Glucotoxicity in Insulin-Producing β-Cells

HANNA K NYBLOM
Dissertation presented at Uppsala University to be publicly examined in B21, BMC, Husargatan 3, Uppsala, Saturday, December 8, 2007 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Background and aims: Type 2 diabetes mellitus is connected with elevated glucose levels, which cause impaired glucose-stimulated insulin secretion (GSIS) and degeneration of β-cells. Mechanisms for such glucotoxic effects were explored in the present study.

Materials and methods: INS-1E cells were cultured for 5 days in 5.5, 11, 20 or 27 mM glucose in the presence or absence of AMPK-agonist AICAR. GSIS was determined from INS-1E cells and islets obtained from type 2 diabetes and control donors. Human islets and INS-1E cells were functionally characterized (GSIS) and protein profiled (SELDI-TOF MS). Glucose-induced de novo synthesis of fatty acyls (HR-MAS NMR spectroscopy), fatty acid composition (GC-MS), triglyceride content and specific proteins (Western blotting) were determined in INS-1E cells.

Results: Impaired GSIS was observed from INS-1E cells exposed to chronic hyperglycaemia and islets isolated from type 2 diabetics compared to INS-1E cells cultured at normal glucose levels and control islets, respectively. Several glucose-regulated proteins were found when type 2 diabetes and control islets or mitochondria from INS-1E cells cultured at different glucose concentrations were protein profiled. Glucose induced lipid de novo synthesis of both saturated and unsaturated fatty acids in specific proportions. Glucose-induced impairment of function and mass was reverted by inclusion of AICAR, which lowered levels of pro-apoptotic protein CHOP but left triglyceride content unaffected.

Conclusions: Impaired GSIS and increased apoptosis observed in β-cells after prolonged exposure to elevated glucose concentrations involved accumulation of lipid species in specific proportions, AMPK-inactivation, ER-stress activation and complex, coordinated changes in expression patterns of mitochondrial and human islet proteins.

Keywords: type 2 diabetes, SELDI-TOF MS, glucotoxicity, proteomics, insulin secretion, mitochondria, lipids, INS-1E cells, GC-MS, HR-MAS NMR, metabolomics, human islet, AMPK, AICAR, ER stress, apoptosis

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ISSN 1651-6206
urn:nbn:se:uu:diva-8309 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-8309)
There is nothing like looking, if you want to find something. You certainly usually find something, if you look, but it is not always quite the something you were after.

JR. Tolkien
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


III  **H K Nyblom**, E Sargsyan, P Bergsten. Improved mass and function in glucotoxic β-cells by AICAR is dose-dependent not affecting triglyceride content. Manuscript.

IV  **H K Nyblom**, M Bugliani, S Torri, P Marchetti, P Bergsten. Pancreatic islet protein expression in human type 2 diabetes correlating with β-cell function. Submitted

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<tr>
<td>2DGE</td>
<td>two-dimensional gel electrophoresis</td>
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<tr>
<td>ACC</td>
<td>acetyl-CoA carboxylase</td>
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<td>AICAR</td>
<td>5-aminoimidazole-4-carboxamide ribonucleoside</td>
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<td>AMPK</td>
<td>AMP-activated protein kinase</td>
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<td>CoA</td>
<td>coenzyme A</td>
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<tr>
<td>CPT1</td>
<td>carnitine palmitoyltransferase-1</td>
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<td>ER</td>
<td>endoplasmic reticulum</td>
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<td>FA</td>
<td>fatty acyl</td>
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<td>FAME</td>
<td>fatty acid methyl esters</td>
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<td>FAS</td>
<td>fatty acid synthase</td>
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<td>FFA</td>
<td>free fatty acid</td>
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<td>GC</td>
<td>gas chromatography</td>
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<td>GPAT</td>
<td>glycerol-3-phosphate acetyltransferase</td>
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<td>GSIS</td>
<td>glucose-stimulated insulin secretion</td>
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<tr>
<td>K&lt;sub&gt;ATP&lt;/sub&gt;</td>
<td>ATP sensitive potassium</td>
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<tr>
<td>KRB</td>
<td>Krebs-Ringer bicarbonate</td>
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<tr>
<td>LC</td>
<td>liquid chromatography</td>
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<tr>
<td>LCFA</td>
<td>long chain fatty acid</td>
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<tr>
<td>MALDI-TOF</td>
<td>matrix-assisted laser desorption/ionization time of flight</td>
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<td>MAS</td>
<td>magic-angle-spinning</td>
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<td>MCD</td>
<td>malonyl-CoA decarboxylase</td>
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<tr>
<td>MS</td>
<td>mass spectrometry</td>
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<td>mtDNA</td>
<td>mitochondrial DNA</td>
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<td>NMR</td>
<td>nuclear magnetic resonance</td>
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<td>PTM</td>
<td>post-translational modification</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>SELDI</td>
<td>surface enhanced laser desorption/ionization</td>
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<td>T2DM</td>
<td>type 2 diabetes mellitus</td>
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<td>TG</td>
<td>triglyceride</td>
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<tr>
<td>UCP2</td>
<td>uncoupling protein 2</td>
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<td>UPR</td>
<td>unfolded protein response</td>
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Introduction

Diabetes mellitus has probably existed as long as mankind. Descriptions of the symptoms have been found on the Egyptian Ebers papyrus dated as early as 1550 B.C [1]. Currently, diabetes affects 246 million of the adult population worldwide [2]. Each year a further 7 million people develop diabetes, reaching epidemic proportions. With 3.8 million deaths attributed to the disease it is the fourth leading cause of global death by disease [2]. Therefore, further understanding of the development of the condition is highly motivated. To date, the development of the disease is still an enigma for thousands of researchers all over the world.

Diabetes mellitus: a group of diseases

Diabetes mellitus is a heterogeneous group of diseases with hyperglycaemia and glucose intolerance, due to inadequate supply of insulin, as the main characteristics [3]. On the basis of aetiology and clinical presentation the disorder is divided into four categories: type 1 diabetes mellitus, type 2 diabetes mellitus, gestational diabetes and other specific types [2]. In the present thesis focus is on exploring causes of type 2 diabetes mellitus.

