.

5 THESIS SUMMARY

Paper I – This study demonstrates that *Ac3* is over-expressed not only in pancreatic islets but also similarly in striatum/hypothalamus of GK rats, supporting the existence of a functional link between the CNS and pancreatic islets via AC3 regulation. The increased levels of *Ac3* mRNA expression in these tissues is partially a primary and inherited defect and not solely secondary to hyperglycaemia. AC3 may participate in regulating glucose homeostasis via insulin secretion and the CNS. Also, AC3 may have a primary role in body weight regulation through CNS control.

Paper II – This study suggests defects in the expression of PKCα and PKCε in pancreatic islets of GK rats secondary to hyperglycaemia. Also, insulin treatment increased the islet p-PKCζ. In liver, PKCε mRNA expression in liver could be under control of insulin. Furthermore, the capacity of hepatic PKCδ to be phosphorylated is decreased in GK rats.

Paper III – This study further supports the notion that mitochondrial pathways involving SCOT, which supply acetoacetate for export to the cytosol, instead of or in combination with the pathway involving ATPCL that converts citrate exported from mitochondria to acetyl-CoA, are important for the potentiation of insulin secretion by the pancreatic β-cell.

Paper IV – This study provides evidence that *ADRA2A* genetic polymorphisms are mainly associated with obesity and may also relate to T2D in a Swedish population.

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7 REFERENCES

ABATE, N. & CHANDALIA, M. 2003. The impact of ethnicity on type 2 diabetes. *Journal of diabetes and its complications,* 17**,** 39-58.

ABDEL-HALIM, S. M., GUENIFI, A., EFENDIC, S. & OSTENSON, C. G. 1993. Both somatostatin and insulin responses to glucose are impaired in the perfused pancreas of the spontaneously noninsulin-dependent diabetic GK (Goto-Kakizaki) rats. *Acta physiologica Scandinavica,* 148**,** 219-26.

ABDEL-HALIM, S. M., GUENIFI, A., HE, B., YANG, B., MUSTAFA, M., HOJEBERG, B., HILLERT, J., BAKHIET, M. & EFENDIC, S. 1998. Mutations in the promoter of adenylyl cyclase (AC)-III gene, overexpression of AC-III mRNA, and enhanced cAMP generation in islets from the spontaneously diabetic GK rat model of type 2 diabetes. *Diabetes,* 47**,** 498-504.

ABDEL-HALIM, S. M., GUENIFI, A., LUTHMAN, H., GRILL, V., EFENDIC, S. & OSTENSON, C. G. 1994. Impact of diabetic inheritance on glucose tolerance and insulin secretion in spontaneously diabetic GK-Wistar rats. *Diabetes,* 43**,** 281-8.

ABDEL-HALIM, S. M., OSTENSON, C. G., ANDERSSON, A., JANSSON, L. & EFENDIC, S. 1995. A defective stimulus-secretion coupling rather than glucotoxicity mediates the impaired insulin secretion in the mildly diabetic F1 hybrids of GK-Wistar rats. *Diabetes,* 44**,** 1280-4.

ADEYEMO, A. & ROTIMI, C. 2010. Genetic variants associated with complex human diseases show wide variation across multiple populations. *Public health genomics,* 13**,** 72-9.

AGARDH, E. E., AHLBOM, A., ANDERSSON, T., EFENDIC, S., GRILL, V., HALLQVIST, J., NORMAN, A. & OSTENSON, C. G. 2003. Work stress and low sense of coherence is associated with type 2 diabetes in middle-aged Swedish women. *Diabetes care,* 26**,** 719-24.

AHREN, B. 2009. Islet G protein-coupled receptors as potential targets for treatment of type 2 diabetes. *Nature reviews. Drug discovery,* 8**,** 369-85.

AHRÉN, B. 2000. Autonomic regulation of islet hormone secretion – Implications for health and disease. *Diabetologia,* 43**,** 393-410.

AHREN, B. & LUNDQUIST, I. 1981. Effects of selective and non-selective beta-adrenergic agents on insulin secretion in vivo. *European journal of pharmacology,* 71**,** 93-104.

ALBERTI, K. G. & ZIMMET, P. Z. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabetic medicine : a journal of the British Diabetic Association,* 15**,** 539-53.

ALBERTI, K. G. M. M. 2010. The Classification and Diagnosis of Diabetes Mellitus. *Textbook of Diabetes.* Wiley-Blackwell.

ALI, M. K., WEBER, M. B. & NARAYAN, K. M. V. 2010. The Global Burden of Diabetes. *Textbook of Diabetes.* Wiley-Blackwell.

ALI, O. 2013. Genetics of type 2 diabetes. *World journal of diabetes,* 4**,** 114-23.

ALSAHLI, M. & GERICH, J. E. 2010. Abnormalities of Insulin Secretion and β-Cell Defects in Type 2 Diabetes. *Textbook of Diabetes.* Wiley-Blackwell.

AMED, S., DANEMAN, D., MAHMUD, F. H. & HAMILTON, J. 2010. Type 2 diabetes in children and adolescents. *Expert review of cardiovascular therapy,* 8**,** 393-406.

AMERICAN DIABETES ASSOCIATION 2013. Diagnosis and Classification of Diabetes Mellitus. *Diabetes care,* 36**,** S67-S74.

APPLIED BIOSYSTEMS 2010. TaqMan allelic discrimination demonstration kit protocol.

ARNER, P. 2005. Resistin: yet another adipokine tells us that men are not mice. *Diabetologia,* 48**,** 2203-5.

ARSLANIAN, S. A., BACHA, F., SAAD, R. & GUNGOR, N. 2005. Family history of type 2 diabetes is associated with decreased insulin sensitivity and an impaired balance between insulin sensitivity and insulin secretion in white youth. *Diabetes care,* 28**,** 115-9.

ASHCROFT, F. M., PROKS, P., SMITH, P. A., ÄMMÄLÄ, C., BOKVIST, K. & RORSMAN, P. 1994. Stimulus–secretion coupling in pancreatic β cells. *Journal of Cellular Biochemistry,* 55**,** 54-65.

ASHCROFT, S. J., BUNCE, J., LOWRY, M., HANSEN, S. E. & HEDESKOV, C. J. 1978. The effect of sugars on (pro)insulin biosynthesis. *Biochem. J.,* 174**,** 517-526.

ASHCROFT, S. J. H. 1980. Glucoreceptor mechanisms and the control of insulin release and biosynthesis. *Diabetologia,* 18**,** 5-15.

BAILES, B. K. 2002. Diabetes Mellitus and its Chronic Complications. *AORN Journal,* 76**,** 265-282.

BARROSO, I. 2005. Genetics of Type 2 diabetes. *Diabetic Medicine,* 22**,** 517-535.

BEGIN-HEICK, N. 1994. Liver beta-adrenergic receptors, G proteins, and adenylyl cyclase activity in obesity-diabetes syndromes. *The American journal of physiology,* 266**,** C1664-72.

BELFER, I., BUZAS, B., HIPP, H., PHILLIPS, G., TAUBMAN, J., LORINCZ, I., EVANS, C., LIPSKY, R. H., ENOCH, M. A., MAX, M. B. & GOLDMAN, D. 2005. Haplotype-based analysis of alpha 2A, 2B, and 2C adrenergic receptor genes captures information on common functional loci at each gene. *Journal of human genetics,* 50**,** 12-20.

BERENDS, L. M. & OZANNE, S. E. 2012. Early determinants of type-2 diabetes. *Best Practice & Research Clinical Endocrinology & Metabolism,* 26**,** 569-580.

BERGMAN, M. 2010. Inadequacies of absolute threshold levels for diagnosing prediabetes. *Diabetes/Metabolism Research and Reviews,* 26**,** 3-6.

BERGSTEN, P. 2000. Pathophysiology of impaired pulsatile insulin release. *Diabetes/Metabolism Research and Reviews,* 16**,** 179-91.

BERGSTEN, P., LIN, J. & WESTERLUND, J. 1998. Pulsatile insulin release: role of cytoplasmic Ca2+ oscillations. *Diabetes & metabolism,* 24**,** 41-5.

BEZY, O., TRAN, T. T., PIHLAJAMAKI, J., SUZUKI, R., EMANUELLI, B., WINNAY, J., MORI, M. A., HAAS, J., BIDDINGER, S. B., LEITGES, M., GOLDFINE, A. B., PATTI, M. E., KING, G. L. & KAHN, C. R. 2011. PKCdelta regulates hepatic insulin sensitivity and hepatosteatosis in mice and humans. *The Journal of clinical investigation,* 121**,** 2504-17.

BIDEN, T. J., SCHMITZ-PEIFFER, C., BURCHFIELD, J. G., GURISIK, E., CANTLEY, J., MITCHELL, C. J. & CARPENTER, L. 2008. The diverse roles of protein kinase C in pancreatic beta-cell function. *Biochemical Society transactions,* 36**,** 916-9.

BISBIS, S., BAILBE, D., TORMO, M. A., PICAREL-BLANCHOT, F., DEROUET, M., SIMON, J. & PORTHA, B. 1993. Insulin resistance in the GK rat: decreased receptor number but normal kinase activity in liver. *The American journal of physiology,* 265**,** E807-13.

BODEN, G. 2001. Free fatty acids-the link between obesity and insulin resistance. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists,* 7**,** 44-51.

BODEN, G. & SHULMAN, G. I. 2002. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and β-cell dysfunction. *European Journal of Clinical Investigation,* 32**,** 14-23.

BRATANOVA-TOCHKOVA, T. K., CHENG, H., DANIEL, S., GUNAWARDANA, S., LIU, Y.-J., MULVANEY-MUSA, J., SCHERMERHORN, T., STRAUB, S. G., YAJIMA, H. & SHARP, G. W. G. 2002. Triggering and Augmentation Mechanisms, Granule Pools, and Biphasic Insulin Secretion. *Diabetes,* 51**,** S83-S90.

BRAY, G. A. 2010. Control of Weight: How Do We Get Fat? *Textbook of Diabetes.* Wiley-Blackwell.

BRODDE, O. E. & BOCK, K. D. 1984. Changes in platelet alpha 2-adrenoceptors in human phaeochromocytoma. *European journal of clinical pharmacology,* 26**,** 265-7.

BUSCH, C. P. & HEGELE, R. A. 2001. Genetic determinants of type 2 diabetes mellitus. *Clinical Genetics,* 60**,** 243-254.

BUTEAU, J., FOISY, S., RHODES, C. J., CARPENTER, L., BIDEN, T. J. & PRENTKI, M. 2001. Protein kinase Czeta activation mediates glucagon-like peptide-1-induced pancreatic beta-cell proliferation. *Diabetes,* 50**,** 2237-43.

BUTLER, A. E., JANSON, J., BONNER-WEIR, S., RITZEL, R., RIZZA, R. A. & BUTLER, P. C. 2003. β-Cell Deficit and Increased β-Cell Apoptosis in Humans With Type 2 Diabetes. *Diabetes,* 52**,** 102-110.

