UPPSALA
UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1476

Free fatty acids and insulin hypersecretion studied in human islets

JING CEN

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2018

ISSN 1651-6206
ISBN 978-91-513-0380-2
urn:nbn:se:uu:diva-355090

Dissertation presented at Uppsala University to be publicly examined in C4:301, BMC, Husargatan 3, Uppsala, Friday, 7 September 2018 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Miriam Cnop (Université Libre de Bruxelles, ULB Center for Diabetes Research).

Abstract

Cen, J. 2018. Free fatty acids and insulin hypersecretion studied in human islets. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1476. 43 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-513-0380-2.

Free fatty acid (FFA) levels are increased in many obese subjects. High FFA levels stimulate the pancreatic beta-cells but have negative long-term effects. In obese children with high FFA levels circulating insulin concentration is high early in life but decline with age precipitating the development of type 2 diabetes mellitus (T2DM). The present study aims at preventing this development of T2DM by defining underlying mechanisms of insulin hypersecretion. Such mechanisms will be identified by studying regulation of insulin secretion from human pancreatic islets and human EndoC-βH1 cells exposed to elevated FFA levels.

We found that elevated concentrations of FFAs acutely stimulate insulin from human pancreatic islets at fasting blood glucose level, with mono-unsatured being more potent than saturated fatty acids. Enhanced secretion was associated with increased glycolytic flux and mitochondrial respiration. Continued exposure to elevated palmitate levels for up to 2 days accentuated insulin secretion, whereas 7 days' exposure caused secretory decline. Metformin prevented insulin hypersecretion from human islets treated with palmitate for 2 days by decreasing mitochondrial metabolism. In islets exposed to palmitate for 7 days metformin improved insulin secretion by enhancing calcium binding protein sorcin levels and thereby reducing ER stress and apoptosis. Downregulation of sorcin had negative effects on insulin secretion, mitochondrial metabolism and ER stress in human islets and EndoC-βH1 cells. Specific cellular pathways involved in insulin hypersecretion and secretory decline were identified by microarray expression analysis and subsequent bioinformatics in human islets cultured with palmitate for 0, 4, 12 hours, 1, 2, and 7 days.

In conclusion, beta-cells respond to elevated levels of FFAs by initially augmenting insulin release followed by declining secretory levels after prolonged exposure. Metformin normalizes these secretory aberrations. Specific signaling pathways and proteins including sorcin contribute to the secretory alterations induced by palmitate. When developing strategies for prevention of T2DM in obese children with elevated FFA levels, metformin should be considered as well as novel strategies involving sorcin and the identified specific pathways.

Keywords: free fatty acids, palmitate, human islets, EndoC-βH1 cells, metformin, sorcin, insulin secretion, mitochondrial respiration, ER stress, human transcriptome array

Jing Cen, Department of Medical Cell Biology, Box 571, Uppsala University, SE-75123 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-513-0380-2
urn:nbn:se:uu:diva-355090 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-355090)

No pains, no gains

一份耕耘，一份收获

List of Papers

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

I **Cen, J.**, Sargsyan, E., Bergsten, P. (2016) Fatty acids stimulate insulin secretion from human pancreatic islets at fasting glucose concentrations via mitochondria-dependent and -independent mechanisms. *Nutrition & Metabolism* 13: 59–67.

II **Cen, J.**, Sargsyan, E., Forslund, A., Bergsten, P. (2018) Mechanisms of beneficial effects of metformin on fatty acid-treated human islets. *Journal of Molecular Endocrinology, accepted.*

III **Cen, J.,** Sargsyan, E., Bergsten. P. (2018) Sorcin counteracts lipotoxicity in palmitate-exposed human beta-cells. *Manuscript.*

IV Sargsyan, E., **Cen, J.,** Roomp, K., Schneider, R., Bergsten, P. (2018) Identification of early biological changes in palmitate-treated isolated human islets. *Submitted manuscript*.

Reprints were made with permission from the respective publishers.

Contents

Abbreviations

ATF6	Activating transcription factor 6
ATP	Adenosine triphosphate
BMI	Body mass index
CPT-1	Carnitine palmitoyltransferase I
DAG	Diacylglycerol
DEG	Differentially expressed gene
ER	Endoplasmic reticulum
ELISA	Enzyme-linked immunosorbent assay
ECAR	Extracellular acidification rate
FFA	Free fatty acid
GSIS	Glucose-stimulated insulin secretion
HTA	Human transcriptome array
IL-6	Interleukin 6
IP_3	Inositol (3,4,5)-trisphosphate
IRE1	Inositol Requiring 1
KEGG	Kyoto Encyclopedia of Genes and Genomes
LC-CoA	Long-chain acyl-CoA
MUFA	Mono-unsaturated fatty acid
OGTT	Oral glucose tolerance test
OCR	Oxygen consumption rate
PIP_2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PERK	PKR-like ER kinase
PKC	Protein kinase C
ROS	Reactive oxygen species
RMA	Robust multi-array average
SFA	Saturated fatty acid
shRNA	Short hairpin RNA
T2DM	Type 2 diabetes mellitus
TNF alpha	Tumor necrosis factor alpha
UCP2	Uncoupling protein 2
UPR	Unfolded protein response

Introduction

The free fatty acid (FFA) concentration varies widely in the circulation from hour to hour [1]. Fluctuations in FFA concentration are meal-related with rises during the fasting state [1], when the FFAs function as the main energy source. Such FFA fluctuations affect multiple cellular functions including regulation of insulin release in the pancreatic beta-cells [2, 3]. Elevated fasting FFA levels are an independent risk factor for incident type 2 diabetes mellitus (T2DM) [4-6]. We have observed a wide range in fasting FFA levels in young obese children [7], which may explain why meta-analysis failed to show a clear association between adiposity and plasma FFA levels [8]. Among the obese children we observed that those with high FFA levels have accentuated insulin responses compared with lean controls [7]. In obese adolescents with high FFA levels the high insulin levels were replaced by lower levels both at fasting and during oral glucose tolerance test (OGTT) [7]. Importantly, first phase insulin secretion was delayed [7], which is a hallmark of T2DM development [9]. In the isolated human islets these characteristics were replicated by exposing the islets for elevated FFA levels for different time periods [7, 10]. Underlying cellular mechanisms for islet insulin hypersecretion have not been defined, however. We have proposed that obese children that demonstrate accentuated insulin secretory responses early in life are more prone to develop complications [7]. Consequently, normalizing insulin secretion in these children may be a way to prevent them from developing T2DM [11, 12].

This thesis aims at identifying mechanisms that underlie the regulation of insulin secretion from human beta-cells after short- and long-term exposure to FFAs, with special focus on mechanisms of insulin hypersecretion studied in human islets and human derived EndoC-βH1 cells.

Background

Obesity

Obesity is defined as a chronic, relapsing disease process [13]. Obesity in childhood and adolescence is becoming increasingly prevalent worldwide [14]. In some of these subjects, impaired metabolic profile is already observed during childhood and often lasts for the whole lifespan if no intervention is carried out [14, 15]. This poses an increasing future health problem of premature chronic metabolic diseases such as hypertension, cardiovascular diseases and T2DM as well as their related complications [16, 17]. In the state of chronic excess energy, fat mass accumulates with the enlargement of the fat cells and increase in the cell number to adapt to the extra fat storage [18, 19]. Ectopic fat distribution in non-adipose tissues, with infiltration and activation of macrophages, occurs once beyond the maximal storage capacity of the adipocytes. When reaching this state increased release of pro-inflammatory cytokine such as tumor necrosis factor alpha (TNF alpha) and interleukin 6 (IL-6), and metabolites such as FFAs from the adipocytes are observed, which can generate impaired tissue function and metabolic damage [20].

Type 2 diabetes mellitus

T2DM is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, and/or insulin action [21]. As T2DM can promote long-term damage, dysfunction and failure of various organs, it is a cause of significant mortality and morbidity [21, 22]. Global prevalence is increasing, which has been coupled to rise in obesity [23]. Even though not all obese subject develop T2DM, excessive weight is one of the strongest predictors of the disease [13]. The interactions between these two diseases are complex but one proposed linking factor is the increased circulating concentrations of non-esterified FFAs [7, 23]. Evidence suggests that T2DM in youth is different from adults and has unique features, such as a more rapidly progressive decline in beta-cell function and accelerated development of diabetes complications [24, 25]. Therefore, it's very important to understand the early pathogenesis of childhood obesity, and to design effective prevention strategies.

Human pancreatic islets

Human pancreatic islets, scattered throughout the whole pancreas, constitute approximately 1-2% of the pancreatic mass and receive 10-15% of its blood flow [26]. At least five hormone-producing cell types make up the complex micro-organs of the pancreatic islets of Langerhans: beta-cells (50-60%) producing insulin with the effect of lowering glucose concentrations, alpha-cells (20–30%) secreting glucagon with glucose-elevating action, delta-cells (~10%) secreting somatostatin as a potent inhibitor of glucagon and insulin release, gamma-cells (~5%) secreting pancreatic polypeptide, and epsilon-cells (~3%) producing ghrelin [26, 27]. These cells are randomly distributed throughout the human islets, which is different from murine islets where clear topographical separation of beta and alpha-cell mass are observed [27]. In addition, small amounts of connective tissues, nerve fibers, pericytes, vessels and endothelial cells and blood cells including macrophages can also be found in human pancreatic islets [28].