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, afflicting 85-95 % of all diabetic individuals [4]. It has a polygenic basis in combination with a strong environmental impact [5]. Recently, several genes like TCF7L2, FTO, CDKAL1, HHEX/IDE implicated in the development of the disease, have been identified [6, 7]. The risk of developing T2DM increases with age, obesity, and sedentary lifestyle [8]. Although obesity itself causes insulin resistance [9] not all obese individuals develop diabetes since the insulin-producing β-cell adapts by increasing insulin secretion [10]. Thus, a progressive loss of β-cell function exerts a key role in the pathogenesis of T2DM. In fact, impaired β-cell function often precedes manifestations of insulin resistance [11]. It is therefore essential to further investigate the molecular basis of defective β-cell function to understand the development of T2DM.
Glucose handling of the pancreatic β-cell

Glucose is the main stimulator for insulin release from the pancreatic β-cell. Normal glucose-stimulated insulin secretion (GSIS) requires glucose sensing, where oxidative mitochondrial metabolism plays a key role [12, 13]. In the ATP sensitive potassium (K\textsubscript{ATP}) channel dependent pathway of glucose metabolism, glucose is via the glycolysis transformed to pyruvate that enters the mitochondria. Mitochondrial oxidation increases the intracellular ATP/ADP ratio, which closes K\textsubscript{ATP} channels that in turn cause depolarization of the plasma membrane, opening of voltage-gated calcium channels, calcium influx and insulin secretion (Fig 1) [14]. Efficient coupling of glucose recognition to insulin secretion is ensured by the mitochondrion [15]. In addition to glucose, fatty acids and amino acids are also metabolized in the mitochondria [16].

![Figure 1](image_url)

**Figure 1.** Glucose-stimulated insulin secretion in the β-cell. Glucose is transported into the cell through the transporter GLUT2 and via glycolysis transformed to pyruvate, which enters the mitochondria. In the citric acid cycle, through several steps, ATP is yielded. Increase in the ATP/ADP ratio closes K\textsubscript{ATP} channels. The subsequent depolarization of the membrane causes opening of calcium channels, leading to an increase in cytoplasmic calcium concentrations and insulin release. At chronic hyperglycaemia citric acid metabolites leaves the mitochondria via cataplerosis. Through a series of reactions malonyl-CoA is formed, which is used by the fatty acid synthase (FAS) for lipid synthesis.

Mechanisms involved in progression of T2DM are thought to involve elevated levels of glucose as part of a vicious cycle causing impaired GSIS [17-19] and degeneration of β-cells [20]. Manifestations of decreased function
and reduction in mass due to elevated glucose levels are collectively called glucotoxicity [21]. Several mechanisms for the glucotoxic damage have been proposed of which increased production of reactive oxygen species (ROS) [12, 22] affects the mitochondria. ROS are generated as a by-product of oxidative phosphorylation in the mitochondria [23-25]. Elevated ROS levels may cause damage to the mitochondrial DNA (mtDNA), which is highly sensitive to oxidative stress since its repair mechanisms are poor [12, 26]. Irreversible damage caused by ROS interferes with mitochondrial function. In an attempt to decrease ROS levels activation of the inner mitochondrial protein uncoupling protein 2 (UCP2) occurs [27]. The increased ROS levels have been associated with impaired GSIS in animal models of the disease [28]. Increased UCP2 redirects oxidation of glucose and free fatty acids (FFAs) from ATP to heat production. With less ATP available GSIS will be impaired [27]. The relevance to T2DM is evident from reports that ROS production is increased in T2DM in response to prolonged exposure of elevated glucose concentrations [12]. Increased formation of ROS is also an important part of aging [29], which might explain the increased prevalence of T2DM with age. The connection between mitochondrial dysfunction and diabetes is further exemplified by maternally inherited diabetes and deafness, which is caused by point mutations in the mtDNA [30-32]. This form of mitochondrial diabetes accounts for about 1% of all diabetes cases [32]. However, changes in other mitochondrial genes or proteins will also cause changes in GSIS [25, 26]. Thus, mitochondrial dysfunction has severe consequences that range from defects in energy metabolism to complex diseases like T2DM [33].

Metabolism of glucose to pyruvate can be further processed via pyruvate carboxylase into the anaplerosis/cataplerosis pathway. Especially at high glucose levels, when ATP/ADP ratio is high, cataplerosis of tricarboxylic cycle intermediates occurs and they are converted into malonyl-CoA [34]. Via a series of reactions, catalyzed by the enzyme complex fatty acid synthase (FAS), malonyl-CoA molecules are synthesized into fatty acids. Rise in malonyl-CoA will inhibit the mitochondrial fatty acid transporter carnitine palmitoyltransferase-1 (CPT1) and thus fatty acid oxidation (Fig 1) [34]. As a consequence fatty acids will be directed towards esterification and the amount of triglycerides will increase in the cell. The impaired GSIS from insulin-producing cells containing elevated triglyceride levels has been coupled to attenuation of insulin gene transcription [35] or production of ceramide [36, 37]. However, the inverse relationship between triglyceride accumulation and cytotoxicity [38] was interpreted as incorporation of FFAs into triglycerides may serve a protective mechanism to avoid reaching too high levels of FFAs. Mechanisms of how rise in cellular triglycerides in insulin-producing cells cause impaired GSIS has mainly been studied in the context of prolonged exposure to elevated levels of FFAs and glucose, which cause reduction in GSIS (glucolipotoxicity) [39-42]. Indeed, it has been suggested
that chronically elevated fatty acids do not harm the cell unless glucose concentrations are elevated as well [43]. Augmented intracellular formation of lipids observed at prolonged hyperglycaemia could in this perspective be regarded as a lipotoxic part of glucotoxicity.

T2DM is often associated with or preceded by central obesity and dyslipidemia [44, 45]. Chronic exposure of β-cells to high levels of FFA have been associated with impaired insulin secretion and reduced mass (lipotoxicity) [39, 46, 47]. Typical features for insulin-producing cells exposed to FFA for an extended time-period are increased basal insulin secretion and decreased insulin levels released at a stimulatory glucose concentration [48]. The lipotoxic effects of FFAs have been linked to mechanisms such as reduced mitochondrial function [49, 50], increased endoplasmic reticulum (ER) stress [51, 52] (see below) and promotion of cell death through apoptosis [38, 53]. Different fatty acids appear to have different influences, though. For example, apoptosis seems to be induced by saturated fatty acids, like palmitic acid, but not by unsaturated fatty acids such as oleic acid [37].

As discussed above, a rise in malonyl-CoA will inhibit CPT1 and thus fatty acid oxidation [34]. AMP-activated protein kinase (AMPK) also plays a major role in fatty acid oxidation. AMPK senses cellular energy status and is activated by an increase in the AMP/ATP ratio induced by fasting or exercise [54]. Activation of AMPK directs the cell away from ATP-consuming pathways such as fatty acid and cholesterol synthesis [55] and promotes ATP production by stimulating fatty acid oxidation [56]. AMPK phosphorylates acetyl-CoA carboxylase and malonyl-CoA decarboxylase, the enzymes that regulate malonyl-CoA synthesis and degradation, respectively, with the effect of lowering malonyl-CoA (Fig 2) [54, 57].

![Figure 2. Simultaneous regulation of acetyl-CoA carboxylase (ACC), malonyl-CoA decarboxylase (MCD) and glycerol-3-phosphate acetyltransferase (GPAT) by AMP-activated protein kinase results in increased β-oxidation of fatty acids and decreased esterified lipid synthesis. Carnitine palmitoyl transferase 1 (CPT1), long-chain fatty acid-CoA (LCFA-CoA), triglycerides (TG).](image-url)
Chronically reduced activity of β-cell AMPK and increased malonyl-CoA levels could lead to lipotoxic effects on the cell [58]. By activating AMPK with pharmacological agents like 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) or metformin such negative effects might be reverted [59, 60].