BUYSSCHAERT, M. & BERGMAN, M. 2011. Definition of prediabetes. *The Medical clinics of North America,* 95**,** 289-97, vii.

CALLE, R., GANESAN, S., SMALLWOOD, J. I. & RASMUSSEN, H. 1992. Glucose-induced phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) in isolated rat pancreatic islets. *The Journal of biological chemistry,* 267**,** 18723-7.

CAMPION, J., MILAGRO, F. I. & MARTINEZ, J. A. 2009. Individuality and epigenetics in obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity,* 10**,** 383-92.

CARULLI, L., RONDINELLA, S., LOMBARDINI, S., CANEDI, I., LORIA, P. & CARULLI, N. 2005. Review article: diabetes, genetics and ethnicity. *Alimentary Pharmacology & Therapeutics,* 22**,** 16-19.

CASES, J. A., GABRIELY, I., MA, X. H., YANG, X. M., MICHAELI, T., FLEISCHER, N., ROSSETTI, L. & BARZILAI, N. 2001. Physiological increase in plasma leptin markedly inhibits insulin secretion in vivo. *Diabetes,* 50**,** 348-52.

CAUCHI, S. & FROGUEL, P. 2008. TCF7L2 genetic defect and type 2 diabetes. *Current diabetes reports,* 8**,** 149-55.

CERASI, E. 1975a. Insulin secretion: mechanism of the stimulation by glucose. *Quarterly reviews of biophysics,* 8**,** 1-41.

CERASI, E. 1975b. Mechanisms of glucose stimulated insulin secretion in health and in diabetes: Some re-evaluations and proposals. *Diabetologia,* 11**,** 1-13.

CHEN, C. K., BURNS, M. E., HE, W., WENSEL, T. G., BAYLOR, D. A. & SIMON, M. I. 2000. Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature,* 403**,** 557-60.

CHEN, R., CORONA, E., SIKORA, M., DUDLEY, J. T., MORGAN, A. A., MORENO-ESTRADA, A., NILSEN, G. B., RUAU, D., LINCOLN, S. E., BUSTAMANTE, C. D. & BUTTE, A. J. 2012. Type 2 diabetes risk alleles demonstrate extreme directional differentiation among human populations, compared to other diseases. *PLoS genetics,* 8**,** e1002621.

COCCHERI, S. 2007. Approaches to prevention of cardiovascular complications and events in diabetes mellitus. *Drugs,* 67**,** 997-1026.

COHN, G. S., KITTLESON, M. M. & BLUMENTHAL, R. S. 2005. Toward an Improved Diagnosis of the Metabolic Syndrome: Other Clues to the Presence of Insulin Resistance. *American Journal of Hypertension,* 18**,** 1099-1103.

COOPER, D. M. 2003. Regulation and organization of adenylyl cyclases and cAMP. *The Biochemical journal,* 375**,** 517-29.

CORKEY, B. E., GLENNON, M. C., CHEN, K. S., DEENEY, J. T., MATSCHINSKY, F. M. & PRENTKI, M. 1989. A role for malonyl-CoA in glucose-stimulated insulin secretion from clonal pancreatic beta-cells. *The Journal of biological chemistry,* 264**,** 21608-12.

COSGROVE, M. P., SARGEANT, L. A. & GRIFFIN, S. J. 2008. Does depression increase the risk of developing type 2 diabetes? *Occupational medicine,* 58**,** 7-14.

CRYER, P. E. & GERICH, J. E. 1985. Glucose Counterregulation, Hypoglycemia, and Intensive Insulin Therapy in Diabetes Mellitus. *New England Journal of Medicine,* 313**,** 232-241.

DAYEH, T. A., OLSSON, A. H., VOLKOV, P., ALMGREN, P., RONN, T. & LING, C. 2013. Identification of CpG-SNPs associated with type 2 diabetes and differential DNA methylation in human pancreatic islets. *Diabetologia,* 56**,** 1036-46.

DE VOS, A., HEIMBERG, H., QUARTIER, E., HUYPENS, P., BOUWENS, L., PIPELEERS, D. & SCHUIT, F. 1995. Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *The Journal of clinical investigation,* 96**,** 2489-95.

DEURENBERG, P., YAP, M. & VAN STAVEREN, W. A. 1998. Body mass index and percent body fat: a meta analysis among different ethnic groups. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* 22**,** 1164-71.

DEVEDJIAN, J. C., PUJOL, A., CAYLA, C., GEORGE, M., CASELLAS, A., PARIS, H. & BOSCH, F. 2000. Transgenic mice overexpressing α2A-adrenoceptors in pancreatic beta-cells show altered regulation of glucose homeostasis. *Diabetologia,* 43**,** 899-906.

DEZAKI, K., SONE, H. & YADA, T. 2008. Ghrelin is a physiological regulator of insulin release in pancreatic islets and glucose homeostasis. *Pharmacology & therapeutics,* 118**,** 239-49.

DEZAKI, K. & YADA, T. 2012. Islet beta-cell ghrelin signaling for inhibition of insulin secretion. *Methods in enzymology,* 514**,** 317-31.

DONNELLY, K. L., SMITH, C. I., SCHWARZENBERG, S. J., JESSURUN, J., BOLDT, M. D. & PARKS, E. J. 2005. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *The Journal of clinical investigation,* 115**,** 1343-51.

DRONG, A. W., LINDGREN, C. M. & MCCARTHY, M. I. 2012. The genetic and epigenetic basis of type 2 diabetes and obesity. *Clinical pharmacology and therapeutics,* 92**,** 707-15.

DRUCKER, D. J. 2006. The biology of incretin hormones. *Cell Metabolism,* 3**,** 153-65.

DYACHOK, O., IDEVALL-HAGREN, O., SAGETORP, J., TIAN, G., WUTTKE, A., ARRIEUMERLOU, C., AKUSJARVI, G., GYLFE, E. & TENGHOLM, A. 2008. Glucose-induced cyclic AMP oscillations regulate pulsatile insulin secretion. *Cell Metabolism,* 8**,** 26-37.

EFENDIC, S. & PORTWOOD, N. 2004. Overview of incretin hormones. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme,* 36**,** 742-6.

ERIKSSON, A. K., EKBOM, A., GRANATH, F., HILDING, A., EFENDIC, S. & OSTENSON, C. G. 2008. Psychological distress and risk of pre-diabetes and Type 2 diabetes in a prospective study of Swedish middle-aged men and women. *Diabetic medicine : a journal of the British Diabetic Association,* 25**,** 834-42.

FARFARI, S., SCHULZ, V., CORKEY, B. & PRENTKI, M. 2000. Glucose-regulated anaplerosis and cataplerosis in pancreatic beta-cells: possible implication of a pyruvate/citrate shuttle in insulin secretion. *Diabetes,* 49**,** 718-726.

FINUCANE, M. M., STEVENS, G. A., COWAN, M. J., DANAEI, G., LIN, J. K., PACIOREK, C. J., SINGH, G. M., GUTIERREZ, H. R., LU, Y., BAHALIM, A. N., FARZADFAR, F., RILEY, L. M. & EZZATI, M. 2011. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet,* 377**,** 557-67.

FIORY, F., ORIENTE, F., MIELE, C., ROMANO, C., TRENCIA, A., ALBEROBELLO, A. T., ESPOSITO, I., VALENTINO, R., BEGUINOT, F. & FORMISANO, P. 2004. Protein kinase C-zeta and protein kinase B regulate distinct steps of insulin endocytosis and intracellular sorting. *The Journal of biological chemistry,* 279**,** 11137-45.

FLORDELLIS, C., MANOLIS, A., SCHEININ, M. & PARIS, H. 2004. Clinical and pharmacological significance of alpha2-adrenoceptor polymorphisms in cardiovascular diseases. *International journal of cardiology,* 97**,** 367-72.

FLOREZ, J. C. 2008. Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: Where are the insulin resistance genes? *Diabetologia,* 51**,** 1100-1110.

FORBES, J. M. & COOPER, M. E. 2013. Mechanisms of Diabetic Complications. *Physiological Reviews,* 93**,** 137-188.

FRANGIOUDAKIS, G., BURCHFIELD, J. G., NARASIMHAN, S., COONEY, G. J., LEITGES, M., BIDEN, T. J. & SCHMITZ-PEIFFER, C. 2009. Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: regulation of lipogenesis by protein kinase C delta. *Diabetologia,* 52**,** 2616-20.

FRAYN, K., ARNER, P. & YKI-JÄRVINEN, H. 2006. Fatty acid metabolism in adipose tissue, muscle and liver in health and disease. *Essays in Biochemistry,* 042**,** 89-103.

FREEMAN, K., FARROW, S., SCHMAIER, A., FREEDMAN, R., SCHORK, T. & LOCKETTE, W. 1995. Genetic polymorphism of the alpha 2-adrenergic receptor is associated with increased platelet aggregation, baroreceptor sensitivity, and salt excretion in normotensive humans. *American journal of hypertension,* 8**,** 863-9.

FULTON-KEHOE, D., HAMMAN, R. F., BAXTER, J. & MARSHALL, J. 2001. A case-control study of physical activity and non-insulin dependent diabetes mellitus (NIDDM). the San Luis Valley Diabetes Study. *Annals of epidemiology,* 11**,** 320-7.

FURMAN, B., ONG, W. K. & PYNE, N. J. 2010. Cyclic AMP signaling in pancreatic islets. *Advances in experimental medicine and biology,* 654**,** 281-304.

GANESAN, S., CALLE, R., ZAWALICH, K., GREENAWALT, K., ZAWALICH, W., SHULMAN, G. I. & RASMUSSEN, H. 1992. Immunocytochemical

localization of alpha-protein kinase C in rat pancreatic beta-cells during glucose-induced insulin secretion. *The Journal of cell biology,* 119**,** 313-24.

GANESAN, S., CALLE, R., ZAWALICH, K., SMALLWOOD, J. I., ZAWALICH, W. S. & RASMUSSEN, H. 1990. Glucose-induced translocation of protein kinase C in rat pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America,* 87**,** 9893-7.

GAO, C. L., ZHAO, D. Y., QIU, J., ZHANG, C. M., JI, C. B., CHEN, X. H., LIU, F. & GUO, X. R. 2009. Resistin induces rat insulinoma cell RINm5F apoptosis. *Molecular biology reports,* 36**,** 1703-8.

GARENC, C., PERUSSE, L., CHAGNON, Y. C., RANKINEN, T., GAGNON, J., BORECKI, I. B., LEON, A. S., SKINNER, J. S., WILMORE, J. H., RAO, D. C. & BOUCHARD, C. 2002. The alpha 2-adrenergic receptor gene and body fat content and distribution: the HERITAGE Family Study. *Molecular medicine,* 8**,** 88-94.

GERICH, J. E. 1993. Control of glycaemia. *Baillière's Clinical Endocrinology and Metabolism,* 7**,** 551-586.