Free fatty acids and insulin secretion

Insulin, the only glucose-lowering hormone in the body, is secreted from pancreatic beta-cells and carefully regulated by a variety of factors including nutrients such as glucose and FFAs available in the circulation [29]. Glucose is the main regulator of insulin secretion [30]. During fasting, when glucose concentration is low, and exercise, when large amounts of glucose are required, FFAs function as the main energy source for most of the tissues in the body expect for the brain [31-33]. FFAs in the circulation display differences in chain length (short-, medium-, and long-chain FFAs) and degrees of saturation (saturated and unsaturated FFAs) [34, 35]. The relationships between FFAs and beta-cell function have long been addressed both *in vitro* and *in vivo* but with mixed results. Reduction of plasma FFA levels both in isolated rat islets and humans severely impairs glucose-stimulated insulin secretion (GSIS) [2, 3, 36], implying that the presence of certain levels of FFAs is essential for GSIS. Effects of high concentrations of FFAs *in vitro* and *in vivo* depend on the exposure period. *In vitro* short exposure of beta-cells and islets to elevated FFA levels stimulates insulin secretion [37-40]. Prolonged exposure inhibits insulin secretion and impairs beta-cell function and induces apoptosis [41-43]. These patterns were supported by *in vivo* studies carried out in healthy subjects, where a short-term lipid infusion increased insulin secretion, while this effect disappeared when the infusion was prolonged [44-47]. Obese non-diabetic subjects are susceptible to long-term inhibitory effect of high FFA levels [44].

The mechanisms underlying acute stimulation of insulin secretion by fatty acids are both via intracellular metabolism [37, 48, 49] and membrane receptors [50, 51]. Fatty acid metabolism is mainly controlled by substrate supply. Fasting and starvation (low glucose levels) lead to elevated FFA levels and thus increased fatty acid oxidation [33, 52, 53]. During oxidation fatty acids are converted to long-chain acyl-CoA (LC-CoA) and enter the mitochondria for energy production [52]. Increased intracellular adenosine triphosphate (ATP) production results in closure of ATP-sensitive K^+ channels, depolarization of the plasma membrane, opening of voltage-dependent Ca^{2+} channels, and Ca^{2+} triggering of insulin granule exocytosis, similar to GSIS [54, 55]. On the other hand, at high glucose levels, malonyl-CoA derived from glucose metabolism inhibits fatty acid oxidation by allosteric inhibition of carnitine palmitoyltransferase I (CPT-1), thereby increasing the cytosolic LC-CoA for signaling purposes [56]. The synthesis of lipid signaling molecules such as diacylglycerol (DAG) and phospholipids due to triglyceride/fatty acid cycle is also important for exocytosis [56]. In addition, exogenous FFAs affect beta-cells by acting as ligands for the G-protein-coupled receptor GPR40 (FFAR1) [50, 51]. Fatty acid binding to the receptor leads to the activation of phospholipase C (PLC)-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into DAG and inositol (3,4,5)-trisphosphate (IP_3), which respectively activate protein kinase C (PKC) and mobilize Ca^{2+} from the endoplasmic reticulum (ER), thus stimulating insulin secretion [57].

Pancreatic islets chronically exposed to high concentrations of FFAs show impaired insulin secretory response to glucose stimulation [7, 10, 43], which is one of the main features of T2DM [58]. The underlying mechanisms have been extensively studied. Chronic palmitate exposure impairs beta-cell function by inhibiting insulin biosynthesis [59, 60], inducing mitochondrial uncoupling [61], inhibiting exocytosis by disrupting the coupling between Ca^{2+} channels and insulin granules [62], depletion of stored insulin [63] and inhibition of glucose metabolism [64]. The production of reactive oxygen species (ROS) and ceramides [65] and the induction of ER stress [66-70] and apoptosis [70, 71] in beta-cells have also been implicated in palmitate-induced beta-cell dysfunction and death.

Mitochondrial function in beta-cells

Proper function of mitochondria in beta-cells is important for insulin secretion in face of nutrition stimuli [72]. Mitochondria not only generate ATP as the energy source for insulin secretion, which is regulated by mitochondrial Ca^{2+} [73], but also synthesize metabolites coupling nutrient sensing to insulin granule exocytosis [74]. Metabolism-secretion coupling depends on respiration. An estimated 98% of beta-cell ATP production depends on mitochondrial oxidative processes [75]. Respiration is increased during nutrient stimulation

[76]. Results from beta-cell lines depleted of mitochondrial DNA and patients with mitochondrial diabetes showed impaired mitochondrial protein synthesis involved in the respiratory chain, concomitant with loss of glucose-induced increase of intracellular Ca^{2+} and insulin secretion [77, 78]. Furthermore, the metabolic imbalance caused by chronic hyperlipidemia severely affects mitochondrial metabolism, leading to the development of impaired GSIS in T2DM [79]. Exposure of pancreatic islets to a high concentration of fatty acids not only stimulates respiratory-chain activity but also stimulates expression of uncoupling protein 2 (UCP2) and production of ROS [61]. Beta-cells are prone to oxidative damage because they express limited levels of antioxidant enzymes [80]. Compared with non-diabetic individuals, pancreatic beta-cells from T2DM subjects showed alteration of mitochondrial morphology and function including impaired GSIS, increased UCP2 expression, lower ATP production, decreased metabolic enzymes, dysregulation of Ca^{2+} homeostasis, and decreased expression of oxidative phosphorylation genes [81-83].

ER stress in beta-cells

The ER is the place where membrane and secretory proteins are synthesized and folded [84]. ER is also responsible for several important cellular functions such as Ca^{2+} storage and cell signaling [84]. The main task of the pancreatic beta-cells is to secrete insulin, which represents approximately 50% of the total protein synthesized [69]. Therefore, to meet the high demand of folding and processing of newly synthesized insulin, beta-cells have a highly developed ER [69, 84]. Only properly folded proteins can be transported from the rough ER to the Golgi apparatus. When unfolded or misfolded proteins are synthesized due to the imbalance between protein folding demand and ER capacity, they are accumulated in the ER lumen and then the unfolded protein response (UPR) also known as ER stress occurs [85]. In order to protect against ER stress, the UPR can be activated by three ER stress sensors PKR-like ER kinase (PERK), Inositol Requiring 1 (IRE1), and Activating Transcription Factor 6 (ATF6) [85]. The UPR attenuates protein translation to alleviate ER stress and restores ER homeostasis by relieving the load on the ER and inducing ER chaperones, ER-associated degradation, and ER expansion [68, 85]. The UPR is an adaptive response but also promotes apoptosis in the fate of conditions when ER stress is prolonged or exaggerated [67]. Metabolic stress like obesity, when excess nutrients such as FFAs can persistently induce insulin secreted from beta-cells, causes a disruption of the ER homeostasis and leads to ER stress [69, 85]. In line with this, *in vitro* studies indicate that saturated FFAs induce ER stress in beta-cells and contributes to beta-cell death [70, 86, 87].

Calcium binding protein, sorcin

Sorcin (soluble resistance-related calcium-binding protein), a 22-kDa Ca^{2+}-binding protein belonging to the penta EF-hand family [88], was first identified in multidrug-resistant cells [89, 90]. Sorcin is widely expressed in a variety of mammalian tissues including heart, skeletal muscles, neurons, and primary mouse islets [91, 92]. It is localized in the cytosolic and membranous compartments e.g., ER and mitochondria [93]. Sorcin is involved in and controlling Ca^{2+} homeostasis and regulating the activity of several Ca^{2+} transporters [91, 94]. Studies in mouse islets showed that sorcin plays a role in linking beta-cell lipotoxicity to ER calcium and ER stress, representing a mechanism for dysregulation of beta-cell function under conditions of metabolic stress [92]. Sorcin modulates mitochondrial Ca^{2+} handling [95]. However, the function of sorcin in mitochondrial function has not been defined in beta-cells.

Metformin

Metformin (1, 1-dimethylbiguanide hydrochloride), derived from guanidine, is a historic drug as its link to glucose-lowering effect can be traced back to the 18th century [96]. Metformin was first introduced to clinical use to treat patients with T2DM in 1957 and has been widely used over many decades [96]. Currently, metformin is the first-line treatment for patients with newly diagnosed T2DM due to a variety of clear benefits [97-99]. In patients with T2DM, metformin treatment reduces fasting insulin concentrations, ameliorates insulin resistance, and does not increase the risk of hypoglycemia or weight gain [97-99]. Lipid-lowering effects and potential cardiovascular benefits were evident at least in some obese individuals [100, 101]. Many studies showed that metformin is connected to prevention or delay of T2DM onset in persons who are at increased risk [102, 103]. Some epidemiological studies also provided evidence that metformin can reduce cancer incidence and mortality [104]. Therefore, recently metformin is increasingly introduced for treatment of obese children and adolescents with or without T2DM [105-108]. The drug is found to be both effective and safe in obese children due to its beneficial effects on body mass index (BMI) and various metabolic outcomes as observed in adults including fasting glucose, fasting insulin, and insulin resistance [105-108].

Even though metformin has been used for some decades, the mechanisms of its action are still not fully clarified. It is a complex drug with multiple sites of action and multiple molecular mechanisms [109, 110]. Metformin has been extensively studied *in vitro* in relation to its beneficial effects on different organs [111-113]. Physiologically, it is widely reported that metformin acts on the liver via AMPK-dependent and independent mechanisms to lower glucose production [110, 114], and on the gut to increase glucose utilization [115],

GLP-1 secretion [116] and alter the microbiome [117, 118]. However, the effects of metformin on beta-cells remain unclear. It is reported that metformin restores insulin secretion from human and mouse islets and rat insulinoma cells altered by chronic exposure to high levels of FFAs [119-121] or glucose [122]. It is not known if metformin affects insulin hypersecretion from human islets induced by palmitate.