The mitochondrion is not the only cellular organelle involved in the detrimental effects of chronic hyperglycaemia. Prolonged elevated glucose concentrations [61] as well as obesity and T2DM [62] have been linked to ER stress. The ER is the site of synthesis, folding and modification of secretory and cell-surface proteins, as well as the resident proteins of the secretory pathway [63]. Pancreatic β-cells have a highly developed ER since these cells need to synthesize and secrete large amounts of insulin [64, 65]. ER stress is induced under conditions of enhanced protein synthesis load. If the protein load surpasses the capacity of the ER to handle cargo proteins accumulation of unfolded or misfolded proteins in ER occurs, which elicits the unfolded protein response (UPR). The UPR is a cellular program by which the cell attempts to alleviate ER stress [63]. If not alleviated, signalling pathways are initiated leading to apoptosis, where CHOP is a component protein [64]. The saturated fatty acid palmitate has been described to elicit ER stress [51, 52], which may be related to the altered morphology of the ER observed in β-cells exposed to the fatty acid [66].

Measurements of complex expression changes

The prevalence of T2DM is rapidly increasing much due to the fact that obesity and a sedentary lifestyle are more common in the modern world [2]. Environmental factors will in principal only give rise to diabetes in genetically susceptible individuals, however. The familial nature of T2DM has led to intense research on its genetic risk factors [67]. Genetic causes of many monogenic forms of diabetes (e.g. maturity onset of diabetes in the young [68] and maternally inherited diabetes and deafness [30, 31]) have been revealed. Recently, several reports describe results of genome-wide association studies, which identified novel risk loci for T2DM and obesity in the broader population [6, 7, 69, 70]. T2DM is a complex disease with altered expression of many genes and their products [71]. This has prompted system-oriented research strategies to investigate complex changes in the pancreatic islet gene expression.

Transcriptomics

Alterations in gene expression measured at the RNA level is based on genome sequencing. The usage of complementary immobilized DNA-strands can deliver a comprehensive view on actively transcribed genes at a given
time in a given sample (transcriptome). Such microarrays have become a reference technology for gene expression profiling with broad application areas like genetic screening for mutations [72], detection of allele-specific gene expression [73] and disease diagnostics [74]. The mRNA array technique benefits from its inherent specificity and immediate identification of differentially expressed genes. Furthermore, it is possible to design mRNA arrays to measure specific genes and thus address specific questions. Transcriptomics has many advantages but the technology also has intrinsic limitations, which include the inability to detect post-translational modifications (PTMs) of proteins. PTMs are decisive for protein activity or function [75]. Although generally there is good correlation between mRNA levels and the corresponding protein levels this is not always the case [76].

**Proteomics**

The proteome can be defined as the protein complement that is present in a cell, tissue or biological fluid under a given set of conditions [77]. Proteins are key mediators of all cellular processes. Due to alternative splicing and PTMs, a protein, coded for by a single gene, can occur in several different forms (Fig 3). The number of genes in a human cell has been calculated to be 30,000-40,000, the corresponding number of proteins and their isoforms after PTMs has been estimated to be as many as 1 million [78].

![Figure 3. Relation between gene and protein species, functional proteins, derived from the gene.](image)

Several key organs and tissues known to contribute to the development of diabetes mellitus have been studied with a variety of proteomic approaches [79]. Two-dimensional gel electrophoresis (2DGE) in combination with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is the classical proteomic method. It has been used to detect and identify proteins in human and mouse islets [80, 81]. Despite the fact that 2DGE has excellent protein resolution and relatively easy protein identification [80-82], it has poor detection of proteins smaller than 10 kDa. Surface enhanced laser desorption/ionization time of flight mass spectrometry-
try (SELDI-TOF MS) [83] is effective at profiling low molecular weight proteins (<20 kDa) and can therefore be used as a complementary protein profiling methodology [84]. In addition, its higher sensitive substantially reduces the amounts of sample required. A number of studies have been performed using the SELDI-TOF MS approach to investigate differences in protein profiles between diabetic and non-diabetic groups [85-87]. The major limitation of the SELDI technique is the identification process, which still relies on other protein separation and enrichment methodologies [88]. Also, a selection of sample proteins is measured based on the surface of the protein array used [89].

Other proteomic approaches, like liquid chromatography (LC) MS/MS, have been used to study the pancreatic β-cell [90, 91]. LC-MS/MS allows for high-throughput analysis with identification of hundreds of proteins from a complex sample. However, this approach is not quantitative and does not allow for identification of splice variants and PTMs in complex samples.

Metabolomics

Metabolites are the products of cellular regulatory processes and their levels can be considered as the response of biological systems to genetic and environmental changes [92]. In the field of metabolomics, changes in the distribution and concentration of a broad range of metabolites are measured. Metabolite expression changes have been measured in single cells all the way to whole organisms [93]. The techniques most frequently used for metabolic studies are nuclear magnetic resonance (NMR) spectroscopy and MS [94]. NMR spectroscopy requires little or no sample preparation [93]. A unique feature of NMR is that it allows studies on intact tissues and cells using a technique known as magic-angle-spinning (MAS) NMR [95]. MS is considerably more sensitive but requires extensive sample preparation and it is generally necessary to couple different separation techniques like gas chromatography (GC) to the MS for detection of certain compounds [93].
The overall aim of the present study was to delineate mechanisms involved in glucotoxicity in insulin-producing β-cells. The specific aims were to identify such mechanisms by measuring:

I. complex changes in protein patterns of mitochondria isolated from INS-1E cells cultured at normal and elevated glucose concentrations.

II. complex changes in levels of metabolites from INS-1E cells cultured at normal and elevated glucose concentrations.

III. effects of AMPK activator AICAR on INS-1E cell cultured at elevated glucose concentrations.

IV. protein patterns in islets isolated from individuals with type 2 diabetes mellitus and control individuals and correlating islet cell properties.
Materials and Methods

Cell culture (papers I, II and III)
INS-1E cells (passage 75-92) were cultured for 24 hours in flasks and 24-well plates at 37°C in a humidified atmosphere containing 5% CO₂ in RPMI 1640 medium containing 11 mM glucose and supplemented with 10 mM HEPES, 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 units/ml penicillin and 100 μg/ml streptomycin. After the initial culture period, the culture medium was replaced with medium containing 5.5, 11, 20 or 27 mM glucose, with or without AICAR (paper III) and culture continued for five days. In experiments of cellular uptake and metabolism of extra-cellular glucose, 5.5 or 20 mM (1-¹³C)glucose was used during the five-day culture (paper II).