GERICH, J. E. 1998. The Genetic Basis of Type 2 Diabetes Mellitus: Impaired Insulin Secretion versus Impaired Insulin Sensitivity. *Endocrine Reviews,* 19**,** 491-503.

GERICH, J. E. 2000. Physiology of glucose homeostasis. *Diabetes, Obesity and Metabolism,* 2**,** 345-350.

GERICH, J. E. 2003. Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. *Mayo Clinic proceedings. Mayo Clinic,* 78**,** 447-56.

GHOSH, A., LIU, T., KHOURY, M. J. & VALDEZ, R. 2010. Family history of diabetes and prevalence of the metabolic syndrome in U.S. adults without diabetes: 6-year results from the National Health and Nutrition Examination Survey (1999-2004). *Public health genomics,* 13**,** 353-9.

GIL-CAMPOS, M., CANETE, R. R. & GIL, A. 2004. Adiponectin, the missing link in insulin resistance and obesity. *Clinical nutrition,* 23**,** 963-74.

GILON, P. & HENQUIN, J.-C. 2001. Mechanisms and Physiological Significance of the Cholinergic Control of Pancreatic β-Cell Function. *Endocrine Reviews,* 22**,** 565-604.

GOTO, Y., KAKIZAKI, M. & MASAKI, N. 1976. Production of spontaneous diabetic rats by repetition of selective breeding. *The Tohoku journal of experimental medicine,* 119**,** 85-90.

GRILL, V., PERSSON, G., CARLSSON, S., NORMAN, A., ALVARSSON, M., OSTENSSON, C. G., SVANSTROM, L. & EFENDIC, S. 1999. Family history of diabetes in middle-aged Swedish men is a gender unrelated factor which associates with insulinopenia in newly diagnosed diabetic subjects. *Diabetologia,* 42**,** 15-23.

GROOP, L. & LYSSENKO, V. 2009. Genetic basis of beta-cell dysfunction in man. *Diabetes, obesity & metabolism,* 11 Suppl 4**,** 149-58.

GU, H. F. 2010. Ac3: A novel gene plays a role in the regulation of body weight. *Open Diabetes Journal,* 3**,** 11-13.

GU, W., LI, X., LIU, C., YANG, J., YE, L., TANG, J., GU, Y., YANG, Y., HONG, J., ZHANG, Y., CHEN, M. & NING, G. 2006. Globular adiponectin augments insulin secretion from pancreatic islet beta cells at high glucose concentrations. *Endocrine,* 30**,** 217-21.

GUARIGUATA, L., WHITING, D., WEIL, C. & UNWIN, N. 2011. The International Diabetes Federation diabetes atlas methodology for estimating global and national prevalence of diabetes in adults. *Diabetes research and clinical practice,* 94**,** 322-332.

GUAY, C., MADIRAJU, S. R., AUMAIS, A., JOLY, E. & PRENTKI, M. 2007. A role for ATP-citrate lyase, malic enzyme, and pyruvate/citrate cycling in glucose-induced insulin secretion. *The Journal of biological chemistry,* 282**,** 35657-65.

GUENIFI, A., PORTELA-GOMES, G. M., GRIMELIUS, L., EFENDIC, S. & ABDEL-HALIM, S. M. 2000. Adenylyl cyclase isoform expression in non-diabetic and diabetic Goto-Kakizaki (GK) rat pancreas. Evidence for distinct overexpression of type-8 adenylyl cyclase in diabetic GK rat islets. *Histochemistry and cell biology,* 113**,** 81-9.

HABER, E. P., PROCOPIO, J., CARVALHO, C. R., CARPINELLI, A. R., NEWSHOLME, P. & CURI, R. 2006. New insights into fatty acid modulation of pancreatic beta-cell function. *International review of cytology,* 248**,** 1-41.

HABER, E. P., XIMENES, H. M., PROCOPIO, J., CARVALHO, C. R., CURI, R. & CARPINELLI, A. R. 2003. Pleiotropic effects of fatty acids on pancreatic beta-cells. *Journal of cellular physiology,* 194**,** 1-12.

HALLS, M. L. & COOPER, D. M. 2011. Regulation by Ca2+-signaling pathways of adenylyl cyclases. *Cold Spring Harbor perspectives in biology,* 3**,** a004143.

HANOUNE, J. & DEFER, N. 2001. Regulation and role of adenylyl cyclase isoforms. *Annual review of pharmacology and toxicology,* 41**,** 145-74.

HANSEN, L. 2003. Candidate genes and late-onset type 2 diabetes mellitus. Susceptibility genes or common polymorphisms? *Danish medical bulletin,* 50**,** 320-46.

HANSEN, T. K. & MØLLER, N. 2010. Acute Metabolic Complications of Diabetes: Diabetic Ketoacidosis and Hyperosmolar Hyperglycemia. *Textbook of Diabetes.* Wiley-Blackwell.

HARDER, T., RODEKAMP, E., SCHELLONG, K., DUDENHAUSEN, J. W. & PLAGEMANN, A. 2007. Birth Weight and Subsequent Risk of Type 2 Diabetes: A Meta-Analysis. *American Journal of Epidemiology,* 165**,** 849-857.

HASAN, N. M., LONGACRE, M. J., STOKER, S. W., BOONSAEN, T., JITRAPAKDEE, S., KENDRICK, M. A., WALLACE, J. C. & MACDONALD, M. J. 2008. Impaired Anaplerosis and Insulin Secretion in Insulinoma Cells Caused by Small Interfering RNA-mediated Suppression of Pyruvate Carboxylase. *Journal of Biological Chemistry,* 283**,** 28048-28059.

HASLAM, D. W. & JAMES, W. P. 2005. Obesity. *Lancet,* 366**,** 1197-209.

HAUNER, H. 2010. Obesity and Diabetes. *Textbook of Diabetes.* Wiley-Blackwell.

HEIDEMAN, W. H., MIDDELKOOP, B. J. C., NIERKENS, V., STRONKS, K., VERHOEFF, A. P., VAN ESCH, S. C. M. & SNOEK, F. J. 2011. Changing the odds. What do we learn from prevention studies targeted at people with a positive family history of type 2 diabetes? *Primary Care Diabetes,* 5**,** 215-221.

HENNIGE, A. M., RANTA, F., HEINZELMANN, I., DUFER, M., MICHAEL, D., BRAUMULLER, H., LUTZ, S. Z., LAMMERS, R., DREWS, G., BOSCH, F., HARING, H. U. & ULLRICH, S. 2010. Overexpression of kinase-negative protein kinase Cdelta in pancreatic beta-cells protects mice from diet-induced glucose intolerance and beta-cell dysfunction. *Diabetes,* 59**,** 119-27.

HENQUIN, J. C. 2000. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes,* 49**,** 1751-1760.

HENQUIN, J. C. 2009. Regulation of insulin secretion: a matter of phase control and amplitude modulation. *Diabetologia,* 52**,** 739-751.

HENQUIN, J. C. 2011. The dual control of insulin secretion by glucose involves triggering and amplifying pathways in beta-cells. *Diabetes research and clinical practice,* 93 Suppl 1**,** S27-31.

HENQUIN, J. C. & MEISSNER, H. P. 1986. Cyclic adenosine monophosphate differently affects the response of mouse pancreatic beta-cells to various amino acids. *The Journal of physiology,* 381**,** 77-93.

HERBERT, V., LAU, K. S., GOTTLIEB, C. W. & BLEICHER, S. J. 1965. Coated charcoal immunoassay of insulin. *The Journal of clinical endocrinology and metabolism,* 25**,** 1375-84.

HERDER, C. & RODEN, M. 2011. Genetics of type 2 diabetes: pathophysiologic and clinical relevance. *European Journal of Clinical Investigation,* 41**,** 679-692.

HERMANN, L. S. & DECKERT, T. 1977. The effect of epinephrine and isoproterenol on insulin secretion and glucose utilization in isolated islets of Langerhans from mice. *Acta endocrinologica,* 84**,** 105-14.

HERRERA, B. M., KEILDSON, S. & LINDGREN, C. M. 2011. Genetics and epigenetics of obesity. *Maturitas,* 69**,** 41-9.

HILDING, A., ERIKSSON, A. K., AGARDH, E. E., GRILL, V., AHLBOM, A., EFENDIC, S. & OSTENSON, C. G. 2006. The impact of family history of diabetes and lifestyle factors on abnormal glucose regulation in middle-aged Swedish men and women. *Diabetologia,* 49**,** 2589-98.

HINNEY, A., VOGEL, C. I. & HEBEBRAND, J. 2010. From monogenic to polygenic obesity: recent advances. *European child & adolescent psychiatry,* 19**,** 297-310.

HOHMEIER, H. E., MULDER, H., CHEN, G., HENKEL-RIEGER, R., PRENTKI, M. & NEWGARD, C. B. 2000. Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes,* 49**,** 424-30.

HOLLAND, P. M., ABRAMSON, R. D., WATSON, R. & GELFAND, D. H. 1991. Detection of specific polymerase chain reaction product by utilizing the 5'----3' exonuclease activity of Thermus aquaticus DNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America,* 88**,** 7276-80.

HOTTA, K., FUNAHASHI, T., ARITA, Y., TAKAHASHI, M., MATSUDA, M., OKAMOTO, Y., IWAHASHI, H., KURIYAMA, H., OUCHI, N., MAEDA, K., NISHIDA, M., KIHARA, S., SAKAI, N., NAKAJIMA, T., HASEGAWA, K., MURAGUCHI, M., OHMOTO, Y., NAKAMURA, T., YAMASHITA, S., HANAFUSA, T. & MATSUZAWA, Y. 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arteriosclerosis, thrombosis, and vascular biology,* 20**,** 1595-9.

HOU, J. C., MIN, L. & PESSIN, J. E. 2009. Insulin granule biogenesis, trafficking and exocytosis. *Vitamins and hormones,* 80**,** 473-506.

HUGHES, S. J., SUZUKI, K. & GOTO, Y. 1994. The role of islet secretory function in the development of diabetes in the GK Wistar rat. *Diabetologia,* 37**,** 863-70.

IN'T VELD, P. & MARICHAL, M. 2010. Microscopic anatomy of the human islet of Langerhans. *Advances in experimental medicine and biology,* 654**,** 1-19.

ISHIYAMA, N., RAVIER, M. A. & HENQUIN, J. C. 2006. Dual mechanism of the potentiation by glucose of insulin secretion induced by arginine and tolbutamide in mouse islets. *American journal of physiology. Endocrinology and metabolism,* 290**,** E540-9.

JAPAN DIABETES SOCIETY, C. O. D. T. 1988. Diabetes mellitus in twins: a cooperative study in Japan. *Diabetes research and clinical practice,* 5**,** 271-280.

JENSEN, M. V., JOSEPH, J. W., RONNEBAUM, S. M., BURGESS, S. C., SHERRY, A. D. & NEWGARD, C. B. 2008. Metabolic cycling in control of glucose-stimulated insulin secretion. *American journal of physiology. Endocrinology and metabolism,* 295**,** E1287-97.