Aims

The overall aim of the work presented in this thesis is to define and explore possible mechanisms underlying the short- and long-term effects of free fatty acids (FFAs) on insulin secretion from human pancreatic islets and EndoC-βH1 cells. The specific aims of the studies are:

1. To evaluate the ability of long-chain FFAs to acutely stimulate insulin secretion from isolated human pancreatic islets at fasting glucose concentrations and to explore the role of mitochondrial-dependent and independent mechanisms in this action.

2. To investigate how metformin influences increased insulin secretion after short-term and decreased insulin secretion after long-term exposure of isolated human islets to elevated levels of FFA palmitate and to explore underlying mechanisms.

3. To explore the link between sorcin levels and insulin secretion, ER stress and mitochondrial respiration from palmitate-treated human islets and human EndoC-βH1 cells.

4. To identify biological events preceding the failure of beta-cells in fatty acid-treated human islets by using human transcriptome array in isolated human islets exposed to fatty acid palmitate for various time periods.

Material and Methods

Human pancreatic islet culture

Human pancreatic islets were obtained from brain-dead and non-diabetic donors from the Nordic Network for Clinical Islet Transplantation (Uppsala University Hospital, Uppsala, Sweden) and from Prodo Laboratories (Prodo Laboratories, Inc., CA). Human islets were cultured in CMRL medium at 37°C in humidified air containing 5% CO_2. Ethical permission to use human islets isolated from donors has been obtained from the Regional Ethical Review Board in Uppsala (EPN number 2010/006; date: 2010-02-10). Experiments were started within 10 days after islet isolation.

EndoC-βH1 cell culture

EndoC-βH1 cells are a human beta-cell line generated from human fetal pancreatic buds transduced with a lentiviral vector expressing SV40LT and further grafted into SCID mice [123]. EndoC-βH1 cells have physiological characteristics resembling primary human beta-cells [124, 125]. Cells were grown on 1% extracellular matrix gel and 2 µg/mL fibronectin coated culture vessels and cultured in DMEM containing 5.5 mM glucose, 2% fatty acid free bovine serum albumin (BSA) fraction V (Roche Diagnostics, Mannheim, Germany), and supplemented with other compounds as previously described [124].

Sorcin shRNA down-regulation

The short hairpin RNA (shRNA) of *sri* was used to inhibit sorcin expression for both human islets and EndoC-βH1 cells and was administered with lentiviral transduction particles SHCLNV VSV-G (Mission transduction particles, Sigma Aldrich). Non-target shRNA control plasmid DNA (Sigma Aldrich) was administrated as negative control. Based on the estimated reduced sorcin expression palmitate treatment was started four days after initiation of transfection.

Fatty acid and metformin preparation and treatment

Fatty acids were prepared as previously described [126]. Briefly, 100 mM stock solutions containing palmitate, stearate, or oleate (all from Sigma Aldrich) were prepared by dissolving fatty acids in 50% ethanol. Stock solution of palmitoleate (Sigma Aldrich) was prepared in 100% ethanol to a concentration of 200 mM. The stock solution was then diluted in incubation medium containing 0.5% fatty acid-free BSA to a final concentration of 0.5 mM for human islet treatment. The final concentration of 1.5 mM palmitate with 2% fatty acid-free BSA was prepared for treatment of EndoC-βH1 cells. FFA was allowed to complex with BSA at 37 °C for at least 30 min. Metformin (Sigma Aldrich) was prepared in 2 mM stock in distilled water and diluted in incubation medium to a final concentration as stated.

Insulin secretion measurements

Human islets were hand-picked and placed into a perifusion chamber for perifusion experiments, or culture plates for static incubation. In perifusion experiments, islets were perifused for 60 min at 37 °C in KRBH buffer supplemented with different components (paper I: 0 or 5.5 mM glucose with 0.5% FFA-free BSA; papers II-IV: 2 mM glucose with 0.1% BSA). Samples were collected every 5 min for 20 min at the same concentration of glucose, followed by another 20 min perifusion with the same buffer containing 0.5% FFA-free BSA and 0.5 mM palmitate (16:0), palmitoleate (16:1), stearate (18:0), or oleate (18:1), or with 10 μM triacsin C (Sigma Aldrich) to inhibit long-chain fatty acyl CoA synthetase or 10 μM DC260126 (Tocris Bioscience, Bristol, UK) to inhibit FFAR1/GPR40 signaling, respectively (paper I), or containing 0.1% BSA and 20 mM glucose (paper II-IV). The perifusates were collected for 20 min. The perifusion rate was 170 μl/min and collected perifusates were used to measure the amounts of secreted insulin.

For static incubation, human pancreatic islets were statically incubated for 60 min in KRBH buffer supplemented with or without 5.5 mM glucose in the presence of 0.5% FFA-free BSA, then changed to the same glucose- and BSA-containing buffer supplemented with 0.5 mM of the different fatty acids for 20 min (paper I). For EndoC-βH1 cells, glucose was changed from 1 mM after 120 min to either 1 or 20 mM in KRBH with 0.1% BSA for another 60 min. Aliquots of the buffer were collected for insulin measurement.

After secretion assessment, islets or cells were washed with DPBS and lysed for measurement of intracellular insulin content and protein content. Insulin was measured by enzyme-linked immunosorbent assay (ELISA) as previously described [127] and total protein content was measured by DC protein assay (Bio-Rad, California, USA).

Measurements of oxygen consumption and extracellular acidification rates

The dynamic changes of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) from isolated human pancreatic islets and EndoC-βH1 cells were measured simultaneously by Extracellular Flux Analyzer XFe96 (Seahorse Bioscience, MA, USA), using non-invasive and optical fluorescent biosensor [128]. OCR is an indicator of mitochondrial respiration, and ECAR is largely the result of glycolysis. Mitochondrial function was assessed by serial injections of different compounds that specifically target components of the electron transport chain to reveal key parameters of metabolic function. The compounds, oligomycin (Sigma Aldrich), FCCP (Sigma Aldrich), and a mix of rotenone (Sigma Aldrich) and antimycin A (Sigma Aldrich), are sequentially injected to measure ATP-coupled and proton leak respiration, maximal respiration, and non-mitochondrial respiration, respectively [129].

Human islets were pre-incubated with assay medium (Seahorse Bioscience) supplemented with 0.5% BSA and 0.5 mM different FFAs (paper I), or directly transferred from the treatment medium (paper II and III), in the presence or absence of 5.5 mM glucose (pH adjusted to 7.4) for 1 hour at 37 °C in the air before insert into the machine for real-time measurement, followed by serial injection of the different compounds. All OCR measurements were corrected for non-mitochondrial OCR. Data of human islets were normalized to total islet area calculated by the Image J software (National Institutes of Health, USA) from pictures (40×) taken with camera (Olympus, Tokyo, Japan) mounted onto an inverted Olympus CKX41 microscope (Olympus). Results from EndoC-βH1 cells were normalized to total protein content measured by DC protein assay.

Sample preparation for protein profiling

Expression of specific proteins in human islets and EndoC-βH1 cells was determined by western blot analysis. Samples were prepared by washing the human islets with DPBS, followed by sonication in the lysis buffer. Then the samples were electrophoresed and transferred onto PVDF-membrane (Bio-Rad). Immunoblotting was performed with primary antibodies against phospho-AMPKα (Cell Signaling, 1:500), AMPKα (Cell Signaling, 1:1000), phospho-eIF2α (Cell Signaling 1:500), CHOP/GADD153 (Santa Cruz 1:500), cleaved caspase-3 (Cell Signaling, 1:500), sorcin (Thermo Fisher Scientific, 1:500), and β-actin (Cell Signaling, 1:1000) and secondary antibody, mouse anti-rabbit IgG-HRP (Santa Cruz, 1:1000). Immunoreactive bands were detected by enhanced chemiluminescence (ECL) detection kit (Amersham Biosciences GE, Little Chalfont, UK) and imaged with ChemiDoc XRS+ (Bio-

Rad). Signals were quantified with Image Lab 4.0.1 (Bio-Rad). Quantitative analysis of bands densities was normalized to β-actin unless otherwise stated.

Human transcriptome array analysis

To understand the involvement of biological pathways and molecular mechanisms in time-dependent changes of GSIS from human islets after palmitate exposure, the high-throughput sequencing of human transcriptome, human transcriptome array (HTA) was applied in this study. Human transcriptome array has been designed to interrogate all transcript isoforms in the human transcriptome with >6 million probes targeting coding transcripts, non-coding transcripts, and exon-exon junctions. The array allows to estimate the unbiased abundance of a transcript and the analysis of differential exon usage between sample groups [130]. After palmitate treatment for 0, 4, 12 hours, 1, 2, and 7 days, 50 human islets were individually collected for each time point and washed with PBS three times. mRNA was isolated using mRNA isolation kit (Macherey-Nagel, Duren, Germany). RNA concentration was measured with ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and RNA quality was evaluated using the Agilent 2100 Bioanalyzer system (Agilent Technologies Inc, Palo Alto, CA). Total RNA, 100 nanograms from each sample, was used to generate amplified and biotinylated sense-strand cDNA from the entire expressed genome according to the GeneChip® WT PLUS Reagent Kit User Manual (P/N 703174 Rev 1 Affymetrix Inc., Santa Clara, CA). GeneChip® ST Arrays (GeneChip® Human Transcriptome Array (HTA) 2.0) were hybridized for 16 hours in a 45 °C incubator, rotated at 60 rpm. According to the GeneChip® Expression Wash, Stain and Scan Manual (PN 702731 Rev 3, Affymetrix Inc., Santa Clara, CA) the arrays were then washed and stained using the Fluidics Station 450 and finally scanned using the GeneChip® Scanner 3000 7G. The HTA array covers almost 68000 genes of which 27000 are annotated.