Human study subjects and islet isolation (paper IV)
Islets of Langerhans were isolated from five individuals with type 2 diabetes and 10 healthy control donors by collagenase digestion and density gradient purification [96-98]. The two groups were age and weight matched. The cause of death was cardiovascular-related in all type 2 diabetes individuals and in 6 of the control individuals. The deaths of the remaining control individuals were trauma-related. In type 2 diabetes patients, known duration of diabetes ranged from 4 to 9 years, and oral treatment was based on glibenclamide plus metformin in three cases and metformin alone in the remaining two. Islets were prepared with the approval of the local Ethics Committee at the University of Pisa, Italy.

Insulin secretion studies
INS-1E cells (papers I and III)
INS-1E cells were cultured for another 60 minutes under identical conditions as during the five-day culture period with the exception of the glucose concentration, which was 1 mM. The cells were subsequently washed and pre-incubated for 30 minutes in glucose-free buffer supplemented with 0.1%
BSA and containing (in mM): NaCl 125, KCl 5.9, MgCl$_2$ 1.2, CaCl$_2$ 1.3, HEPES 25 titrated to pH 7.4 with NaOH. The glucose-free buffer was replaced by the same buffer supplemented with either 3 or 15 mM glucose and the cells were incubated for 30 min at 37°C. Insulin in the buffer samples was determined by a competitive ELISA [99].

Human islets (paper IV)
Following a 45 min pre-incubation period in M199 culture medium at 3.3 mM glucose, groups of approximately 30 islets of comparable size were kept at 37°C for 45 min in Krebs-Ringer bicarbonate solution (KRB), 0.5% albumin, pH 7.4, containing 3.3 mM glucose. At the end of this period, buffer was removed and replaced with KRB, which contained either 3.3 mM glucose, 16.7 mM glucose, 3.3 mM glucose plus 20 mM arginine or 3.3 mM glucose plus 100 μM glibenclamide for 45 minutes. Insulin was determined by IRMA [96-98].

Protein expression measurements

Surface enhanced laser desorption/ionization time-of-flight mass spectrometry measurements

Mitochondria (paper I)
The mitochondria obtained from INS-1E cells cultured for five days at 5.5, 11, 20 or 27 mM glucose were lysed with a buffer containing 50 mM TrizmaBase, 7 M Urea and 3% CHAPS. INS-1E mitochondrial samples for protein profiling were diluted to a protein concentration of 75 μg/ml. Protein profiling was performed by strong anion exchange protein arrays (SAX2) at pH 7.0 and SELDI-TOF MS [84]. Mass accuracy was calibrated externally using the All-in-1 peptide and All-in-1 Protein molecular mass standards. Generated mass spectrograms were analyzed with supplied software. Settings for cluster formation were first pass s/n=3 in 20% of all spectra and second pass s/n=2. Cluster mass window was 0.7%.

Human islets (paper IV)
Each sample, containing approximately 100 islets, were lysed with 30 μl of a buffer containing 50 mM TrizmaBase, 8 M urea, 2% CHAPS, 1% ASB-14 and 5 mM PEFA-block. Profiling of islet samples was conducted by allowing samples with equal (5 μg) total protein amounts, in binding buffer containing 0.1 M phosphate buffer, pH 6.0 and 0.1% Triton, to bind to the Q10 (strong anion exchange) protein array [85, 100]. Mass spectra of the different
samples were generated by the SELDI-TOF MS reader. Generated mass spectrograms were analyzed using the supplied software. Mass accuracy was calibrated externally using the All-in-1 peptide and All-in-1 protein molecular mass standards. Settings for cluster formation were first pass s/n=4 in 15% of all spectra and second pass s/n=2. Cluster mass window was 0.7%.

Western blot (paper III)

INS-1E cells were lysed and total protein content was determined by the DC Protein Assay. The samples were separated by SDS-PAGE (10%), electrophoretically transferred to PVDF-membranes, and probed with primary and secondary anti-body. Antibody against phospho-AMPK, AMPK, phosphorylated eIF2α, BiP or CHOP was used as primary antibody and the secondary antibody was a horseradish peroxidise-conjugated anti-rabbit immunoglobulin G prepared in goat. Signal detection was performed using chemiluminescence and a CCD-camera. Signals were quantified using supplied software. Subsequently, PVDF membranes were stained with Coomassie, imaged scanned and quantified with supplied software. The expression level of each protein was normalised to the corresponding Coomassie stained lane.

Isolation of mitochondria (paper II)

After the five-day culture in the presence of the different glucose concentrations, INS-1E cells cultured in flasks were detached with a cell scraper and suspended in 5 ml homogenization buffer consisting of 0.25 M sucrose, 0.25 mM EGTA, 5 mM HEPES, 1 mM dithiothreitol and protease inhibitor cocktail, pH was adjusted to 7.4 with KOH. Cells were disrupted by 15 strokes of a Teflon pestle in a 10-ml glass homogenizer. For subcellular fractionation studies, the homogenates were subjected to differential centrifugation [101]. A discontinuous density gradient consisting of three layers of 36, 30 and 10% iodixanol in homogenization buffer was used in the final step [102]. Mitochondria banded just above the 10-30% interface and were collected by aspiration using a syringe. Fractions from the top layer (10%) and bottom layer (36%) were also collected. The purity of the fractions was analyzed using different markers. Whereas mitochondrial concentration was measured by cytochrome c activity, the presence of insulin granule and lysosomes were measured by insulin and acid phosphatase, respectively.

Nuclear magnetic resonance measurements (paper II)

INS-1E cells cultured at 5.5, 11, 20 or 27 mM glucose for five days were analyzed with HR-MAS NMR [103]. One-dimensional 1H spectra of INS-1E
cells were acquired with a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence \[90-(\tau-180-\tau)_n\text{-acquisition}] [104] as a T_2 filter. The total delay time counted as \(n(2\tau)\) was 21 ms. The data were acquired and processed with standard software. To aid spectral assignments, two-dimensional \(^1\text{H}-^1\text{H}\) total correlation spectroscopy (TOCSY) was performed on selected samples. \(^1\text{H}-^1\text{C}\) correlated heteronuclear single quantum coherence (HSQC) spectra were recorded on cell suspensions of INS-1E cells cultured at 5.5 or 20 mM (1-\(^{13}\text{C}\))glucose.

Gas chromatography-mass spectrometric measurements
(paper II)
For measurements of cellular fatty acids and their derivatives, a mixture of 0.88% KCl and CHCl_3-MeOH (2:1, v:v) [105] was added to INS-1E cells cultured at 5.5, 11, 20 or 27 mM glucose or at 5.5 or 20 mM (1-\(^{13}\text{C}\))glucose. The lipid containing organic phase was transferred to a new vial and evaporated to dryness by N_2. Derivatization to form fatty acid methyl esters (FAMEs) of the extracted lipids was carried out by addition of toluene and 1% H_2SO_4 (1:2, v:v) [106]. The FAMEs were extracted by hexane and analysed by GC-MS. Retention times and mass spectra of the FAMEs of the samples were compared to FAME reference standards.