JITRAPAKDEE, S., WUTTHISATHAPORNCHAI, A., WALLACE, J. C. & MACDONALD, M. J. 2010. Regulation of insulin secretion: role of mitochondrial signalling. *Diabetologia,* 53**,** 1019-1032.

JONES, P. M. & PERSAUD, S. J. 1998. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. *Endocrine Reviews,* 19**,** 429-61.

JONES, P. M. & PERSAUD, S. J. 2010. Islet Function and Insulin Secretion. *Textbook of Diabetes.* Wiley-Blackwell.

JOSEPH, J. W., ODEGAARD, M. L., RONNEBAUM, S. M., BURGESS, S. C., MUEHLBAUER, J., SHERRY, A. D. & NEWGARD, C. B. 2007. Normal flux through ATP-citrate lyase or fatty acid synthase is not required for glucose-stimulated insulin secretion. *The Journal of biological chemistry,* 282**,** 31592-600.

KAHN, S. E. 2001. The Importance of β-Cell Failure in the Development and Progression of Type 2 Diabetes. *Journal of Clinical Endocrinology & Metabolism,* 86**,** 4047-4058.

KAHN, S. E., HULL, R. L. & UTZSCHNEIDER, K. M. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature,* 444**,** 840-846.

KAHN, S. E., ZRAIKA, S., UTZSCHNEIDER, K. M. & HULL, R. L. 2009. The beta cell lesion in type 2 diabetes: there has to be a primary functional abnormality. *Diabetologia,* 52**,** 1003-1012.

KANASHIRO, C. A. & KHALIL, R. A. 1998. Signal transduction by protein kinase C in mammalian cells. *Clinical and Experimental Pharmacology and Physiology,* 25**,** 974-985.

KAPRIO, J., TUOMILEHTO, J., KOSKENVUO, M., ROMANOV, K., REUNANEN, A., ERIKSSON, J., STENGARD, J. & KESANIEMI, Y. A. 1992. Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. *Diabetologia,* 35**,** 1060-7.

KASAI, H., HATAKEYAMA, H., OHNO, M. & TAKAHASHI, N. 2010. Exocytosis in islet beta-cells. *Advances in experimental medicine and biology,* 654**,** 305-38.

KATAGIRI, H., IMAI, J. & OKA, Y. 2009. Neural relay from the liver induces proliferation of pancreatic beta cells: a path to regenerative medicine using the self-renewal capabilities. *Communicative & integrative biology,* 2**,** 425-7.

KAUL, K., TARR, J. M., AHMAD, S. I., KOHNER, E. M. & CHIBBER, R. 2012. Introduction to diabetes mellitus. *Advances in experimental medicine and biology,* 771**,** 1-11.

KELLY, T., YANG, W., CHEN, C. S., REYNOLDS, K. & HE, J. 2008. Global burden of obesity in 2005 and projections to 2030. *International journal of obesity,* 32**,** 1431-7.

KHAN, A. H. & PESSIN, J. E. 2002. Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia,* 45**,** 1475-83.

KHARROUBI, I., RASSCHAERT, J., EIZIRIK, D. L. & CNOP, M. 2003. Expression of adiponectin receptors in pancreatic beta cells. *Biochemical and biophysical research communications,* 312**,** 1118-22.

KIEFFER, T. J., HELLER, R. S., LEECH, C. A., HOLZ, G. G. & HABENER, J. F. 1997. Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes,* 46**,** 1087-93.

KITABCHI, A. E., UMPIERREZ, G. E., MURPHY, M. B. & KREISBERG, R. A. 2006. Hyperglycemic Crises in Adult Patients With Diabetes: A consensus statement from the American Diabetes Association. *Diabetes care,* 29**,** 2739-2748.

KOLEVA, D. I., ORBETZOVA, M. M. & ATANASSOVA, P. K. 2013. Adipose tissue hormones and appetite and body weight regulators in insulin resistance. *Folia medica,* 55**,** 25-32.

KORNER, A., KIESS, W., STUMVOLL, M. & KOVACS, P. 2008. Polygenic contribution to obesity: genome-wide strategies reveal new targets. *Frontiers of hormone research,* 36**,** 12-36.

KOTA, S. K., MEHER, L. K., JAMMULA, S. & MODI, K. D. 2012. Genetics of type 2 diabetes mellitus and other specific types of diabetes; its role in treatment modalities. *Diabetes & metabolic syndrome,* 6**,** 54-8.

KROOK, A., KAWANO, Y., SONG, X. M., EFENDIC, S., ROTH, R. A., WALLBERG-HENRIKSSON, H. & ZIERATH, J. R. 1997. Improved glucose tolerance restores insulin-stimulated Akt kinase activity and glucose transport in skeletal muscle from diabetic Goto-Kakizaki rats. *Diabetes,* 46**,** 2110-4.

KROOK, A., WALLBERG-HENRIKSSON, H. & ZIERATH, J. R. 2004. Sending the signal: molecular mechanisms regulating glucose uptake. *Medicine and science in sports and exercise,* 36**,** 1212-7.

KUO, W. N., HODGINS, D. S. & KUO, J. F. 1973. Adenylate cyclase in islets of Langerhans. Isolation of islets and regulation of adenylate cyclase activity by various hormones and agents. *The Journal of biological chemistry,* 248**,** 2705-11.

KURNIK, D., MUSZKAT, M., LI, C., SOFOWORA, G. G., SOLUS, J., XIE, H. G., HARRIS, P. A., JIANG, L., MCMUNN, C., IHRIE, P., DAWSON, E. P., WILLIAMS, S. M., WOOD, A. J. & STEIN, C. M. 2006. Variations in the alpha2A-adrenergic receptor gene and their functional effects. *Clinical pharmacology and therapeutics,* 79**,** 173-85.

KWAK, S. & PARK, K. 2013. Genetics of type 2 diabetes and potential clinical implications. *Archives of Pharmacal Research,* 36**,** 167-177.

KVEZERELI, M., VALLENTIN, A., MOCHLY-ROSEN, D., BUSQUE, S. & FONTAINE, M. J. 2008. Islet cell survival during isolation improved through protein kinase C epsilon activation. *Transplantation proceedings,* 40**,** 375-8.

LAMBERT, G. W., STRAZNICKY, N. E., LAMBERT, E. A., DIXON, J. B. & SCHLAICH, M. P. 2010. Sympathetic nervous activation in obesity and the metabolic syndrome--causes, consequences and therapeutic implications. *Pharmacology & therapeutics,* 126**,** 159-72.

LANDER, E. & SCHORK, N. 1994. Genetic dissection of complex traits. *Science,* 265**,** 2037-2048.

LANGBERG, E. C., GU, H. F., NORDMAN, S., EFENDIC, S. & OSTENSON, C. G. 2007. Genetic variation in receptor protein tyrosine phosphatase sigma is associated with type 2 diabetes in Swedish Caucasians. *European journal of endocrinology / European Federation of Endocrine Societies,* 157**,** 459-64.

LI, J. L., CANHAM, R. M., VONGPATANASIN, W., LEONARD, D., AUCHUS, R. J. & VICTOR, R. G. 2006. Do allelic variants in alpha2A and alpha2C adrenergic receptors predispose to hypertension in blacks? *Hypertension,* 47**,** 1140-6.

LIMA, J. J., FENG, H., DUCKWORTH, L., WANG, J., SYLVESTER, J. E., KISSOON, N. & GARG, H. 2007. Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations. *Metabolism: clinical and experimental,* 56**,** 757-65.

LOCKETTE, W., GHOSH, S., FARROW, S., MACKENZIE, S., BAKER, S., MILES, P., SCHORK, A. & CADARET, L. 1995. Alpha 2-adrenergic receptor gene polymorphism and hypertension in blacks. *American journal of hypertension,* 8**,** 390-4.

LU, J. Y., HUANG, K. C., CHANG, L. C., HUANG, Y. S., CHI, Y. C., SU, T. C., CHEN, C. L. & YANG, W. S. 2008. Adiponectin: a biomarker of obesity-induced insulin resistance in adipose tissue and beyond. *Journal of biomedical science,* 15**,** 565-76.

LUPI, R., MARCHETTI, P., MAFFEI, M., DEL GUERRA, S., BENZI, L., MARSELLI, L., BERTACCA, A. & NAVALESI, R. 1999. Effects of acute or prolonged exposure to human leptin on isolated human islet function. *Biochemical and biophysical research communications,* 256**,** 637-41.

LUTHRA, A., BORKOWSKI, K. R. & CARRUTHERS, S. G. 1991. Genetic aspects of variability in superficial vein responsiveness to norepinephrine. *Clinical pharmacology and therapeutics,* 49**,** 355-61.

LYAMICHEV, V., BROW, M. A. & DAHLBERG, J. E. 1993. Structure-specific endonucleolytic cleavage of nucleic acids by eubacterial DNA polymerases. *Science,* 260**,** 778-83.

MA, R. C. W. & TONG, P. C. Y. 2010. Epidemiology of Type 2 Diabetes. *Textbook of Diabetes.* Wiley-Blackwell.

MACDONALD, M. J., FAHIEN, L. A., BROWN, L. J., HASAN, N. M., BUSS, J. D. & KENDRICK, M. A. 2005. Perspective: emerging evidence for signaling roles of mitochondrial anaplerotic products in insulin secretion. *American journal of physiology. Endocrinology and metabolism,* 288**,** E1-15.

MACDONALD, M. J., LONGACRE, M. J. & KENDRICK, M. A. 2009a. Mitochondrial malic enzyme (ME2) in pancreatic islets of the human, rat and mouse and clonal insulinoma cells. *Archives of biochemistry and biophysics,* 488**,** 100-4.

MACDONALD, M. J., LONGACRE, M. J., LANGBERG, E. C., TIBELL, A., KENDRICK, M. A., FUKAO, T. & OSTENSON, C. G. 2009b. Decreased levels of metabolic enzymes in pancreatic islets of patients with type 2 diabetes. *Diabetologia,* 52**,** 1087-91.

MACDONALD, M. J., SMITH, A. D., 3RD, HASAN, N. M., SABAT, G. & FAHIEN, L. A. 2007. Feasibility of pathways for transfer of acyl groups from mitochondria to the cytosol to form short chain acyl-CoAs in the pancreatic beta cell. *The Journal of biological chemistry,* 282**,** 30596-606.

MACGREGOR, A. J., SNIEDER, H., SCHORK, N. J. & SPECTOR, T. D. 2000. Twins: novel uses to study complex traits and genetic diseases. *Trends in Genetics,* 16**,** 131-134.

MAECHLER, P., LI, N., CASIMIR, M., VETTERLI, L., FRIGERIO, F. & BRUN, T. 2010. Role of Mitochondria in β-cell Function and Dysfunction. *In:* ISLAM, M. S. (ed.) *The Islets of Langerhans.* Springer Netherlands.