Microarray data analysis and KEGG pathway enrichment analysis

The raw data was normalized in Expression Console, provided by Affymetrix (http://www.affymetrix.com), using the robust multi-array average (RMA) method as previously described [131, 132]. Genes with more than 1.3-fold change after palmitate exposure compared to untreated islets were defined as differentially expressed genes (DEGs). DEGs were selected for bioinformatics analysis. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway over-

representation analysis was done using ConsensusPathDB (http://consensuspathdb.org/) [133]. In the further analysis, DEGs in the pathways of interest were manually annotated using literature survey and UniProt database. Results for Gene's expression are expressed as mean ± SD. KEGG pathway over-representation analysis in ConsensusPathDB was carried out using input gene lists that were compared to functional modules derived from KEGG pathway definitions. A p-value cut-off of < 0.01 and a minimum overlap with the input list of two genes were used. The calculated p-value reflects the significance of the observed overlap between an input gene list and a module's members, as compared to random expectations. Therefore, small p-values indicate that of the genes in the input list, more are present in a module (pathway) than would be expected by chance alone and this may indicate dysregulated pathways [134].

Statistical analysis

All the other results are presented as means ± SEM. Statistical analysis was performed using GraphPad Prism Version 6.0c (GraphPad software, CA, USA). Statistical significance was analyzed by using Student's t-test analysis or one-way ANOVA followed by Holm-Sidak multiple comparisons. $P<0.05$ was considered statistically significant.

Results and Discussion

Acute effects of FFAs on human islets (paper I)

The opinion that long-chain FFAs acutely potentiate insulin secretion at high glucose concentrations but have no or little effect on insulin secretion at low glucose levels, with SFAs being more potent than MUFAs is generally accepted [38-40, 135]. However, in this study we found that short-term static incubation of human islets with different long-chain FFAs at fasting glucose levels enhanced insulin secretion. Also, degree of saturation of FFAs played a positive role on insulin secreted with MUFAs being more potent than SFAs. Given these discrepant findings from the previous reports, dynamic changes of insulin secretion from human islets were assessed by perifusing the human islets. The results were in line with the static findings. In addition, perifusion data indicated that FFAs stimulated biphasic insulin secretion from human islets [136], much like glucose. The rises in insulin secretion caused by SFAs and MUFAs were accounted for by rises in both first and second phases of insulin secretion.

Beta-cell mitochondrial metabolism is known to play a critical role in maintaining nutrient-induced insulin secretion [137]. We therefore explored the contribution of mitochondrial metabolism to the effects of SFAs and MUFAs on insulin secretion. At fasting glucose concentrations FFAs significantly elevated OCR with MUFAs being more potent than SFAs, which was in line with the insulin secretion data. And ECAR analysis reveals that higher oxygen consumption rate (OCR) was observed in the presence of MUFAs compared with SFAs, which was due to higher glycolysis. When glucose was omitted from the medium introduction of FFAs did not affect OCR, supporting the role of glycolysis on increased OCR induced by FFAs. In the absence of glucose FFAs still stimulated insulin secretion from the islets although secretion was reduced. The same pattern, with MUFAs being more potent than SFAs, was detected in the absence of glucose. Contribution of mitochondria-independent mechanisms to the reduced insulin secretion was then tested [138, 139]. When either intracellular metabolism or GPR40/FFAR1 signaling was inhibited, FFAs no longer stimulated insulin secretion suggesting that these two pathways are interrelated and act synergistically. Interestingly, whereas OCR was reduced by approximately 10% in islets incubated in the absence of glucose, insulin secretion was lowered by almost 60%. It seems that beta-cells may efficiently use different sources for ATP generation [75] and, thereby

maintaining normal cell function. However, to efficiently enhance insulin secretion glucose is required.

Chronic effects of palmitate on human islets and EndoC-βH1 cells

Metformin has beneficial effects on human islets chronically treated with palmitate (paper II)

Metformin is recommended as first-line oral drug in treatment of T2DM in adults with clear benefits in relation to glucose metabolism and diabetes-related complications [97-99]. We therefore investigated whether metformin has protective effects on isolated human islets treated with palmitate for 2 and 7 days resulting in hyper- and hypo-secretion of insulin, respectively. Palmitate caused time-dependent changes in GSIS and insulin content as previously demonstrated [7, 10]. Compared with control islets, GSIS was almost doubled after treatment with the fatty acids for 2 days but halved after treatment for 7 days. Introduction of metformin during palmitate exposure restored insulin secretion to the control levels both after 2 and 7 days. Insulin content was reduced by 25% after 2 and 75% after 7 days' exposure to palmitate, respectively. Introduction of metformin had no effect on insulin content after 2 days' exposure to palmitate but improved the level after 7 days' exposure.

Palmitate enhances mitochondrial function [140]. In contrast, metformin has been related to mild and transient inhibition of mitochondrial respiratory chain and further reduction of ATP production [110, 111, 141, 142]. We therefore explored the role of the drug in mitochondrial metabolism in palmitate-treated human islets. Metformin inhibited ATP-coupled OCR but not proton leak OCR after 2 days' palmitate exposure suggesting that reduced generation of ATP production is a potential mechanism by which the drug attenuated GSIS. However, 7 days' exposure to palmitate with or without metformin had no effect on OCR from human islets. AMPK phosphorylation is a sensitive indicator of the cellular energy status [143]. As there was a change in ATP-related mitochondrial respiration we investigated AMPK phosphorylation. Surprisingly, addition of metformin had no effect on p-AMPK/AMPK ratio after 2 days' palmitate treatment. When human islets were cultured with palmitate for 7 days p-AMPK/AMPK was reduced by ~50% compared with the control islets. The addition of metformin in the presence of palmitate restored this ratio to the control level after 7 days. The fact that changes in insulin secretion from islet exposed to palmitate and metformin follow the changes in AMPK phosphorylation after 7 but not 2 days suggests that this mechanism

plays an important role in regulation of GSIS from human islets after prolonged exposure to the fatty acid.

Metformin has been shown to attenuate palmitate-induced ER stress and apoptosis in human islets [120]. In our study, after 2 days' exposure of human islets to palmitate, there were no changes in the expression level of pro-apoptotic markers of the ER stress response, phosphorylated eIF2α and CHOP, and apoptotic marker, cleaved caspase-3. After 7 days' palmitate treatment, the markers of ER stress and apoptosis were upregulated and normalized when metformin was included during culture. Proper activation of adaptive pathways of ER stress can protect beta-cells from palmitate-induced ER stress and apoptosis [144]. Therefore, we also measured the levels of sorcin, a Ca^{2+}-binding protein that relocates calcium from the cytoplasm to the ER and, in such way, protects cells from ER stress [92, 145]. The substantial increase of sorcin after 2-day palmitate culture of human islets reflects an adaptation against palmitate-induced ER stress. But this compensatory effect failed in a long-term period resulting in upregulation of ER stress makers. The introduction of metformin during palmitate culture delayed the development of ER stress and, as a result, the adaptive effects were also postponed. Our data indicate that the reduction in ER stress and apoptosis by metformin treatment may contribute to restoration of insulin secretion and intracellular insulin content from human islets after 7 days' culture with palmitate.

Sorcin counteracts lipotoxicity in palmitate-exposed human beta-cells (papers II and III)

Sorcin has been linked to the protective ability against ER stress (paper II). To further test the proposed explanation of rise in sorcin levels found in paper II, sorcin expression in human islets and EndoC-βH1 cells was inhibited by shRNA. Human islets and EndoC-βH1 cells were cultured in the absence and presence of palmitate for 2 days. Sorcin expression, GSIS, mitochondrial respiration, and glycolysis were induced by palmitate but ER stress and apoptosis were not affected. When sorcin was knocked down palmitate-induced upregulation of sorcin was reduced, which led to reduction in GSIS, mitochondrial respiration and glycolysis but rise in ER stress and apoptosis.