Triglyceride content (paper III)
INS-1E cells from two wells in a 6-well plate were scraped in total 100 \(\mu\)l buffer containing 20 mM Tris, 150 mM NaCl, 2 mM EDTA, and 1% Triton, pH 7.5. Triglycerides were extracted in 3 ml chloroform:methanol (2:1, v/v). Samples were resuspended in 50 \(\mu\)l chloroform, 20 \(\mu\)l in duplicate were transferred to microtubes and air-dried. Thesit (5 \(\mu\)l, 10%) was added to the dry pellet. After the Thesit had dried, 10 \(\mu\)l H_2O was added [35]. Triglycerides were measured using a commercial kit and the TG content was correlated to total protein determined by the DC Protein Assay. The triolein standard curve, used to determine the TG content, was treated in parallel with the samples.

Apoptosis assay (papers III and IV)
Apoptosis was determined by an ELISA-based assay measuring levels of mono- and oligonucleosomes. INS-1E cells from a 24-well plate or aliquots of approximately 15 islets of comparable size were lysed with 200 \(\mu\)l of the
supplied lysis buffer. After 30 min incubation at room-temperature the lysates were centrifuged at 1200 rpm for 10 min. The assay was performed using 20 μl of supernatant in the ELISA. Absorbances of samples were read spectrophotometrically at 405 nm and reflect differences in the amounts of apoptotic mono- and oligonucleosomes in the samples.

Immunocytochemistry studies (paper IV)

Pancreatic samples were taken at the region between the pancreas neck and body before islet isolation. Blocks were fixed in 10% buffered formalin for 24-48 hours and then paraffin embedded and processed for light microscopy analysis [98]. Slides were incubated overnight at 4°C in a humidified chamber with prediluted insulin rabbit polyclonal antibody, followed by sequential 15-min incubations with biotinylated-linked antibody and alkaline phosphatase-labelled avidin. Diaminobenzidine tetrahydrochloride solution was used as a chromogen, yielding brown staining of the cytoplasm. At least 15 islets (diameter ≥ 50 μm) per pancreas section were examined blindly by two independent persons.

Data analysis (papers I-IV)

In paper I, II and III differences in insulin secretion, protein expression NMR signals, triglyceride content and apoptosis were evaluated using ANOVA with different post-hoc tests. Signals in NMR spectra and gas chromatograms were calculated by integration followed by normalization to total integrated area of the spectra or chromatograms, respectively. In paper IV protein expression was evaluated using the Mann-Whitney U test. The two-tailed Student’s t-test was used to compare control and type 2 diabetes islet properties. Linear correlation studies between the peak intensities and different islet characteristics were also performed.

P-values lower than 0.05 were considered significant. Values were expressed as means ± SEM.
Results and Discussion

Glucotoxicity in INS-1E cells after prolonged exposure to elevated glucose concentrations

Glucose-stimulated insulin secretion is the main glucose-lowering principle essential for maintaining euglycemia. Extended hyperglycaemia causes impaired GSIS [17-19] and degeneration of β-cells [20]. Manifestations of decreased function and reduction in mass due to elevated glucose levels are collectively called glucotoxicity [21]. The manifestations of elevated glucose concentrations are also evident in the insulin-producing cell line INS-1 [107]. In the present thesis a glucotoxic phenotype was established in the insulin producing INS-1E cell line [108] to delineate mechanisms of impaired GSIS after prolonged exposure of elevated glucose levels. INS-1E cells were cultured for five days at 5.5, 11, 20 or 27 mM and insulin secretion in response to 3 or 15 mM glucose was measured. When the glucose concentration was raised to 15 mM, insulin release increased 8 or 13–fold from INS-1E cells cultured in the presence of 5.5 or 11 mM glucose, respectively. No significant change in insulin release was observed from cells cultured at 20 or 27 mM glucose. There was no change in insulin secretion at basal (3 mM) glucose concentration. Similar observations have been made in islets [17, 18] and have been ascribed to imbalance between insulin synthesis and release leading to reduction in β-cell insulin content [109], which was also observed in this study. A characteristic of primary β-cells after exposure to extended hyperglycaemia is increased levels of basal insulin secretion [109], which was not seen in INS-1E cells in the present and a previous study [110]. The discrepancy may reflect different effects of elevated glucose on insulin gene transcription, which is elevated in primary β-cells [111] but decreased in INS-1E cells [110]. Increase of basal insulin secretory rate after prolonged exposure to elevated glucose concentrations has been recorded from the parental INS-1 cell line [112], however.

Glucose-induced enhanced ROS production causes irreversible damage by interfering with mitochondrial function [12, 26, 97, 113]. We therefore hypothesized that elevated levels of glucose would affect the levels of several mitochondrial proteins important for proper function of the β-cell (Hypothesis 1). Mitochondria were isolated from INS-1E cells showing GSIS, which ranged from normal to impaired. In order to obtain a mitochondrial fraction with high purity, differential gradient centrifugation was employed.
The isolated mitochondrial fraction was lysed and the proteins were allowed to bind to a protein array with a strong anion exchange (SAX2) surface at pH 7.0. Mitochondrial protein profiles were subsequently generated using SELDI-TOF MS. The mass spectra contained approximately 100 peaks with the chosen conditions of analysis (s/n=3, peak percentage=20%). Out of these peaks, 34 proteins were differentially expressed (p<0.05) when mass spectra obtained from INS-1E cells cultured at different glucose concentrations were compared. The isolated mitochondrial fraction was also analyzed for mitochondrial content using a cytochrome c oxidase assay, which showed a more than 100-fold increase in mitochondrial concentration compared to the other layers of the gradient. Two other organelles, insulin granules and lysosomes, were also found in the mitochondrial fraction, which is consistent with previous results [101]. Accordingly, the differentially expressed proteins may or may not originate from mitochondria, which will be clarified once the proteins are identified. An important aspect of the present work is consistent preparation of fractions, which will prevent erroneous assumptions when different fractions are compared [114].