MAECHLER, P. & WOLLHEIM, C. B. 2001. Mitochondrial function in normal and diabetic [beta]-cells. *Nature,* 414**,** 807-812.

MAEDA, N., SHIMOMURA, I., KISHIDA, K., NISHIZAWA, H., MATSUDA, M., NAGARETANI, H., FURUYAMA, N., KONDO, H., TAKAHASHI, M., ARITA, Y., KOMURO, R., OUCHI, N., KIHARA, S., TOCHINO, Y., OKUTOMI, K., HORIE, M., TAKEDA, S., AOYAMA, T., FUNAHASHI, T. & MATSUZAWA, Y. 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nature medicine,* 8**,** 731-7.

MAEDLER, K., SCHULTHESS, F. T., BIELMAN, C., BERNEY, T., BONNY, C., PRENTKI, M., DONATH, M. Y. & RODUIT, R. 2008. Glucose and leptin induce apoptosis in human beta-cells and impair glucose-stimulated insulin secretion through activation of c-Jun N-terminal kinases. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* 22**,** 1905-13.

MALECKI, M. T. 2005. Genetics of type 2 diabetes mellitus. *Diabetes research and clinical practice,* 68, Supplement 1**,** S10-S21.

MANOLIO, T. A., COLLINS, F. S., COX, N. J., GOLDSTEIN, D. B., HINDORFF, L. A., HUNTER, D. J., MCCARTHY, M. I., RAMOS, E. M., CARDON, L. R., CHAKRAVARTI, A., CHO, J. H., GUTTMACHER, A. E., KONG, A., KRUGLYAK, L., MARDIS, E., ROTIMI, C. N., SLATKIN, M., VALLE, D., WHITTEMORE, A. S., BOEHNKE, M., CLARK, A. G., EICHLER, E. E., GIBSON, G., HAINES, J. L., MACKAY, T. F., MCCARROLL, S. A. & VISSCHER, P. M. 2009. Finding the missing heritability of complex diseases. *Nature,* 461**,** 747-53.

MARCHETTI, P., BUGLIANI, M., BOGGI, U., MASINI, M. & MARSELLI, L. 2012. The pancreatic beta cells in human type 2 diabetes. *Advances in experimental medicine and biology,* 771**,** 288-309.

MARCHETTI, P., LUPI, R., DEL GUERRA, S., BUGLIANI, M., MARSELLI, L. & BOGGI, U. 2010. The beta-cell in human type 2 diabetes. *Advances in experimental medicine and biology,* 654**,** 501-14.

MARROQUI, L., GONZALEZ, A., NECO, P., CABALLERO-GARRIDO, E., VIEIRA, E., RIPOLL, C., NADAL, A. & QUESADA, I. 2012. Role of leptin in the pancreatic beta-cell: effects and signaling pathways. *Journal of molecular endocrinology,* 49**,** R9-17.

MASSARSKY, A., TRUDEAU, V. L. & MOON, T. W. 2011. beta-blockers as endocrine disruptors: the potential effects of human beta-blockers on aquatic organisms. *Journal of experimental zoology. Part A, Ecological genetics and physiology,* 315**,** 251-65.

MATSUMOTO, M., OGAWA, W., HINO, Y., FURUKAWA, K., ONO, Y., TAKAHASHI, M., OHBA, M., KUROKI, T. & KASUGA, M. 2001. Inhibition of insulin-induced activation of Akt by a kinase-deficient mutant of the epsilon isozyme of protein kinase C. *The Journal of biological chemistry,* 276**,** 14400-6.

MATSUZAWA, Y. 2010. Adiponectin: a key player in obesity related disorders. *Current pharmaceutical design,* 16**,** 1896-901.

MATTHEWS, D. R., HOSKER, J. P., RUDENSKI, A. S., NAYLOR, B. A., TREACHER, D. F. & TURNER, R. C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia,* 28**,** 412-9.

MAYO, O. 2008. A century of Hardy-Weinberg equilibrium. *Twin research and human genetics : the official journal of the International Society for Twin Studies,* 11**,** 249-56.

MCALLISTER, E. J., DHURANDHAR, N. V., KEITH, S. W., ARONNE, L. J., BARGER, J., BASKIN, M., BENCA, R. M., BIGGIO, J., BOGGIANO, M. M., EISENMANN, J. C., ELOBEID, M., FONTAINE, K. R., GLUCKMAN, P., HANLON, E. C., KATZMARZYK, P., PIETROBELLI, A., REDDEN, D. T., RUDEN, D. M., WANG, C., WATERLAND, R. A., WRIGHT, S. M. & ALLISON, D. B. 2009. Ten putative contributors to the obesity epidemic. *Critical reviews in food science and nutrition,* 49**,** 868-913.

MCCARTHY, J. J., MEYER, J., MOLITERNO, D. J., NEWBY, L. K., ROGERS, W. J. & TOPOL, E. J. 2003. Evidence for substantial effect modification by gender in a large-scale genetic association study of the metabolic syndrome among coronary heart disease patients. *Human genetics,* 114**,** 87-98.

MCCULLOCH, L. J., VAN DE BUNT, M., BRAUN, M., FRAYN, K. N., CLARK, A. & GLOYN, A. L. 2011. GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications for understanding

genetic association signals at this locus. *Molecular genetics and metabolism,* 104**,** 648-53.

MEDICI, F., HAWA, M., IANARI, A., PYKE, D. A. & LESLIE, R. D. G. 1999. Concordance rate for Type II diabetes mellitus in monozygotic twins: actuarial analysis. *Diabetologia,* 42**,** 146-150.

MELLOR, H. & PARKER, P. J. 1998. The extended protein kinase C superfamily. *The Biochemical journal,* 332 (Pt 2)**,** 281-92.

MICHEL, M. C., MINDERMANN, G., DAUL, A. & BRODDE, O. E. 1991. Effects of antihypertensive therapy on human alpha- and beta-adrenoceptors. *Journal of hypertension,* 9**,** 601-6.

MICHEL, M. C., PLOGMANN, C., PHILIPP, T. & BRODDE, O. E. 1999. Functional correlates of alpha(2A)-adrenoceptor gene polymorphism in the HANE study. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association,* 14**,** 2657-63.

MINN, A. H., PATTERSON, N. B., PACK, S., HOFFMANN, S. C., GAVRILOVA, O., VINSON, C., HARLAN, D. M. & SHALEV, A. 2003. Resistin is expressed in pancreatic islets. *Biochemical and biophysical research communications,* 310**,** 641-5.

MIRALLES, F. & PORTHA, B. 2001. Early development of beta-cells is impaired in the GK rat model of type 2 diabetes. *Diabetes,* 50**,** S84.

MITRAKOU, A. 2011. Kidney: Its impact on glucose homeostasis and hormonal regulation. *Diabetes research and clinical practice,* 93, Supplement 1**,** S66-S72.

MOJIMINIYI, O. A., ABDELLA, N. A., AL AROUJ, M. & BEN NAKHI, A. 2007. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. *International journal of obesity,* 31**,** 213-20.

MORIOKA, T., ASILMAZ, E., HU, J., DISHINGER, J. F., KURPAD, A. J., ELIAS, C. F., LI, H., ELMQUIST, J. K., KENNEDY, R. T. & KULKARNI, R. N. 2007. Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *The Journal of clinical investigation,* 117**,** 2860-8.

MOVASSAT, J., SAULNIER, C., SERRADAS, P. & PORTHA, B. 1997. Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. *Diabetologia,* 40**,** 916-25.

MUECKLER, M. & THORENS, B. 2013. The SLC2 (GLUT) family of membrane transporters. *Molecular Aspects of Medicine,* 34**,** 121-138.

MUREA, M., MA, L. & FREEDMAN, B. I. 2012. Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. *The review of diabetic studies : RDS,* 9**,** 6-22.

MUZUMDAR, R., MA, X., YANG, X., ATZMON, G., BERNSTEIN, J., KARKANIAS, G. & BARZILAI, N. 2003. Physiologic effect of leptin on insulin secretion is mediated mainly through central mechanisms. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* 17**,** 1130-2.

NAKATA, M., OKADA, T., OZAWA, K. & YADA, T. 2007. Resistin induces insulin resistance in pancreatic islets to impair glucose-induced insulin release. *Biochemical and biophysical research communications,* 353**,** 1046-51.

NATHAN, D. M., DAVIDSON, M. B., DEFRONZO, R. A., HEINE, R. J., HENRY, R. R., PRATLEY, R. & ZINMAN, B. 2007. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes care,* 30**,** 753-9.

NATIONAL HEART, L., AND BLOOD INSTITUTE 1998. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. *Obesity research,* 6 Suppl 2**,** 51S-209S.

NESHER, R. & CERASI, E. 2002. Modeling phasic insulin release: immediate and time-dependent effects of glucose. *Diabetes,* 51 Suppl 1**,** S53-9.

NESHER, R., WARWAR, N., KHAN, A., EFENDIC, S., CERASI, E. & KAISER, N. 2001. Defective stimulus-secretion coupling in islets of Psammomys obesus, an animal model for type 2 diabetes. *Diabetes,* 50**,** 308-14.

NEWMAN, B., SELBY, J. V., KING, M. C., SLEMENDA, C., FABSITZ, R. & FRIEDMAN, G. D. 1987. Concordance for Type 2 (non-insulin-dependent) diabetes mellitus in male twins. *Diabetologia,* 30**,** 763-768.

NEWSHOLME, P., GAUDEL, C. & MCCLENAGHAN, N. H. 2010. Nutrient regulation of insulin secretion and beta-cell functional integrity. *Advances in experimental medicine and biology,* 654**,** 91-114.

NILSSON, T., ARKHAMMAR, P., RORSMAN, P. & BERGGREN, P. O. 1989. Suppression of insulin release by galanin and somatostatin is mediated by a G-protein. An effect involving repolarization and reduction in cytoplasmic free Ca2+ concentration. *The Journal of biological chemistry,* 264**,** 973-80.

NISHIZUKA, Y. 1986. Studies and perspectives of protein kinase C. *Science,* 233**,** 305-12.

NISHIZUKA, Y. 1988. The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature,* 334**,** 661-5.

NOLAN, C. J., MADIRAJU, M. S., DELGHINGARO-AUGUSTO, V., PEYOT, M. L. & PRENTKI, M. 2006a. Fatty acid signaling in the beta-cell and insulin secretion. *Diabetes,* 55 Suppl 2**,** S16-23.

NOLAN, C. J. & PRENTKI, M. 2008. The islet beta-cell: fuel responsive and vulnerable. *Trends in endocrinology and metabolism: TEM,* 19**,** 285-91.

NOLAN, J. J., O'HALLORAN, D., MCKENNA, T. J., FIRTH, R. & REDMOND, S. 2006b. The cost of treating type 2 diabetes (CODEIRE). *Irish medical journal,* 99**,** 307-10.