Sorcin modulates mitochondrial Ca^{2+} handling and plays a critical role in the mitochondrial antiapoptotic pathways [95]. The relationship of sorcin to mitochondrial respiration was also explored in this study and, for the first time, we showed that there is a positive association between expression level of sorcin and mitochondrial metabolism. As the mitochondria are the major site of oxidative metabolism and mitochondrial Ca^{2+} plays an important role in ATP synthesis [73, 146], we assumed that increased sorcin expression after short-term palmitate treatment may increase mitochondrial Ca^{2+}, thereby increasing mitochondrial respiration and at the same time, play an antiapoptotic

role. Sorcin also modulates ER Ca^{2+} levels with implications for cytoplasmic Ca^{2+} levels [92]. Palmitate depletes ER Ca^{2+} levels [70], which is suggested to be counteracted by upregulation of sorcin. We found that silencing sorcin in the presence of palmitate accentuates ER stress, which is consistent with other studies showing that sorcin overexpression increased ER Ca^{2+} stores from mouse and human islets [92]. Silencing of sorcin activated ER stress and apoptosis [92]. RNA sequencing study showed that there is a significant positive correlation between *sri* mRNA levels and GSIS in both diabetic and non-diabetic islets, and a tendency toward lower sorcin levels in islets from patients with T2DM versus islets obtained from healthy subjects [92, 147]. One of the interesting findings of the current study is the apparent inhibition of GSIS and insulin secreted from the culture medium after sorcin silencing in human islets and EndoC-βH1 cells regardless of presence of palmitate treatment. This is in agreement with other studies carried out in sorcin silenced MIN6 cells and overexpressed mouse and human islets, where intracellular Ca^{2+} was changed [92, 148]. The observed decrease in GSIS in our shRNA beta-cell models is most likely secondary to the decrease in glucose-induced intracellular Ca^{2+} fluxes [149].

We identified that rise in sorcin expression was necessary for rise in mitochondrial metabolism and insulin secretion and prevention of ER stress and apoptosis in palmitate-exposed human islets and human EndoC-βH1 cells. We propose that sorcin upregulation in response to palmitate increases mitochondrial respiration and protects beta-cells from ER stress, thus playing a role in palmitate potentiated GSIS.

Protective and deleterious pathways identified by transcript profiling of palmitate-exposed human islets (paper IV)

Mechanisms underlying impaired GSIS from beta-cells exposed to long-term FFAs have been intensively studied [41-43, 150]. Such impaired GSIS may explain the development of obesity-related T2DM [7, 151, 152]. To protect beta-cells from lipotoxicity and to further develop strategies to revert islet dysfunction, identifying early biological events triggered by the chronic fatty acid exposure is important. Therefore, in human islets exposed to palmitate we introduced early time points 1 hour, 4 hours and 1 day, in addition to 2 and 7 days (Paper II). Compared with control islets, GSIS was not changed after 4 hours but gradually increased reaching the maximal level after 1 day and then decreased to 70% of control level after 7 days. Intracellular insulin content was not changed up to 1 day's culture with palmitate but gradually decreased to approximately 75 and 30% of control level after 2 and 7 days, respectively.

To gain insights into biological events potentially underlying this development in GSIS genome-wide analysis of gene expression by Affymetrix HTA2.0 in isolated human islets exposed to fatty acid palmitate for the different time periods was performed. DEGs were defined as change in gene expression more than 1.3-fold after palmitate treatment compared with control human islets. We found 903 unique genes to be differentially expressed in at least one of the culture time points. All DEGs were mapped onto KEGG pathways and enrichment analysis was performed yielding 15 significantly enriched pathways related to human islet biology in at least one culture time point.

Four pathways were enriched prior to the decline of islet function defined. All the metallothioneins (MTs) in the "Mineral absorption" pathway (enriched at 4 hours) were elevated in the presence of palmitate after 4 hours' exposure. MTs regulate the intracellular level of free zinc, which is essential for the fundamental beta-cell functions including insulin biosynthesis and insulin storage [153, 154]. Dysregulation or dysfunction of zinc-transporting proteins *in vitro* [155] or polymorphisms in MT encoding genes *in vivo* [156] are related to impaired insulin processing and glucose metabolism. Overexpression or exogenous introduction of MT preserved insulin production from beta-cells [157, 158]. The "Aminoacyl-tRNA biosynthesis" pathway (enriched at 12 hours) was predicted based on 6 tRNA synthetases elevated after 12 hours of palmitate exposure. Protein synthesis occurs in the cytosol and requires tRNAs loaded with the appropriate amino acid [159]. Presumably, increased levels of tRNA synthetases enhance insulin biosynthesis, which allows to maintain insulin hypersecretion from palmitate-treated islets at this time point. Thus, an initial transient increase in levels of MTs transcript and tRNA synthetases in palmitate-treated human islets could be an adaptive mechanism to support insulin synthesis during insulin hypersecretion. The "PPAR signaling pathway" (enriched at 4 hours and 1 day) and the "Adipocytokine signaling pathway" (enriched at 4 hours and 1 day) included elevated gene transcripts with a function in fatty acid and glucose metabolism. The increased metabolism and beta-oxidation of fatty acids are beneficial for beta-cells [160, 161]. The PPAR1 pathway plays a protective role in palmitate-treated beta-cells [162]. Therefore, activation of these pathways may be an adaptive response to fatty acid exposure.

The "TNF signalling pathway" was predicted based on 8 genes elevated only after 1 and 2 days' exposure to palmitate. Most transcripts in the "TNF signalling" pathway encode chemokines, which can be synthesized and secreted by pancreatic beta-cells to recruit leukocytes into pancreatic tissues [163]. Chemokines play a role in inflammatory responses and impair beta-cell function [164]. Some chemokines promote beta-cell health, either by recruiting specific immune cells or through direct effects on beta-cells, however [165]. Furthermore, chemokines can also trigger pathway signaling by interacting with receptors expressed on human islets [164, 166]. Activation of the

"TNF signaling pathway" may contribute to insulin hypersecretion at these earlier time points.

Three pathways were enriched both before (at time points 1 day and 2 days) and after (at time point 7 days) the decline of islet function. The remaining seven pathways were enriched only after 2 and/or 7 days of palmitate exposure. The "Metabolism of xenobiotics by P450" pathway was the top pathway after 1 and 2 days and the second top pathway after 7 days of palmitate exposure. Decreased expression was observed of genes encoding the detoxification enzymes that play a role in metabolism of fatty acids via omega-oxidation, a process almost identical to xenobiotic detoxification [167, 168]. Dicarboxylic acid, which are converted from FFAs in the ER during omega-oxidation [168], enters mitochondria or peroxisomes for further metabolism via beta-oxidation [169]. Omega-oxidation, therefore, is regarded as a rescue pathway that allows to eliminate toxic levels of fatty acids that accumulate in the cells when the main beta-oxidation pathway is overwhelmed [170]. The inhibition of the "Metabolism of xenobiotics by P450" pathway in human islets upon prolonged palmitate exposure may therefore be an early deleterious event, causing accumulation of toxic amounts of fatty acids which contributes to islet beta-cell failure.

These results propose that in palmitate-treated human islets, at early time points, protective events, including upregulation of metallothioneins, tRNA synthetases and fatty acid-metabolizing proteins, dominate over deleterious events, including inhibition of fatty acid detoxification enzymes, which contribute to enhanced GSIS. After prolonged exposure of islets to palmitate, the protective events are outweighed by the deleterious events, which contribute to impaired GSIS.

Summary and Conclusions

1. Long-chain FFAs acutely induced insulin secretion from human pancreatic islets at physiologically fasting blood glucose level, with MUFAs being more potent than SFAs, and this effect is associated with increased glycolytic flux and mitochondrial respiration.

2. Metformin prevents early insulin hypersecretion from chronically palmitate-treated human islets by decreasing mitochondrial metabolism and improves later decrease in insulin secretion by reducing ER stress and apoptosis.

3. Sorcin counteracts lipotoxicity in beta-cells exposed to palmitate involving insulin secretion, ER stress and mitochondrial respiration. Overexpression or activation of sorcin may be beneficial for beta-cells under metabolic stress.

4. In human islets treated with palmitate, protective events dominate over deleterious events at early time points which contributes to potentiation of GSIS whereas prolonged exposure impaired GSIS when protective events are outweighed by the deleterious events.

In conclusion, isolated beta-cells respond to elevated levels of FFAs by initially augmenting insulin release followed by declining secretory levels after prolonged exposure. Metformin normalizes these secretory aberrations. In the islet beta-cell changes in specific signaling pathways and proteins including sorcin contribute to these secretory manifestations. We propose the elevated FFA levels in obese children may lead to insulin hypersecretion early in life and precipitate secretory decline and T2DM later in life. When developing strategies for prevention of T2DM in obese children with elevated FFA levels, metformin should be considered as well as novel strategies involving sorcin and the identified specific pathways.

Acknowledgements

This thesis work was carried out at the Department of Medical Cell Biology, Uppsala University, Sweden. Many people have contributed to this thesis and to the work behind it in many different ways. I would therefore like to take the opportunity to express my sincere gratitude to all of you who in any way guided and supported me throughout this thesis work.

I would like to first thank my supervisor Professor **Peter Bergsten** for providing me this great opportunity to do my PhD study in your research group. I am thankful you for taking me as a project student in the beginning when I was in China looking for an opportunity to study abroad. Thank you for always giving me a lot of support, patience, encouragement, and positive attitude during all my study period, keeping inspiring me to different research projects and for allowing me to progress in many different aspects of research and personal issues.

My co-supervisor Associate Professor **Anders Forslund**, for your advice and support on research. Your enthusiasm and warm smiles give me a lot of positive energy.

Thanks to all the current and former members of the **Bergsten research group** during my time. **Ernest Sargsyan** for sharing the office with me, and for your guidance, collaborations and many interesting discussions on scientific and non-scientific topics. To **Hjalti Kristinsson** for all the kind advice and suggestions about research and for sharing with me a lot of experience as being a good parent. **Hannes Manell** for all the nice time we spent together and a lot of positive views, especially for always encouraging me keeping on learning Swedish. **Levon Manukyan** for all the technical helps in the lab work and nice time we spent together. **Johan Staaf** for all the help and good times. I still remember clearly when I was Skype interviewed by Peter and you before I came to Sweden and joined this group. **Azazul Chowdhury** for all your help and good times when we had a lot of scientific and non-scientific discussions. **Anna Drzazga** for good times and the invitation to your defense in Poland even though I cannot make it in the end. **Ismael Rojas Vazquez** for good times. And to all the students doing projects in our group, especially **Rasmus Stenlid**, **Dalal Saaid** and **Lina Juzokaite**.