Glucose, especially at high concentrations, promotes incorporation of glucose-derived carbon into fatty acids [107, 115, 116]. Elevation of cellular fatty acids is connected with ablated GSIS [117, 118]. Different fatty acids appear to have different influences, however. For example, apoptosis seems to be induced by saturated fatty acids like palmitic acid but not by unsaturated fatty acids such as oleic acid [37, 119, 120]. Therefore glucose-induced de novo lipid synthesis was hypothesized to consist of preferential synthesis of saturated fatty acids including palmitate (Hypothesis 2). To address the hypothesis HR-MAS NMR spectroscopy [103] and GC-MS were used. The methods allowed us not only to measure amounts of fatty acyls (FAs) in INS-1E cells cultured for 5 days at different glucose concentrations but also to determine what fatty acid species were subject to de novo synthesis. Our experiments were designed to minimize the time between ending the cell culture and initiation of the HR-MAS NMR measurements. This was achieved by directly applying living cells into the rotor of the spectrometer after harvesting and washing the cells. The use of an insert in the rotor allowed us to generate NMR spectra from as little as 1.5 million cells [103]. When INS-1E cells cultured for 5 days at 5.5 mM glucose were compared to cells cultured at 20 or 27 mM glucose, a 5-fold increase in FA was observed in cells cultured at the elevated glucose concentrations. The contribution of extra-cellular glucose for this build-up of cellular lipids was assessed by culturing INS-1E cells for 5 days at 20 mM (1-13C)glucose. After culture, cells were analyzed by HR-MAS 13C NMR spectrometry. In the 2D 1H-13C NMR spectrum a signal was observed at δ 22.87–30.05 ppm, which corresponds to the methylene group of the FA [121].

De novo synthesis of fatty acids from glucose has previously been demonstrated using 14C-labeled glucose [107, 115, 116]. Via a series of reactions
catalyzed by FAS and other enzymes, malonyl-CoA molecules are synthesized into fatty acids. The induction of lipogenic genes including FAS by elevated glucose levels in INS-1E cells is under the control of SREBP-1c [110, 122, 123]. The fate of these FFAs could either be incorporation into membranes, triglycerides or oxidation. The latter alternative was found to be minimal probably due to the inhibitory effect of malonyl-CoA on CPT1 [34]. Although there is a constant turnover of membrane lipids, as demonstrated by the incorporation of newly formed FFAs into membranes, the total membrane pool of FAs is relatively constant [124]. It can therefore be projected that at least part of the observed rise in total FAs reflects a rise in triglycerides. Mechanisms of how such increase in cellular triglycerides in insulin-producing cells causes impaired GSIS have mainly been studied in the context of prolonged exposure to elevated levels of FFAs and glucose, which cause reduction in GSIS [36, 39-41, 50]. The impaired GSIS from insulin-producing cells containing elevated triglyceride levels has been coupled to attenuation of insulin gene transcription [35, 39] or production of ceramide [37]. However, an inverse relationship between triglyceride accumulation and cytotoxicity was interpreted as incorporation of FFAs into triglycerides may serve a protective mechanism to avoid reaching too high levels of FFAs [38]. Also, it has been suggested that the detrimental effect of lipids on GSIS is related to pyruvate metabolism rather than build-up of cellular triglyceride stores [50]. In a recent study, gene expression changes induced by high glucose included up-regulation of pro-apoptotic genes [110]. The latter study implies that conditions of glucotoxicity may be translated into glucolipotoxicity. An indication that the two phenomena may also have separate mechanistic backgrounds, at least in part, is demonstrated by the fact that the increase in basal insulin secretion observed after exposure to elevated glucose and FFA levels [50, 125, 126], was not seen after exposure to elevated glucose alone [85, 110].

The NMR FA signal was further analyzed by determining the components of the signal. To address this issue fatty acids and their derivatives were extracted from INS-1E cells cultured at 5.5, 11, 20 or 27 mM glucose. The extracts were analyzed by GC-MS after derivatization to form FAMEs of the fatty acyl containing lipids. The identities of these fatty acids were confirmed by mass spectrometry. The five most abundant fatty acids with their relative percentages in INS-1E cells cultured at 5.5 mM glucose were oleate (33%), palmitate (25%), stearate (19%), octadecenoate (13%) and palmitoleate (4.4%). Together these fatty acids accounted for approximately 95% of the total cellular fatty acid content. Although the FAs as measured by NMR increased as the culture glucose was raised, there was no change in relative composition of the fatty acids when the extracts obtained from the different culture conditions were compared. The contribution of extracellular glucose to the build-up of component fatty acids was analyzed in lipid extracts of INS-1E cells cultured at 5.5 or 20 mM of either glucose or
(1-^{13}C)\text{glucose}. Mass spectra generated from lipid extracts of cells cultured at 5.5 or 20 mM glucose and 5.5 or 20 mM (1-^{13}C)\text{glucose} showed the molecular ion \([M]^+\) and its characteristic fragments for every fatty acid. From the spectra for palmitate we calculated the percentage of palmitate containing zero and up to six \(^{13}\text{C}\) carbons, taking into account naturally occurring \(^{13}\text{C}\). For cells cultured at 5.5 mM glucose these percentages were 44\% for non-labeled palmitate, 18\% (one \(^{13}\text{C}\)), 19\% (two \(^{13}\text{C}\)), 10\% (three \(^{13}\text{C}\)), 6\% (four \(^{13}\text{C}\)), 2\% (five \(^{13}\text{C}\)) and 0\% with six \(^{13}\text{C}\). In the presence of 20 mM glucose these percentages shifted to 29\%, 11\%, 21\%, 20\%, 12\%, 6\% and 1\%, respectively. Thus, incorporation of glucose-derived carbons in palmitate was observed already in the presence of 5.5 mM glucose and enhanced at 20 mM of the hexose.

The GC-MS analysis of the cellular extracts of fatty acids and their derivatives of INS-1E cells cultured at different glucose concentrations revealed the identities of nine different fatty acids. These acids are components of both membrane lipids and triglycerides [127] reinforcing the notion that newly formed FAs can be incorporated as acyls in either of the two compartments. The relative composition of these fatty acids showed that oleate was the most prominent component followed by palmitate and stearate. Despite substantial increase in cellular FAs in response to elevated glucose concentrations, the relative composition of the different component fatty acids was not affected. This is in contrast to when insulin-producing cells are exposed to elevated levels of fatty acids, which cause pronounced intracellular accumulation of the externally applied fatty acid [66]. Deposition of excess fatty acids as triglycerides has been positively and negatively correlated with fatty acid-induced death of \(\beta\)-cells [38, 98, 128, 129]. It appears that the constituent fatty acids incorporated into triglycerides are determinants to what extent the lipid accumulation is detrimental or not. Both chain length and degree of saturation could play roles [119, 130]. Accumulation in response to elevated glucose is less detrimental than that observed in response to elevated, especially saturated, fatty acids. In this context it was observed that \(\beta\)-cell lipid accumulation in the presence of externally applied saturated fatty acid palmitate was clearly harmful affecting the morphology of the ER [66]. Thus, lipid accumulation caused by elevated glucose concentrations alone results in a \(\beta\)-cell lipid profile, which is different from the one caused by elevated levels of both glucose and FFA.