NORBERG, M., STENLUND, H., LINDAHL, B., ANDERSSON, C., ERIKSSON, J. W. & WEINEHALL, L. 2007. Work stress and low emotional support is associated with increased risk of future type 2 diabetes in women. *Diabetes research and clinical practice,* 76**,** 368-77.

NORDLIE, R. C., FOSTER, J. D. & LANGE, A. J. 1999. Regulation of glucose production by the liver. *Annual review of nutrition,* 19**,** 379-406.

NORDMAN, S., ABULAITI, A., HILDING, A., LANGBERG, E. C., HUMPHREYS, K., OSTENSON, C. G., EFENDIC, S. & GU, H. F. 2008. Genetic variation of the adenylyl cyclase 3 (AC3) locus and its influence on type 2 diabetes and obesity susceptibility in Swedish men. *International journal of obesity,* 32**,** 407-12.

NORDMAN, S., DING, B., OSTENSON, C. G., KARVESTEDT, L., BRISMAR, K., EFENDIC, S. & GU, H. F. 2005. Leu7Pro polymorphism in the neuropeptide Y (NPY) gene is associated with impaired glucose tolerance and type 2 diabetes in Swedish men. *Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association,* 113**,** 282-7.

O'DONNELL, C. J., LINDPAINTNER, K., LARSON, M. G., RAO, V. S., ORDOVAS, J. M., SCHAEFER, E. J., MYERS, R. H. & LEVY, D. 1998. Evidence for association and genetic linkage of the angiotensin-converting

enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. *Circulation,* 97**,** 1766-72.

OKAMOTO, M., OHARA-IMAIZUMI, M., KUBOTA, N., HASHIMOTO, S., ETO, K., KANNO, T., KUBOTA, T., WAKUI, M., NAGAI, R., NODA, M., NAGAMATSU, S. & KADOWAKI, T. 2008. Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. *Diabetologia,* 51**,** 827-35.

OKAMOTO, Y., KIHARA, S., FUNAHASHI, T., MATSUZAWA, Y. & LIBBY, P. 2006. Adiponectin: a key adipocytokine in metabolic syndrome. *Clinical science,* 110**,** 267-78.

OPPERT, J. M., TOURVILLE, J., CHAGNON, M., MAURIEGE, P., DIONNE, F. T., PERUSSE, L. & BOUCHARD, C. 1995. DNA polymorphisms in the alpha 2- and beta 2-adrenoceptor genes and regional fat distribution in humans: association and linkage studies. *Obesity research,* 3**,** 249-55.

OSTENSON, C. G. 2001. The pathophysiology of type 2 diabetes mellitus: an overview. *Acta physiologica Scandinavica,* 171**,** 241-7.

OSTENSON, C. G. & EFENDIC, S. 2007. Islet gene expression and function in type 2 diabetes; studies in the Goto-Kakizaki rat and humans. *Diabetes, obesity & metabolism,* 9 Suppl 2**,** 180-6.

OSTENSON, C. G., KHAN, A., ABDEL-HALIM, S. M., GUENIFI, A., SUZUKI, K., GOTO, Y. & EFENDIC, S. 1993. Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. *Diabetologia,* 36**,** 3-8.

PARK, L., NIGG, J. T., WALDMAN, I. D., NUMMY, K. A., HUANG-POLLOCK, C., RAPPLEY, M. & FRIDERICI, K. H. 2005. Association and linkage of alpha-2A adrenergic receptor gene polymorphisms with childhood ADHD. *Molecular psychiatry,* 10**,** 572-80.

PATJA, K., JOUSILAHTI, P., HU, G., VALLE, T., QIAO, Q. & TUOMILEHTO, J. 2005. Effects of smoking, obesity and physical activity on the risk of type 2 diabetes in middle-aged Finnish men and women. *Journal of internal medicine,* 258**,** 356-362.

PERSEGHIN, G., PETERSEN, K. & SHULMAN, G. I. 2003. Cellular mechanism of insulin resistance: potential links with inflammation. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* 27 Suppl 3**,** S6-11.

PERSSON, P. G., CARLSSON, S., SVANSTRÖM, L., ÖSTENSON, C. G., EFENDIC, S. & GRILL, V. 2000. Cigarette smoking, oral moist snuff use and glucose intolerance. *Journal of internal medicine,* 248**,** 103-110.

PESSIN, J. E. & SALTIEL, A. R. 2000. Signaling pathways in insulin action: molecular targets of insulin resistance. *The Journal of clinical investigation,* 106**,** 165-9.

PETERSEN, K. F. & SHULMAN, G. I. 2002. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *The American journal of cardiology,* 90**,** 11G-18G.

PETRIE, J. R., PEARSON, E. R. & SUTHERLAND, C. 2011. Implications of genome wide association studies for the understanding of type 2 diabetes pathophysiology. *Biochemical Pharmacology,* 81**,** 471-477.

POITOUT, V. & ROBERTSON, R. P. 2002. Minireview: Secondary β-Cell Failure in Type 2 Diabetes—A Convergence of Glucotoxicity and Lipotoxicity. *Endocrinology,* 143**,** 339-342.

POITOUT, V. & ROBERTSON, R. P. 2008. Glucolipotoxicity: Fuel Excess and β-Cell Dysfunction. *Endocrine Reviews,* 29**,** 351-366.

POLONSKY, K. S., STURIS, J. & BELL, G. I. 1996. Non-Insulin-Dependent Diabetes Mellitus — A Genetically Programmed Failure of the Beta Cell to Compensate for Insulin Resistance. *New England Journal of Medicine,* 334**,** 777-783.

PORTHA, B., GIROIX, M. H., TOURREL-CUZIN, C., LE-STUNFF, H. & MOVASSAT, J. 2012. The GK rat: a prototype for the study of non-overweight type 2 diabetes. *Methods in molecular biology,* 933**,** 125-59.

PORTHA, B., LACRAZ, G., CHAVEY, A., FIGEAC, F., FRADET, M., TOURREL-CUZIN, C., HOMO-DELARCHE, F., GIROIX, M. H., BAILBE, D., GANGNERAU, M. N. & MOVASSAT, J. 2010. Islet structure and function in the GK rat. *Advances in experimental medicine and biology,* 654**,** 479-500.

PORTHA, B., LACRAZ, G., KERGOAT, M., HOMO-DELARCHE, F., GIROIX, M. H., BAILBE, D., GANGNERAU, M. N., DOLZ, M., TOURREL-CUZIN, C. & MOVASSAT, J. 2009. The GK rat beta-cell: a prototype for the diseased human beta-cell in type 2 diabetes? *Molecular and cellular endocrinology,* 297**,** 73-85.

POY, M. N., YANG, Y., REZAEI, K., FERNSTROM, M. A., LEE, A. D., KIDO, Y., ERICKSON, S. K. & NAJJAR, S. M. 2002. CEACAM1 regulates insulin clearance in liver. *Nature genetics,* 30**,** 270-6.

PRATLEY, R. E. & WEYER, C. 2001. The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. *Diabetologia,* 44**,** 929-945.

PRENTKI, M., JOLY, E., EL-ASSAAD, W. & RODUIT, R. 2002. Malonyl-CoA Signaling, Lipid Partitioning, and Glucolipotoxicity: Role in β-Cell Adaptation and Failure in the Etiology of Diabetes. *Diabetes,* 51**,** S405-S413.

PRENTKI, M., VISCHER, S., GLENNON, M. C., REGAZZI, R., DEENEY, J. T. & CORKEY, B. E. 1992. Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *The Journal of biological chemistry,* 267**,** 5802-10.

PUGAZHENTHI, S. & KHANDELWAL, R. 1995. Regulation of glycogen synthase activation in isolated hepatocytes. *Molecular and Cellular Biochemistry,* 149-150**,** 95-101.

QI, L., HU, F. B. & HU, G. 2008. Genes, environment, and interactions in prevention of type 2 diabetes: a focus on physical activity and lifestyle changes. *Current molecular medicine,* 8**,** 519-32.

RADZIUK, J. & PYE, S. 2001. Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. *Diabetes/Metabolism Research and Reviews,* 17**,** 250-72.

RAKATZI, I., MUELLER, H., RITZELER, O., TENNAGELS, N. & ECKEL, J. 2004. Adiponectin counteracts cytokine- and fatty acid-induced apoptosis in the pancreatic beta-cell line INS-1. *Diabetologia,* 47**,** 249-58.

RAZAK, F., ANAND, S. S., SHANNON, H., VUKSAN, V., DAVIS, B., JACOBS, R., TEO, K. K., MCQUEEN, M. & YUSUF, S. 2007. Defining obesity cut points in a multiethnic population. *Circulation,* 115**,** 2111-8.

RENART, J., REISER, J. & STARK, G. R. 1979. Transfer of proteins from gels to diazobenzyloxymethyl-paper and detection with antisera: a method for studying antibody specificity and antigen structure. *Proceedings of the National Academy of Sciences of the United States of America,* 76**,** 3116-20.

RODUIT, R., NOLAN, C., ALARCON, C., MOORE, P., BARBEAU, A., DELGHINGARO-AUGUSTO, V., PRZYBYKOWSKI, E., MORIN, J., MASSE, F., MASSIE, B., RUDERMAN, N., RHODES, C., POITOUT, V. & PRENTKI, M. 2004. A role for the malonyl-CoA/long-chain acyl-CoA pathway of lipid signaling in the regulation of insulin secretion in response to both fuel and nonfuel stimuli. *Diabetes,* 53**,** 1007-19.

ROGLIC, G. & UNWIN, N. 2010. Mortality attributable to diabetes: Estimates for the year 2010. *Diabetes research and clinical practice,* 87**,** 15-19.

ROGLIC, G., UNWIN, N., BENNETT, P. H., MATHERS, C., TUOMILEHTO, J., NAG, S., CONNOLLY, V. & KING, H. 2005. The Burden of Mortality Attributable to Diabetes: Realistic estimates for the year 2000. *Diabetes care,* 28**,** 2130-2135.

RORSMAN, P. & BRAUN, M. 2013. Regulation of Insulin Secretion in Human Pancreatic Islets. *Annual Review of Physiology,* 75**,** 155-179.

ROSENGREN, A. H., JOKUBKA, R., TOJJAR, D., GRANHALL, C., HANSSON, O., LI, D. Q., NAGARAJ, V., REINBOTHE, T. M., TUNCEL, J., ELIASSON, L., GROOP, L., RORSMAN, P., SALEHI, A., LYSSENKO, V., LUTHMAN, H. & RENSTROM, E. 2010. Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. *Science,* 327**,** 217-20.

ROSMOND, R., BOUCHARD, C. & BJORNTORP, P. 2002. A C-1291G polymorphism in the alpha2A-adrenergic receptor gene (ADRA2A) promoter is associated with cortisol escape from dexamethasone and elevated glucose levels. *Journal of internal medicine,* 251**,** 252-7.

ROTH, J., QIANG, X., MARBÁN, S. L., REDELT, H. & LOWELL, B. C. 2004. The Obesity Pandemic: Where Have We Been and Where Are We Going? *Obesity research,* 12**,** 88S-101S.