Thanks to the staff at **Department of Women's and Children's Health, Uppsala University** and especially **Marie Dahlbom**, **Iris Ciba** and **Malte Lindström** for the collaboration and some interesting discussions.

Thanks to all the colleagues and friends in the **Beta-Judo consortium**, for wonderful collaborations and discussions. I was really enjoying the time during the annual face-to-face meetings with all of you in different cities.

Thanks to all the staff and students at the **Department of Medical Cell Biology, Uppsala University.** Special thanks to the current and former head of department Professor **Nils Welsh** and Professor **Erik Gylfe**.

Thanks to all the senior professors and group leaders during my time especially: **Per-Ola Carlsson**, **Gunilla Westermark**, **Anders Tengholm**, **Michael Welsh**, **Ulf Eriksson**, **Olof Idevall**, **Leif Jansson**, and **Lena Holm**.

Thanks to: **Lina Thorvaldsson, Björn Åkerblom, Martin Blixt, Faranak Azarbayjani, Erik Sandin, Britt Högberg, Shumin Pan, Carl Lundström, Camilla Sävmarker, Antoine Giraud, Monica Sandberg,** and **Göran Ståhl**.

To all the members of Friday Fika Club and to many fellow PhD students during my time, especially to **Nikhil, Kailash, Parham, Ida, Sofia, Marie, Jalal, Carmen, Mediha, Nikos, Natasa, My, Nadine, Gonzalo, Antije, Paul, Eva, Parvin, and Oleg.**

And thanks to all my former and current Chinese friends in the department: **Geng Tian, Jia Li, Xuan Wang, Qian Yu, Xiaohong Gu, Chenxiao Liu, Hongyan Shuai, Ye Wang, Yun Wang, Beichen Xie, Yunjian Xu, Mingyu Yang,** and **Xin Yu**.

Thanks to my former fellow students, teachers and professors from the years I spent in **Medical College of Xiamen University** and to all the doctors at **Zhongshan Hospital, Xiamen University** where I had my internship training. Especially to Professor **Xiangjiu Yang**, **Zhengjie Huang**, and **Yiqun Wu** for the support, guidance, and encouragement.

Thanks to all my former friends, doctors and professors at **Peking Union Medical College Hospital**, especially to my Master supervisor, Professor **Feng Gu**, for the support and encouragement. To Professor **Zimeng Jin**, **Naishi Li**, **Lian Duan**, and **Lin Lu** for all the help in my learning and training during the clinical work, Associate Professor **Min Nie** for the guidance in the research and nice collaborations. To all of my friends for nice time both during work and some spare time we spent together, especially to **Lu Xu, Yufei Dai, Rui Min, Lanwen Han, Zengyi Li, Xiao Zhao, Dan Tian,** and **Xiangqing Wang.**

Many thanks to **my parents** who always support and encourage me whenever I need help or feel like giving up. Especially to **my father**, the most important mentor in my life not only showing me the value of determination and hard work, the importance of persistence, always being prepared for the unknown future, but also encouraging me to pursue my dream of becoming a doctor and doing biomedical research abroad. To **my husband** who inspiring me looking for a PhD position abroad. Last but not least, I would like to thank my little boy **Alex (应有为)** for always reminding me the beauty of life.

The studies were supported by grants from European Commission FP7 Project Beta-JUDO, Swedish Diabetes Association, and Family Ernfors Foundation.

References

1. Singer, P., et al., Postprandial hyperinsulinemia in patients with mild essential hypertension. Hypertension, 1985. **7**(2): p. 182-6.
2. Balasse, E.O. and H.A. Ooms, Role of plasma free fatty acids in the control of insulin secretion in man. Diabetologia, 1973. **9**(2): p. 145-51.
3. Boden, G., X. Chen, and N. Iqbal, Acute lowering of plasma fatty acids lowers basal insulin secretion in diabetic and nondiabetic subjects. Diabetes, 1998. **47**(10): p. 1609-12.
4. Charles, M.A., et al., The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study. Diabetologia, 1997. **40**(9): p. 1101-6.
5. Fraze, E., et al., Ambient plasma free fatty acid concentrations in noninsulin-dependent diabetes mellitus: evidence for insulin resistance. J Clin Endocrinol Metab, 1985. **61**(5): p. 807-11.
6. Stefan, N., et al., Elevated plasma nonesterified fatty acids are associated with deterioration of acute insulin response in IGT but not NGT. Am J Physiol Endocrinol Metab, 2003. **284**(6): p. E1156-61.
7. Staaf, J., et al., Initial hyperinsulinemia and subsequent beta-cell dysfunction is associated with elevated palmitate levels. Pediatr Res, 2016. **80**(2): p. 267-74.
8. Karpe, F., J.R. Dickmann, and K.N. Frayn, Fatty acids, obesity, and insulin resistance: time for a reevaluation. Diabetes, 2011. **60**(10): p. 2441-9.
9. Hayashi, T., et al., Patterns of insulin concentration during the OGTT predict the risk of type 2 diabetes in Japanese Americans. Diabetes Care, 2013. **36**(5): p. 1229-35.
10. Kristinsson, H., et al., FFAR1 is involved in both the acute and chronic effects of palmitate on insulin secretion. Endocrinology, 2013. **154**(11): p. 4078-88.
11. Page, M.M. and J.D. Johnson, Mild Suppression of Hyperinsulinemia to Treat Obesity and Insulin Resistance. Trends Endocrinol Metab, 2018. **29**(6): p. 389-399.
12. Szabat, M., et al., Reduced Insulin Production Relieves Endoplasmic Reticulum Stress and Induces beta Cell Proliferation. Cell Metab, 2016. **23**(1): p. 179-93.
13. Bray, G.A., et al., Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation. Obes Rev, 2017. **18**(7): p. 715-723.
14. Kumar, S. and A.S. Kelly, Review of Childhood Obesity: From Epidemiology, Etiology, and Comorbidities to Clinical Assessment and Treatment. Mayo Clin Proc, 2017. **92**(2): p. 251-265.

15. Csabi, G., et al., Presence of metabolic cardiovascular syndrome in obese children. Eur J Pediatr, 2000. **159**(1-2): p. 91-4.
16. Reilly, J.J. and J. Kelly, Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. Int J Obes (Lond), 2011. **35**(7): p. 891-8.
17. Weiss, R., et al., Obesity and the metabolic syndrome in children and adolescents. N Engl J Med, 2004. **350**(23): p. 2362-74.
18. Halberg, N., I. Wernstedt-Asterholm, and P.E. Scherer, The adipocyte as an endocrine cell. Endocrinol Metab Clin North Am, 2008. **37**(3): p. 753-68, x-xi.
19. Bray, G.A., From farm to fat cell: why aren't we all fat? Metabolism, 2015. **64**(3): p. 349-53.
20. Stefan, N., F. Schick, and H.U. Haring, Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. N Engl J Med, 2014. **371**(23): p. 2236-7.
21. Chatterjee, S., K. Khunti, and M.J. Davies, Type 2 diabetes. Lancet, 2017. **389**(10085): p. 2239-2251.
22. Alberti, K.G. and P.Z. Zimmet, Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med, 1998. **15**(7): p. 539-53.
23. Eckel, R.H., et al., Obesity and type 2 diabetes: what can be unified and what needs to be individualized? J Clin Endocrinol Metab, 2011. **96**(6): p. 1654-63.
24. Copeland, K.C., et al., Characteristics of adolescents and youth with recent-onset type 2 diabetes: the TODAY cohort at baseline. J Clin Endocrinol Metab, 2011. **96**(1): p. 159-67.
25. Nadeau, K.J., et al., Youth-Onset Type 2 Diabetes Consensus Report: Current Status, Challenges, and Priorities. Diabetes Care, 2016. **39**(9): p. 1635-42.
26. In't Veld, P. and M. Marichal, Microscopic anatomy of the human islet of Langerhans. Adv Exp Med Biol, 2010. **654**: p. 1-19.
27. Cabrera, O., et al., The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. Proc Natl Acad Sci U S A, 2006. **103**(7): p. 2334-9.
28. de Koning, E.J., et al., Macrophages and pancreatic islet amyloidosis. Amyloid, 1998. **5**(4): p. 247-54.
29. Hennes, M.M., A. Dua, and A.H. Kissebah, Effects of free fatty acids and glucose on splanchnic insulin dynamics. Diabetes, 1997. **46**(1): p. 57-62.
30. Rorsman, P. and M. Braun, Regulation of insulin secretion in human pancreatic islets. Annu Rev Physiol, 2013. **75**: p. 155-79.
31. Rebelos, E., et al., Influence of endogenous NEFA on beta cell function in humans. Diabetologia, 2015. **58**(10): p. 2344-51.
32. Owen, O.E., et al., Energy metabolism in feasting and fasting. Adv Exp Med Biol, 1979. **111**: p. 169-88.
33. McGarry, J.D., M.E. Boyd, and D.W. Foster, Regulation of hepatic fatty acid oxidation in the rat during feeding and starvation. Biochem Soc Trans, 1981. **9**(4): p. 341.