In an attempt to further evaluate the role of intracellular lipid accumulation in response to elevated glucose concentrations AMPK agonist AICAR was added during culture of INS-1E cells at elevated glucose concentrations. The compound has been reported to inhibit lipogenesis and increase fatty acid oxidation via AMPK activation in hepatocytes and muscle cells [131-133]. Activation of AMPK by metformin is used in the treatment of diabetes. The drug activates AMPK indirectly [134] through inhibition of the respiratory chain [135]. Basal and glucose-stimulated insulin plasma levels are
decreased in metformin-treated patients, an observation usually attributed to the increase in peripheral insulin sensitivity [136]. To what extent metformin or AICAR have protective or detrimental effects on β-cell function and mass is however unclear [60, 96, 137]. We therefore hypothesized that AMPK activation would have positive effects on the impairment in function and mass of β-cells exposed to elevated levels of glucose (Hypothesis 3). Insulin secretion in response to 3 or 15 mM glucose was measured from INS-1E cells cultured for five days at 5.5, 11 or 27 mM glucose in the presence or absence of AICAR (0.3 or 1 mM). High glucose concentrations have been shown to inactivate AMPK in the β-cell [138, 139]. When AMPK activity was determined by relating p-AMPK to total AMPK it was decreased in INS-1E cells cultured in the presence of 11 mM glucose compared to 5.5 mM glucose. No further reduction was observed in cells cultured in the presence of 27 mM glucose. When AMPK agonist AICAR (0.3 mM) was included during culture of INS-1E cells at 27 mM glucose, insulin secretion in response to 15 mM glucose was similar to levels observed in control cells cultured in the presence of 11 mM glucose. Insulin release in the presence of 3 mM glucose was raised, however. When the AICAR concentration was increased to 1 mM, basal insulin release increased further and stimulatory release was curtailed. Similar results were obtained for cells cultured in the presence of 11 mM glucose with enhanced insulin secretion at 15 mM glucose in the presence of 0.3 mM AICAR, which was reversed when 1 mM of the agonist was added to the culture medium. Basal insulin secretion at 3 mM glucose was dose-dependently increased by AICAR also for cells cultured at 11 mM glucose.

Reduced apoptosis was observed in β-cells when AICAR was present at a concentration associated with phosphorylation of AMPK. Lowered apoptosis in the presence of AICAR has been demonstrated in β-cell exposed to elevated levels of glucose and fatty acids [59] and attributed redirection of fatty acids from esterification to oxidation [59, 131-133, 140]. Given the results that AICAR did not affect triglyceride content in the present and a previous similarly designed study [141], the explanation of redirection from esterification to oxidation seems less plausible under conditions of elevated glucose levels alone. The observed decrease in apoptosis without changes in triglyceride content renders further support for the view that lipid accumulation per se is not detrimental for the β-cell [38].

GSIS has been reported to be improved [141-143], inhibited [138, 139] or not affected [144] when AICAR was included during culture. Improved GSIS was observed when AICAR was administered for a short time period to islets and may represent other mechanisms including generation of AICA riboside trisphosphates [143] compared to when the agonist is present for extended time periods. In addition, when β-cells were exposed to AICAR for short time periods, phosphorylation of AMPK and its targets (Fig 2) may not have occurred. Whereas $K_{\text{ATP}}$-channel conductivity has been proposed to be
the principle of the stimulating effect of AICAR [145], inhibition of glucose metabolism with reduction in ATP-generation and Ca\(^{2+}\) influx may be a mechanism of the inhibiting effect of the agonist [139]. In cells over-expressing SREBP-1c, which resulted in enhanced triglyceride accumulation, improved GSIS was observed after exposure to AICAR [141]. In these cells, AICAR decreased triglyceride content. In the present study β-cells were cultured in the presence of elevated glucose concentrations and 1 mM AICAR. Subsequent GSIS was not improved despite a rise in stimulated insulin release in response to 15 mM glucose, since basal insulin release at 3 mM glucose was also elevated. As discussed above we find that INS-1E cells exposed to chronic hyperglycaemia have impaired GSIS and elevated total fatty acyls without increase in basal insulin secretion. In addition, these cells show no change in their lipid profile. Activation of AMPK by AICAR may cause a redistribution of the fatty acid composition in the cell more prone to oxidation. The rise in basal insulin release induced by AICAR has been observed previously [138, 139, 142]. The pattern with elevated basal insulin secretion has been attributed enhanced metabolism also observed when β-cells are exposed to elevated levels of fatty acids [39, 146].

Prolonged elevated glucose concentrations have been associated with ER-stress (20). ER-stress is induced under conditions of enhanced protein synthesis load. If the protein load surpasses the capacity of the ER to handle cargo proteins accumulation of unfolded or misfolded proteins in ER occurs, which elicits the unfolded protein response (UPR). The UPR is a cellular program by which the cell attempts to alleviate ER-stress [63]. If not alleviated, signalling pathways are initiated leading to apoptosis, where the ER-stress related pro-apoptotic protein CHOP is a component part [64]. From the observation that AICAR reduced protein levels of CHOP, it can be proposed that ER-stress alleviation is a mechanism by which AICAR reduces apoptosis under glucotoxic conditions.

In conclusion, although AICAR-induced activation of AMPK reduced apoptosis and improved insulin release in β-cells exposed to high glucose concentrations, these positive effects occurred at different concentrations of the agonist. Indeed, when AICAR at a given concentration positively affected one β-cell parameter, other β-cell parameters deteriorated. These effects of AICAR on β-cell function and mass make the administration of the agonist questionable as strategy to treat individuals with type 2 diabetes mellitus.
Islet protein expression from type 2 diabetes donors correlating with \(\beta\)-cell function

Significant progress has been made in the understanding of the aetiology of the secretory derangements in T2DM. Several mechanisms of how elevated nutrient levels may cause impaired \(\beta\)-cell function and mass have been proposed including accumulation of lipids [38], ceramide formation [98] and induction of ER stress [52, 147]. In most of these studies insulinoma cells or sometimes rodent islets were used and it remains to be demonstrated that the mechanisms are operational in human islets. In addition, the polygenicity of the disease [148] makes it a difficult task to dissect molecular mechanisms of the disease. With this background protein profiles of islets isolated from T2DM and control individuals were generated using SELDI-TOF MS, which may not only reveal mechanisms for impaired \(\beta\)-cell function and mass but also directly addresses the relevance of such mechanisms for type 2 diabetes mellitus in humans (Hypothesis 4).

The proteomic approach SELDI-TOF MS was used to address mechanisms responsible for deterioration of GSIS. The amount of tissue required for this method was an order of magnitude lower than with two-dimensional gel electrophoresis, which has so far been the proteomic approach most widely used in \(\beta\)-cell research by us and others [80, 81, 149-152]. The lower requirement of tissue was of special interest since mitochondrial isolation (paper I) is connected with considerable losses of material during preparation and the rarity of human islets from T2DM donors (paper IV). These advantages of SELDI-TOF MS should be balanced with its disadvantages, which include less effective profiling of proteins with masses higher than 30 kDa [84] and the identification process, which relies on protein separation and enrichment by additional methodologies [88].