SAKO, Y. & GRILL, V. E. 1990. A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely coupled to fatty acid oxidation. *Endocrinology,* 127**,** 1580-9.

SAKURABA, H., MIZUKAMI, H., YAGIHASHI, N., WADA, R., HANYU, C. & YAGIHASHI, S. 2002. Reduced beta-cell mass and expression of oxidative stress-related DNA damage in the islet of Japanese Type II diabetic patients. *Diabetologia,* 45**,** 85-96.

SAVAGE, D. B., PETERSEN, K. F. & SHULMAN, G. I. 2005. Mechanisms of Insulin Resistance in Humans and Possible Links With Inflammation. *Hypertension,* 45**,** 828-833.

SAVONTAUS, E., FAGERHOLM, V., RAHKONEN, O. & SCHEININ, M. 2008. Reduced blood glucose levels, increased insulin levels and improved glucose tolerance in alpha2A-adrenoceptor knockout mice. *European journal of pharmacology,* 578**,** 359-64.

SCHMITZ-PEIFFER, C. & BIDEN, T. J. 2008. Protein kinase C function in muscle, liver, and beta-cells and its therapeutic implications for type 2 diabetes. *Diabetes,* 57**,** 1774-83.

SCHMITZ-PEIFFER, C., LAYBUTT, D. R., BURCHFIELD, J. G., GURISIK, E., NARASIMHAN, S., MITCHELL, C. J., PEDERSEN, D. J., BRAUN, U., COONEY, G. J., LEITGES, M. & BIDEN, T. J. 2007. Inhibition of PKCε Improves Glucose-Stimulated Insulin Secretion and Reduces Insulin Clearance. *Cell Metabolism,* 6**,** 320-328.

SEED AHMED, M., KOVOOR, A., NORDMAN, S., ABU SEMAN, N., GU, T., EFENDIC, S., BRISMAR, K., OSTENSON, C. G. & GU, H. F. 2012. Increased expression of adenylyl cyclase 3 in pancreatic islets and central nervous system of diabetic Goto-Kakizaki rats: a possible regulatory role in glucose homeostasis. *Islets,* 4**,** 343-8.

SEINO, S. 2012. Cell signalling in insulin secretion: the molecular targets of ATP, cAMP and sulfonylurea. *Diabetologia,* 55**,** 2096-2108.

SHARP, G. W. 1996. Mechanisms of inhibition of insulin release. *The American journal of physiology,* 271**,** C1781-99.

SIEMS, W. G., SOMMERBURG, O. & GRUNE, T. 2000. Erythrocyte free radical and energy metabolism. *Clinical nephrology,* 53**,** S9-17.

SIMMONS, R. A. 2013. Programming of DNA methylation in type 2 diabetes. *Diabetologia,* 56**,** 947-8.

STEIN, D. T., ESSER, V., STEVENSON, B. E., LANE, K. E., WHITESIDE, J. H., DANIELS, M. B., CHEN, S. & MCGARRY, J. D. 1996. Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. *The Journal of clinical investigation,* 97**,** 2728-35.

STOLERMAN, E. S. & FLOREZ, J. C. 2009. Genomics of type 2 diabetes mellitus: implications for the clinician. *Nat Rev Endocrinol,* 5**,** 429-436.

STRAUB, S. G. & SHARP, G. W. 1996a. Glucose-dependent insulinotropic polypeptide stimulates insulin secretion via increased cyclic AMP and [Ca2+]1 and a wortmannin-sensitive signalling pathway. *Biochemical and biophysical research communications,* 224**,** 369-74.

STRAUB, S. G. & SHARP, G. W. 1996b. Mechanisms of action of VIP and PACAP in the stimulation of insulin release. *Annals of the New York Academy of Sciences,* 805**,** 607-12.

STRAUB, S. G. & SHARP, G. W. 1996c. A wortmannin-sensitive signal transduction pathway is involved in the stimulation of insulin release by vasoactive intestinal polypeptide and pituitary adenylate cyclase-activating polypeptide. *The Journal of biological chemistry,* 271**,** 1660-8.

STRAUB, S. G. & SHARP, G. W. 2012. Evolving insights regarding mechanisms for the inhibition of insulin release by norepinephrine and heterotrimeric G proteins. *American journal of physiology. Cell physiology,* 302**,** C1687-98.

STRAUB, S. G. & SHARP, G. W. G. 2002. Glucose-stimulated signaling pathways in biphasic insulin secretion. *Diabetes/Metabolism Research and Reviews,* 18**,** 451-463.

STUMVOLL, M., GOLDSTEIN, B. J. & VAN HAEFTEN, T. W. 2005. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet,* 365**,** 1333-46.

STUMVOLL, M., MEYER, C., MITRAKOU, A., NADKARNI, V. & GERICH, J. E. 1997. Renal glucose production and utilization: new aspects in humans. *Diabetologia,* 40**,** 749-757.

SUN, M. K. & ALKON, D. L. 2006. Protein kinase C pharmacology: perspectives on therapeutic potentials as antidementic and cognitive agents. *Recent patents on CNS drug discovery,* 1**,** 147-56.

SURAMPUDI, P. N., JOHN-KALARICKAL, J. & FONSECA, V. A. 2009. Emerging concepts in the pathophysiology of type 2 diabetes mellitus. *The Mount Sinai journal of medicine, New York,* 76**,** 216-26.

SWINBURN, B. A., SACKS, G., HALL, K. D., MCPHERSON, K., FINEGOOD, D. T., MOODIE, M. L. & GORTMAKER, S. L. 2011. The global obesity pandemic: shaped by global drivers and local environments. *Lancet,* 378**,** 804-14.

SZASZAK, M., CHRISTIAN, F., ROSENTHAL, W. & KLUSSMANN, E. 2008. Compartmentalized cAMP signalling in regulated exocytic processes in non-neuronal cells. *Cellular signalling,* 20**,** 590-601.

TAYLOR, S. I., OLEFSKY, J. M. & LEROITH, D. 2004. *Diabetes mellitus : a fundamental and clinical text,* Philadelphia, Pa., Lippincott Williams & Wilkins.

THORENS, B. & MUECKLER, M. 2010. Glucose transporters in the 21st Century. *American journal of physiology. Endocrinology and metabolism,* 298**,** E141-5.

TIRONE, T. A. & BRUNICARDI, F. C. 2001. Overview of Glucose Regulation. *World Journal of Surgery,* 25**,** 461-467.

TOWBIN, H., STAEHELIN, T. & GORDON, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America,* 76**,** 4350-4.

UCHIDA, T., IWASHITA, N., OHARA-IMAIZUMI, M., OGIHARA, T., NAGAI, S., CHOI, J. B., TAMURA, Y., TADA, N., KAWAMORI, R., NAKAYAMA, K. I., NAGAMATSU, S. & WATADA, H. 2007. Protein kinase Cdelta plays a non-redundant role in insulin secretion in pancreatic beta cells. *The Journal of biological chemistry,* 282**,** 2707-16.

UCHIZONO, Y., ALARCÓN, C., WICKSTEED, B. L., MARSH, B. J. & RHODES, C. J. 2007. The balance between proinsulin biosynthesis and insulin secretion: where can imbalance lead? *Diabetes, Obesity and Metabolism,* 9**,** 56-66.

UKKOLA, O., PERUSSE, L., CHAGNON, Y. C., DESPRES, J. P. & BOUCHARD, C. 2001. Interactions among the glucocorticoid receptor, lipoprotein lipase and adrenergic receptor genes and abdominal fat in the Quebec Family Study. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity,* 25**,** 1332-9.

ULLRICH, S. & WOLLHEIM, C. B. 1984. Islet cyclic AMP levels are not lowered during alpha 2-adrenergic inhibition of insulin release. *The Journal of biological chemistry,* 259**,** 4111-5.

UNGER, R. H. 1981. The milieu interieur and the islets of Langerhans. *Diabetologia,* 20**,** 1-11.

UTZSCHNEIDER, K. M., CARR, D. B., TONG, J., WALLACE, T. M., HULL, R. L., ZRAIKA, S., XIAO, Q., MISTRY, J. S., RETZLAFF, B. M., KNOPP, R. H. & KAHN, S. E. 2005. Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. *Diabetologia,* 48**,** 2330-3.

WAJCHENBERG, B. L. 2007. β-Cell Failure in Diabetes and Preservation by Clinical Treatment. *Endocrine Reviews,* 28**,** 187-218.

WAJCHENBERG, B. L. 2010. Clinical approaches to preserve beta-cell function in diabetes. *Advances in experimental medicine and biology,* 654**,** 515-35.

VAN TILBURG, J., VAN HAEFTEN, T. W., PEARSON, P. & WIJMENGA, C. 2001. Defining the genetic contribution of type 2 diabetes mellitus. *Journal of Medical Genetics,* 38**,** 569-578.

WANG, H., WU, M., ZHU, W., SHEN, J., SHI, X., YANG, J., ZHAO, Q., NI, C., XU, Y., SHEN, H., SHEN, C. & GU, H. F. 2010. Evaluation of the association between the AC3 genetic polymorphisms and obesity in a Chinese Han population. *PloS one,* 5**,** e13851.

WANG, Z., LI, V., CHAN, G. C., PHAN, T., NUDELMAN, A. S., XIA, Z. & STORM, D. R. 2009. Adult type 3 adenylyl cyclase-deficient mice are obese. *PloS one,* 4**,** e6979.

WARWAR, N., EFENDIC, S., OSTENSON, C. G., HABER, E. P., CERASI, E. & NESHER, R. 2006. Dynamics of glucose-induced localization of PKC isoenzymes in pancreatic beta-cells: diabetes-related changes in the GK rat. *Diabetes,* 55**,** 590-9.

VASAVADA, R. C., WANG, L., FUJINAKA, Y., TAKANE, K. K., ROSA, T. C., MELLADO-GIL, J. M., FRIEDMAN, P. A. & GARCIA-OCANA, A. 2007. Protein kinase C-zeta activation markedly enhances beta-cell proliferation: an essential role in growth factor mediated beta-cell mitogenesis. *Diabetes,* 56**,** 2732-43.

WAUGH, J. L., CELVER, J., SHARMA, M., DUFRESNE, R. L., TERZI, D., RISCH, S. C., FAIRBROTHER, W. G., NEVE, R. L., KANE, J. P., MALLOY, M. J., PULLINGER, C. R., GU, H. F., TSATSANIS, C., HAMILTON, S. P., GOLD,

S. J., ZACHARIOU, V. & KOVOOR, A. 2011. Association between regulator of G protein signaling 9-2 and body weight. *PloS one,* 6**,** e27984.

VAXILLAIRE, M. & FROGUEL, P. 2010. The Genetics of Type 2 Diabetes: From Candidate Gene Biology to Genome-Wide Studies. *Textbook of Diabetes.* Wiley-Blackwell.