34. Sabin, M.A., et al., Fasting nonesterified fatty acid profiles in childhood and their relationship with adiposity, insulin sensitivity, and lipid levels. Pediatrics, 2007. **120**(6): p. e1426-33.
35. Rise, P., et al., Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. Prostaglandins Leukot Essent Fatty Acids, 2007. **76**(6): p. 363-9.
36. Stein, D.T., et al., Essentiality of circulating fatty acids for glucose-stimulated insulin secretion in the fasted rat. J Clin Invest, 1996. **97**(12): p. 2728-35.
37. Warnotte, C., et al., Mechanisms of the stimulation of insulin release by saturated fatty acids. A study of palmitate effects in mouse beta-cells. Diabetes, 1994. **43**(5): p. 703-11.
38. Stein, D.T., et al., The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. J Clin Invest, 1997. **100**(2): p. 398-403.
39. Opara, E.C., et al., Effect of fatty acids on insulin release: role of chain length and degree of unsaturation. Am J Physiol, 1994. **266**(4 Pt 1): p. E635-9.
40. Gravena, C., P.C. Mathias, and S.J. Ashcroft, Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. J Endocrinol, 2002. **173**(1): p. 73-80.
41. Zhou, Y.P. and V.E. Grill, Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest, 1994. **93**(2): p. 870-6.
42. Zhou, Y.P. and V. Grill, Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. J Clin Endocrinol Metab, 1995. **80**(5): p. 1584-90.
43. Shimabukuro, M., et al., Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. Proc Natl Acad Sci U S A, 1998. **95**(5): p. 2498-502.
44. Carpentier, A., et al., Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. Diabetes, 2000. **49**(3): p. 399-408.
45. Paolisso, G., et al., Opposite effects of short- and long-term fatty acid infusion on insulin secretion in healthy subjects. Diabetologia, 1995. **38**(11): p. 1295-9.
46. Carpentier, A., et al., Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. Am J Physiol, 1999. **276**(6 Pt 1): p. E1055-66.
47. Kashyap, S., et al., A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes, 2003. **52**(10): p. 2461-74.
48. Malaisse, W.J., et al., Participation of endogenous fatty acids in the secretory activity of the pancreatic B-cell. Biochem J, 1985. **227**(3): p. 995-1002.
49. Hamilton, J.A. and F. Kamp, How are free fatty acids transported in membranes? Is it by proteins or by free diffusion through the lipids? Diabetes, 1999. **48**(12): p. 2255-69.

50. Briscoe, C.P., et al., The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem, 2003. **278**(13): p. 11303-11.
51. Itoh, Y., et al., Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature, 2003. **422**(6928): p. 173-6.
52. Berne, C., The metabolism of lipids in mouse pancreatic islets. The biosynthesis of triacylglycerols and phospholipids. Biochem J, 1975. **152**(3): p. 667-73.
53. McGarry, J.D. and D.W. Foster, Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem, 1980. **49**: p. 395-420.
54. Prentki, M., New insights into pancreatic beta-cell metabolic signaling in insulin secretion. Eur J Endocrinol, 1996. **134**(3): p. 272-86.
55. Ashcroft, F.M. and P. Rorsman, ATP-sensitive K+ channels: a link between B-cell metabolism and insulin secretion. Biochem Soc Trans, 1990. **18**(1): p. 109-11.
56. Larsson, O., et al., Activation of the ATP-sensitive K+ channel by long chain acyl-CoA. A role in modulation of pancreatic beta-cell glucose sensitivity. J Biol Chem, 1996. **271**(18): p. 10623-6.
57. Mancini, A.D. and V. Poitout, The fatty acid receptor FFA1/GPR40 a decade later: how much do we know? Trends Endocrinol Metab, 2013. **24**(8): p. 398-407.
58. Seino, S., T. Shibasaki, and K. Minami, Dynamics of insulin secretion and the clinical implications for obesity and diabetes. J Clin Invest, 2011. **121**(6): p. 2118-25.
59. Poitout, V., et al., Regulation of the insulin gene by glucose and fatty acids. J Nutr, 2006. **136**(4): p. 873-6.
60. Bjorklund, A. and V. Grill, Enhancing effects of long-term elevated glucose and palmitate on stored and secreted proinsulin-to-insulin ratios in human pancreatic islets. Diabetes, 1999. **48**(7): p. 1409-14.
61. Carlsson, C., L.A. Borg, and N. Welsh, Sodium palmitate induces partial mitochondrial uncoupling and reactive oxygen species in rat pancreatic islets in vitro. Endocrinology, 1999. **140**(8): p. 3422-8.
62. Hoppa, M.B., et al., Chronic Palmitate Exposure Inhibits Insulin Secretion by Dissociation of Ca(2+) Channels from Secretory Granules. Cell Metab, 2011. **13**(4): p. 487.
63. Bollheimer, L.C., et al., Intracellular depletion of insulin: a comparative study with palmitate, oleate and elaidate in INS-1 cells. Eur J Endocrinol, 2003. **148**(4): p. 481-6.
64. Boden, G., et al., Mechanisms of fatty acid-induced inhibition of glucose uptake. J Clin Invest, 1994. **93**(6): p. 2438-46.
65. Lupi, R., et al., Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes, 2002. **51**(5): p. 1437-42.
66. Harding, H.P. and D. Ron, Endoplasmic reticulum stress and the development of diabetes: a review. Diabetes, 2002. **51 Suppl 3**: p. S455-61.

67. Oyadomari, S., E. Araki, and M. Mori, Endoplasmic reticulum stress-mediated apoptosis in pancreatic beta-cells. Apoptosis, 2002. **7**(4): p. 335-45.
68. Cnop, M., et al., Endoplasmic reticulum stress and eIF2alpha phosphorylation: The Achilles heel of pancreatic beta cells. Mol Metab, 2017. **6**(9): p. 1024-1039.
69. Eizirik, D.L., A.K. Cardozo, and M. Cnop, The role for endoplasmic reticulum stress in diabetes mellitus. Endocr Rev, 2008. **29**(1): p. 42-61.
70. Karaskov, E., et al., Chronic palmitate but not oleate exposure induces endoplasmic reticulum stress, which may contribute to INS-1 pancreatic beta-cell apoptosis. Endocrinology, 2006. **147**(7): p. 3398-407.
71. El-Assaad, W., et al., Saturated fatty acids synergize with elevated glucose to cause pancreatic beta-cell death. Endocrinology, 2003. **144**(9): p. 4154-63.
72. Maechler, P., et al., Role of mitochondria in beta-cell function and dysfunction. Adv Exp Med Biol, 2010. **654**: p. 193-216.
73. Tarasov, A.I., E.J. Griffiths, and G.A. Rutter, Regulation of ATP production by mitochondrial Ca(2+). Cell Calcium, 2012. **52**(1): p. 28-35.
74. Prentki, M., F.M. Matschinsky, and S.R. Madiraju, Metabolic signaling in fuel-induced insulin secretion. Cell Metab, 2013. **18**(2): p. 162-85.
75. Erecinska, M., et al., Energy metabolism in islets of Langerhans. Biochim Biophys Acta, 1992. **1101**(3): p. 273-95.
76. Ortsater, H., et al., Contribution of glycolytic and mitochondrial pathways in glucose-induced changes in islet respiration and insulin secretion. Pflugers Arch, 2002. **444**(4): p. 506-12.
77. Kennedy, E.D., P. Maechler, and C.B. Wollheim, Effects of depletion of mitochondrial DNA in metabolism secretion coupling in INS-1 cells. Diabetes, 1998. **47**(3): p. 374-80.
78. Maassen, J.A., L.M. t Hart, and D.M. Ouwens, Lessons that can be learned from patients with diabetogenic mutations in mitochondrial DNA: implications for common type 2 diabetes. Curr Opin Clin Nutr Metab Care, 2007. **10**(6): p. 693-7.
79. Prentki, M. and C.J. Nolan, Islet beta cell failure in type 2 diabetes. J Clin Invest, 2006. **116**(7): p. 1802-12.
80. Tiedge, M., et al., Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes, 1997. **46**(11): p. 1733-42.
81. Olsson, A.H., et al., Decreased expression of genes involved in oxidative phosphorylation in human pancreatic islets from patients with type 2 diabetes. Eur J Endocrinol, 2011. **165**(4): p. 589-95.
82. Anello, M., et al., Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. Diabetologia, 2005. **48**(2): p. 282-9.
83. MacDonald, M.J., et al., Decreased levels of metabolic enzymes in pancreatic islets of patients with type 2 diabetes. Diabetologia, 2009. **52**(6): p. 1087-91.