Protein arrays exist with different surfaces with different physiochemical properties [100, 153]. By using different protein array surfaces the coverage of the islet proteome can be increased. In the present studies we used the strong anionic exchange surface Q10, previously known as SAX2. The reason for choosing this surface is based on its ability to efficiently bind islet and \(\beta\)-cell proteins compared to other array surfaces [85, 87]. By using the same array for islet or \(\beta\)-cell protein profiling similar mass spectra will be generated in different studies. Knowledge about identities of \(\beta\)-cell proteins obtained in one study can then be used to also identify peaks by inference in other studies. In this way a reference spectra based on the Q10 array is constructed. This reference mass spectrum in which knowledge about identified peaks are accumulated, will be used in a similar way as the islet reference gel [81], which was used to identify human islet proteins [80].

Islet protein expressions from five T2DM individuals were compared with those of ten healthy control individuals. The total number of peaks for the chosen conditions of analysis was about 110. Despite the relatively low
number of expression profiles (due to the rarity of available tissue, in particular with regard to individuals with T2DM) and the variability inherent to human beings, we found that 31 out of these peaks were differentially expressed ($p<0.05$) when mass spectra obtained from islets from T2DM donors were compared to islets from control donors. Several out of these peaks were correlated with changes in islet cell properties. The majority of the peaks were in the 3 to 30 kDa range, where also 90% of the differentially expressed proteins were found. Whereas 22 of the latter proteins were decreased, 9 were increased in individuals with T2DM compared to control individuals. As expected, insulin release in response to 16.7 mM glucose was significantly lower from islets obtained from the T2DM individuals and this defect was observed also with glibenclamide, but not with arginine stimulation. In addition, it was found that the amount of dead islet cells was significantly higher and the number of insulin positive cells per islet was significantly lower in islets from T2DM than control individuals.

Since pancreatic islets are heterogeneous in their cellular composition, with non β-cells representing 20-40% of total cell amount in islets from control individuals [96, 97, 154, 155] and an even higher percentage in islets from T2DM individuals [20], we cannot exclude that some of the observed differentially expressed islet proteins may be of non β-cell origin. In support of a β-cell origin of at least some of the discovered differentially expressed proteins we observed that many peaks, in particular in diabetic islets, were correlated to alterations in insulin secretion. In islets from T2DM individuals there were several positive correlations between protein peaks and insulin release in response to a sulfonylurea. Three of the 5 patients were on chronic sulfonylurea treatment, which can induce the expression of several genes in islet cells [156]. However, two patients were on metformin therapy alone, and therefore previous exposure to oral antidiabetic agents cannot fully explain our findings, and more studies are definitely warranted.

The potential significance of the differentially expressed proteins for understanding mechanisms of β-cell failure in T2DM depends on their identification. The restricted amount of material in the present study limited our possibilities to perform such work at this time. However, with the development of the bioinformatical tool based on the islet reference mass spectrum using the Q10 array such identification will be accelerated. In this context, the proteins with expression patterns correlating with changes in β-cell functional and mass, presented in the study, will be of particular interest.
Conclusions

Prolonged exposure to elevated glucose concentrations cause impaired GSIS and apoptosis in INS-1E cells, which is connected with:

- Lipid de novo synthesis of both saturated and unsaturated fatty acids in specific proportions identical to those present under control conditions
- AMPK-inactivation; activation of the kinase counteracts the glucotoxic effects
- ER-stress activation; alleviation of ER-stress is observed when the glucotoxic effects are reduced
- Complex, coordinated changes in expression patterns of INS-1E mitochondrial- and human islet proteins correlated to impaired function.
Målsättningen med den här avhandlingen har varit att studera olika orsaker till hur höga blodsockernivåer skadar β-cellerna i typ 2 diabetes.


proteinmönster från sjuka och friska celler kunde vi se att de skiljde sig åt. Genom att ta reda på identiteter på de proteiner som är förändrade kan viktiga ledtrådar till vilka mekanismer som är skadade i mitokondrien vid T2D komma fram.

Precis som att människor blir överviktiga om de äter för mycket kommer cellen samla på sig fett om den har tillgång till ett överskott av socker. I den andra artikeln ville vi undersöka i vilken utsträckning våra celler samlar på sig fett och i så fall vilken sorts fett. Mättade fetter har nämligen visat sig vara mer skadliga för β-cellen än omättade fetter. Samma modellsystem som tidigare användes. Vi såg att celler som hade odlats i högt glukos innehöll 5 gånger så mycket fett som celler som odlats i lågt glukos. Däremot var sammansättningen mellan mättade och omättade fetter densamma i celler som odlats i högt och lågt glukos trots att de förstnämnda innehöll totalt sett mer fett. Av det drog vi slutsatsen att den försämring av GSIS som vi kan se i celler som är utsatta för en diabetisk miljö inte beror på ett enbart ökat innehåll av mättade fetter.


Acknowledgements

Jag vill rikta mitt varmaste tack till följande personer:
(Den här avhandlingen hade aldrig blivit till utan någon av er!)

Min handledare, Peter Bergsten för att du alltid är så positiv, inspirerande och hellhjärtat engagerad!

Mina medförfattare, Kristofer Thörn, Meftun Ahmed, Rolf Anderson, Lennart Kenne, Lars Nord, Marco Bugliani, Scilla Torri, Piero Marchetti and Ernest Sargsyan.

Min bihandledare Mikael Welsh för mycket värdefulla kommentarer på avhandlingen.

Prefekterna på institutionen under min tid här, Godfried Roomans och Arne Andersson.

Medarbetare på institutionen för all hjälp. Speciellt tack till Kärstin Flink, Göran Ståhl, Agneta Sandler Bäfwe och Marianne Ljungkvist.

Erik Gylfes grupp.

Mina närmaste (doktorand)kollegor som jag delar labb med, både nuvarande, Tea, Kristofer, E-ri, Meri, Ernest, Johanna, Jenny, Olof, Tian, Anne, Jing och tidigare, Sophia, Elaine och inte minst Henrik, för att det är så roligt att jobba, äta lunch och resa med er. Jag har haft tur!

Övriga doktorander på institutionen för trevliga pratstunder, pizzamöten och kul undervisningstillfällen.

Anja – du är en klippa!

Mina vänner: för att ni är ni och finns i mitt liv!

Min familj: mamma Sonia, pappa Olle, syster Helena, hennes sambo Andreas och min underbara pojkvän Mattias för att ni alltid finns där!
This work was performed at the department of Medical Cell Biology, Uppsala University, Uppsala Sweden.

Grants from the Swedish Medical Research Council (72X-14019), European Foundation for the Study of Diabetes, Swedish Diabetes Association, Novo Nordisk Foundation, Family Ernfors Foundation, Filip Lundbergs Foundation, Marcus and Amalia Wallenberg Foundation, Göran Gustafsson Foundation, Magnus Bergvall Foundation, Swedish Society for Medical Research and Swedish Foundation for Strategic Research supported the study.
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Acta Universitatis Upsaliensis

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Editor: The Dean of the Faculty of Medicine

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