WEIR, B. S. 1996. *Genetic data analysis II : methods for discrete population genetic data,* Sunderland, Mass., Sinauer Associates.

VELAZQUEZ-GARCIA, S., VALLE, S., ROSA, T. C., TAKANE, K. K., DEMIRCI, C., ALVAREZ-PEREZ, J. C., MELLADO-GIL, J. M., ERNST, S., SCOTT, D. K., VASAVADA, R. C., ALONSO, L. C. & GARCIA-OCANA, A. 2011. Activation of protein kinase C-zeta in pancreatic beta-cells in vivo improves glucose tolerance and induces beta-cell expansion via mTOR activation. *Diabetes,* 60**,** 2546-59.

VENABLES, M. C. & JEUKENDRUP, A. E. 2009. Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. *Diabetes/Metabolism Research and Reviews,* 25**,** S18-S23.

WESS, J. 2010. More is not always better: alpha2A-adrenoceptor expression in type 2 diabetes. *Cell Metabolism,* 11**,** 3-5.

WESTERLUND, J., ORTSATER, H., PALM, F., SUNDSTEN, T. & BERGSTEN, P. 2001. Glucose-regulated pulsatile insulin release from mouse islets via the K(ATP) channel-independent pathway. *European journal of endocrinology / European Federation of Endocrine Societies,* 144**,** 667-75.

WHEELER, E. & BARROSO, I. 2011. Genome-wide association studies and type 2 diabetes. *Briefings in Functional Genomics,* 10**,** 52-60.

WHINCUP, P. H., KAYE, S. J., OWEN, C. G., HUXLEY, R., COOK, D. G., ANAZAWA, S., BARRETT-CONNOR, E., BHARGAVA, S. K., BIRGISDOTTIR, B. E., CARLSSON, S., DE ROOIJ, S. R., DYCK, R. F., ERIKSSON, J. G., FALKNER, B., FALL, C., FORSEN, T., GRILL, V., GUDNASON, V., HULMAN, S., HYPPONEN, E., JEFFREYS, M., LAWLOR, D. A., LEON, D. A., MINAMI, J., MISHRA, G., OSMOND, C., POWER, C., RICH-EDWARDS, J. W., ROSEBOOM, T. J., SACHDEV, H. S., SYDDALL, H., THORSDOTTIR, I., VANHALA, M., WADSWORTH, M. & YARBROUGH, D. E. 2008. Birth weight and risk of type 2 diabetes: a systematic review. *JAMA : the journal of the American Medical Association,* 300**,** 2886-97.

WHITING, D. R., GUARIGUATA, L., WEIL, C. & SHAW, J. 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes research and clinical practice,* 94**,** 311-21.

WHO. 2000. *Obesity: preventing and managing the global epidemic. Report of a WHO consultation* [Online]. WHO Technical Report Series 894, Geneva, Switzerland. Available: http://libdoc.who.int/trs/WHO_TRS_894.pdf [Accessed October 14, 2013].

WHO. 2013a. *Diabetes, fact sheet N°312* [Online]. WHO. Available: http://www.who.int/mediacentre/factsheets/fs312/en/ [Accessed April 04, 2013].

WHO. 2013b. *Obesity and overweight, fact sheet N°311* [Online]. WHO. Available: http://www.who.int/mediacentre/factsheets/fs311/en/index.html [Accessed October 14, 2013].

WHO/IDF. 2006. *Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation* [Online]. WHO Document Production Services, Geneva, Switzerland. Available:

http://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/index.html [Accessed October 01, 2013].
VIGNINI, A., RAFFAELLI, F., CESTER, A., IANNILLI, A., CHERUBINI, V., MAZZANTI, L. & NANETTI, L. 2012. Environmental and genetical aspects of the link between pregnancy, birth size, and type 2 diabetes. *Current diabetes reviews,* 8**,** 155-61.
WILD, S. H. & BYRNE, C. D. 2006. ABC of obesity. Risk factors for diabetes and coronary heart disease. *BMJ,* 333**,** 1009-11.
WILLI, C., BODENMANN, P., GHALI, W. A., FARIS, P. D. & CORNUZ, J. 2007. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA : the journal of the American Medical Association,* 298**,** 2654-64.
VOIGHT, B. F., SCOTT, L. J., STEINTHORSDOTTIR, V., MORRIS, A. P., DINA, C., WELCH, R. P., ZEGGINI, E., HUTH, C., AULCHENKO, Y. S., THORLEIFSSON, G., MCCULLOCH, L. J., FERREIRA, T., GRALLERT, H., AMIN, N., WU, G., WILLER, C. J., RAYCHAUDHURI, S., MCCARROLL, S. A., LANGENBERG, C., HOFMANN, O. M., DUPUIS, J., QI, L., SEGRE, A. V., VAN HOEK, M., NAVARRO, P., ARDLIE, K., BALKAU, B., BENEDIKTSSON, R., BENNETT, A. J., BLAGIEVA, R., BOERWINKLE, E., BONNYCASTLE, L. L., BENGTSSON BOSTROM, K., BRAVENBOER, B., BUMPSTEAD, S., BURTT, N. P., CHARPENTIER, G., CHINES, P. S., CORNELIS, M., COUPER, D. J., CRAWFORD, G., DONEY, A. S., ELLIOTT, K. S., ELLIOTT, A. L., ERDOS, M. R., FOX, C. S., FRANKLIN, C. S., GANSER, M., GIEGER, C., GRARUP, N., GREEN, T., GRIFFIN, S., GROVES, C. J., GUIDUCCI, C., HADJADJ, S., HASSANALI, N., HERDER, C., ISOMAA, B., JACKSON, A. U., JOHNSON, P. R., JORGENSEN, T., KAO, W. H., KLOPP, N., KONG, A., KRAFT, P., KUUSISTO, J., LAURITZEN, T., LI, M., LIEVERSE, A., LINDGREN, C. M., LYSSENKO, V., MARRE, M., MEITINGER, T., MIDTHJELL, K., MORKEN, M. A., NARISU, N., NILSSON, P., OWEN, K. R., PAYNE, F., PERRY, J. R., PETERSEN, A. K., PLATOU, C., PROENCA, C., PROKOPENKO, I., RATHMANN, W., RAYNER, N. W., ROBERTSON, N. R., ROCHELEAU, G., RODEN, M., SAMPSON, M. J., SAXENA, R., SHIELDS, B. M., SHRADER, P., SIGURDSSON, G., SPARSO, T., STRASSBURGER, K., STRINGHAM, H. M., SUN, Q., SWIFT, A. J., THORAND, B., et al. 2010. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nature genetics,* 42**,** 579-89.
WOLFARTH, B., RIVERA, M. A., OPPERT, J. M., BOULAY, M. R., DIONNE, F. T., CHAGNON, M., GAGNON, J., CHAGNON, Y., PERUSSE, L., KEUL, J. & BOUCHARD, C. 2000. A polymorphism in the alpha2a-adrenoceptor gene and endurance athlete status. *Medicine and science in sports and exercise,* 32**,** 1709-12.
WOLFORD, J. K. & VOZAROVA DE COURTEN, B. 2004. Genetic basis of type 2 diabetes mellitus: implications for therapy. *Treatments in endocrinology,* 3**,** 257-67.
WU, X. & GARVEY, W. T. 2010. Insulin Action. *Textbook of Diabetes.* Wiley-Blackwell.
YAMADA, T., OKA, Y. & KATAGIRI, H. 2008. Inter-organ metabolic communication involved in energy homeostasis: potential therapeutic targets for obesity and metabolic syndrome. *Pharmacology & therapeutics,* 117**,** 188-98.

YAMAUCHI, T., KAMON, J., WAKI, H., TERAUCHI, Y., KUBOTA, N., HARA, K., MORI, Y., IDE, T., MURAKAMI, K., TSUBOYAMA-KASAOKA, N., EZAKI, O., AKANUMA, Y., GAVRILOVA, O., VINSON, C., REITMAN, M. L., KAGECHIKA, H., SHUDO, K., YODA, M., NAKANO, Y., TOBE, K., NAGAI, R., KIMURA, S., TOMITA, M., FROGUEL, P. & KADOWAKI, T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature medicine,* 7**,** 941-6.

YAMAZAKI, H., ZAWALICH, K. C. & ZAWALICH, W. S. 2010. Physiologic Implications of Phosphoinositides and Phospholipase C in the Regulation of Insulin Secretion. *Journal of Nutritional Science and Vitaminology,* 56**,** 1-8.

YAMAZAKI, S., KATADA, T. & UI, M. 1982. Alpha 2-adrenergic inhibition of insulin secretion via interference with cyclic AMP generation in rat pancreatic islets. *Molecular pharmacology,* 21**,** 648-53.

YANEY, G. C., FAIRBANKS, J. M., DEENEY, J. T., KORCHAK, H. M., TORNHEIM, K. & CORKEY, B. E. 2002. Potentiation of insulin secretion by phorbol esters is mediated by PKC-alpha and nPKC isoforms. *American journal of physiology. Endocrinology and metabolism,* 283**,** E880-8.

YE, J. 2013. Mechanisms of insulin resistance in obesity. *Frontiers of Medicine,* 7**,** 14-24.

YEDOVITZKY, M., MOCHLY-ROSEN, D., JOHNSON, J. A., GRAY, M. O., RON, D., ABRAMOVITCH, E., CERASI, E. & NESHER, R. 1997. Translocation inhibitors define specificity of protein kinase C isoenzymes in pancreatic beta-cells. *The Journal of biological chemistry,* 272**,** 1417-20.

YKI-JÄRVINEN, H. 1992. Glucose Toxicity. *Endocrine Reviews,* 13**,** 415-431.

YKI-JÄRVINEN, H. 2010. Insulin Resistance in Type 2 Diabetes. *Textbook of Diabetes.* Wiley-Blackwell.

YOUNGSON, N. A. & MORRIS, M. J. 2013. What obesity research tells us about epigenetic mechanisms. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 368.

ZAWALICH, W. S., BONNET-EYMARD, M. & ZAWALICH, K. C. 1997. Signal transduction in pancreatic beta-cells: regulation of insulin secretion by information flow in the phospholipase C/protein kinase C pathway. *Frontiers in bioscience : a journal and virtual library,* 2**,** d160-72.

ZHANG, H., NAGASAWA, M., YAMADA, S., MOGAMI, H., SUZUKI, Y. & KOJIMA, I. 2004. Bimodal role of conventional protein kinase C in insulin secretion from rat pancreatic beta cells. *The Journal of physiology,* 561**,** 133-47.

ZHAO, Y., FANG, Q., STRAUB, S. G. & SHARP, G. W. 2008. Both G i and G o heterotrimeric G proteins are required to exert the full effect of norepinephrine on the beta-cell K ATP channel. *The Journal of biological chemistry,* 283**,** 5306-16.

ZHOU, Y. P. & GRILL, V. E. 1994. Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *The Journal of clinical investigation,* 93**,** 870-6.