84. Schwarz, D.S. and M.D. Blower, The endoplasmic reticulum: structure, function and response to cellular signaling. Cell Mol Life Sci, 2016. **73**(1): p. 79-94.
85. Scheuner, D. and R.J. Kaufman, The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. Endocr Rev, 2008. **29**(3): p. 317-33.
86. Cunha, D.A., et al., Initiation and execution of lipotoxic ER stress in pancreatic beta-cells. J Cell Sci, 2008. **121**(Pt 14): p. 2308-18.
87. Sargsyan, E., et al., Diazoxide-induced beta-cell rest reduces endoplasmic reticulum stress in lipotoxic beta-cells. J Endocrinol, 2008. **199**(1): p. 41-50.
88. Van der Bliek, A.M., et al., A 22-kd protein (sorcin/V19) encoded by an amplified gene in multidrug-resistant cells, is homologous to the calcium-binding light chain of calpain. EMBO J, 1986. **5**(12): p. 3201-8.
89. Meyers, M.B. and J.L. Biedler, Increased synthesis of a low molecular weight protein in vincristine-resistant cells. Biochem Biophys Res Commun, 1981. **99**(1): p. 228-35.
90. Meyers, M.B., et al., Gene amplification-associated cytogenetic aberrations and protein changes in vincristine-resistant Chinese hamster, mouse, and human cells. J Cell Biol, 1985. **100**(2): p. 588-97.
91. Meyers, M.B., et al., Association of sorcin with the cardiac ryanodine receptor. J Biol Chem, 1995. **270**(44): p. 26411-8.
92. Marmugi, A., et al., Sorcin Links Pancreatic beta-Cell Lipotoxicity to ER Ca2+ Stores. Diabetes, 2016. **65**(4): p. 1009-21.
93. Pickel, V.M., C.L. Clarke, and M.B. Meyers, Ultrastructural localization of sorcin, a 22 kDa calcium binding protein, in the rat caudate-putamen nucleus: association with ryanodine receptors and intracellular calcium release. J Comp Neurol, 1997. **386**(4): p. 625-34.
94. Meyers, M.B., et al., Sorcin associates with the pore-forming subunit of voltage-dependent L-type Ca2+ channels. J Biol Chem, 1998. **273**(30): p. 18930-5.
95. Suarez, J., et al., Sorcin modulates mitochondrial Ca(2+) handling and reduces apoptosis in neonatal rat cardiac myocytes. Am J Physiol Cell Physiol, 2013. **304**(3): p. C248-56.
96. Bailey, C.J., Metformin: historical overview. Diabetologia, 2017. **60**(9): p. 1566-1576.
97. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. Lancet, 1998. **352**(9131): p. 854-65.
98. Sanchez-Rangel, E. and S.E. Inzucchi, Metformin: clinical use in type 2 diabetes. Diabetologia, 2017. **60**(9): p. 1586-1593.
99. Holman, R.R., et al., 10-year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med, 2008. **359**(15): p. 1577-89.
100. Wulffele, M.G., et al., The effect of metformin on blood pressure, plasma cholesterol and triglycerides in type 2 diabetes mellitus: a systematic review. J Intern Med, 2004. **256**(1): p. 1-14.
101. Carlsen, S.M., et al., Metformin improves blood lipid pattern in nondiabetic patients with coronary heart disease. J Intern Med, 1996. **239**(3): p. 227-33.

102. Herman, W.H., et al., The cost-effectiveness of lifestyle modification or metformin in preventing type 2 diabetes in adults with impaired glucose tolerance. Ann Intern Med, 2005. **142**(5): p. 323-32.
103. American Diabetes, A., D. National Institute of Diabetes, and D. Kidney, The prevention or delay of type 2 diabetes. Caring, 2005. **24**(6): p. 52-64.
104. Gandini, S., et al., Metformin and cancer risk and mortality: a systematic review and meta-analysis taking into account biases and confounders. Cancer Prev Res (Phila), 2014. **7**(9): p. 867-85.
105. Kostev, K. and H. Richter, Unlicensed use of metformin in children and adolescents in Germany and France. Br J Clin Pharmacol, 2012. **73**(2): p. 307-8.
106. Hsia, Y., et al., Unlicensed use of metformin in children and adolescents in the UK. Br J Clin Pharmacol, 2012. **73**(1): p. 135-9.
107. Kendall, D.L., R. Amin, and P.E. Clayton, Metformin in the treatment of obese children and adolescents at risk of type 2 diabetes. Paediatr Drugs, 2014. **16**(1): p. 13-20.
108. Kendall, D., et al., Metformin in obese children and adolescents: the MOCA trial. J Clin Endocrinol Metab, 2013. **98**(1): p. 322-9.
109. Foretz, M., et al., Metformin: from mechanisms of action to therapies. Cell Metab, 2014. **20**(6): p. 953-66.
110. Rena, G., D.G. Hardie, and E.R. Pearson, The mechanisms of action of metformin. Diabetologia, 2017. **60**(9): p. 1577-1585.
111. Musi, N., et al., Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. Diabetes, 2002. **51**(7): p. 2074-81.
112. Leone, A., et al., New perspective for an old antidiabetic drug: metformin as anticancer agent. Cancer Treat Res, 2014. **159**: p. 355-76.
113. Cao, J., et al., Low concentrations of metformin suppress glucose production in hepatocytes through AMP-activated protein kinase (AMPK). J Biol Chem, 2014. **289**(30): p. 20435-46.
114. Miller, R.A., et al., Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature, 2013. **494**(7436): p. 256-60.
115. Koffert, J.P., et al., Metformin treatment significantly enhances intestinal glucose uptake in patients with type 2 diabetes: Results from a randomized clinical trial. Diabetes Res Clin Pract, 2017. **131**: p. 208-216.
116. Mannucci, E., et al., Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. Diabetes Care, 2001. **24**(3): p. 489-94.
117. Forslund, K., et al., Corrigendum: Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature, 2017. **545**(7652): p. 116.
118. Forslund, K., et al., Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature, 2015. **528**(7581): p. 262-266.
119. Patane, G., et al., Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes, 2000. **49**(5): p. 735-40.

120. Lupi, R., et al., Lipotoxicity in human pancreatic islets and the protective effect of metformin. Diabetes, 2002. **51 Suppl 1**: p. S134-7.
121. Simon-Szabo, L., et al., Metformin attenuates palmitate-induced endoplasmic reticulum stress, serine phosphorylation of IRS-1 and apoptosis in rat insulinoma cells. PLoS One, 2014. **9**(6): p. e97868.
122. Moon, J.S., et al., Metformin prevents glucotoxicity by alleviating oxidative and ER stress-induced CD36 expression in pancreatic beta cells. J Diabetes Complications, 2017. **31**(1): p. 21-30.
123. Ravassard, P., et al., A genetically engineered human pancreatic beta cell line exhibiting glucose-inducible insulin secretion. J Clin Invest, 2011. **121**(9): p. 3589-97.
124. Andersson, L.E., et al., Characterization of stimulus-secretion coupling in the human pancreatic EndoC-betaH1 beta cell line. PLoS One, 2015. **10**(3): p. e0120879.
125. Tsonkova, V.G., et al., The EndoC-betaH1 cell line is a valid model of human beta cells and applicable for screenings to identify novel drug target candidates. Mol Metab, 2018. **8**: p. 144-157.
126. Thorn, K. and P. Bergsten, Fatty acid-induced oxidation and triglyceride formation is higher in insulin-producing MIN6 cells exposed to oleate compared to palmitate. J Cell Biochem, 2010. **111**(2): p. 497-507.
127. Bergsten, P. and B. Hellman, Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. Diabetes, 1993. **42**(5): p. 670-4.
128. Wikstrom, J.D., et al., A novel high-throughput assay for islet respiration reveals uncoupling of rodent and human islets. PLoS One, 2012. **7**(5): p. e33023.
129. Brand, M.D. and D.G. Nicholls, Assessing mitochondrial dysfunction in cells. Biochem J, 2011. **435**(2): p. 297-312.
130. Nazarov, P.V., et al., RNA sequencing and transcriptome arrays analyses show opposing results for alternative splicing in patient derived samples. BMC Genomics, 2017. **18**(1): p. 443.
131. Li, C. and W. Hung Wong, Model-based analysis of oligonucleotide arrays: model validation, design issues and standard error application. Genome Biol, 2001. **2**(8): p. RESEARCH0032.
132. Irizarry, R.A., et al., Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics, 2003. **4**(2): p. 249-64.
133. Kamburov, A., et al., The ConsensusPathDB interaction database: 2013 update. Nucleic Acids Res, 2013. **41**(Database issue): p. D793-800.
134. Kamburov, A., et al., ConsensusPathDB--a database for integrating human functional interaction networks. Nucleic Acids Res, 2009. **37**(Database issue): p. D623-8.
135. Warnotte, C., M. Nenquin, and J.C. Henquin, Unbound rather than total concentration and saturation rather than unsaturation determine the potency of fatty acids on insulin secretion. Mol Cell Endocrinol, 1999. **153**(1-2): p. 147-53.
136. Littman, E.D., et al., Role of protein kinase C isoenzymes in fatty acid stimulation of insulin secretion. Pancreas, 2000. **20**(3): p. 256-63.

137. Maechler, P., Mitochondrial function and insulin secretion. Mol Cell Endocrinol, 2013. **379**(1-2): p. 12-8.
138. El-Azzouny, M., et al., Increased glucose metabolism and glycerolipid formation by fatty acids and GPR40 receptor signaling underlies the fatty acid potentiation of insulin secretion. J Biol Chem, 2014. **289**(19): p. 13575-88.
139. Nolan, C.J., et al., Fatty acid signaling in the beta-cell and insulin secretion. Diabetes, 2006. **55 Suppl 2**: p. S16-23.
140. Kristinsson, H., P. Bergsten, and E. Sargsyan, Free fatty acid receptor 1 (FFAR1/GPR40) signaling affects insulin secretion by enhancing mitochondrial respiration during palmitate exposure. Biochim Biophys Acta, 2015. **1853**(12): p. 3248-57.
141. Viollet, B., et al., Cellular and molecular mechanisms of metformin: an overview. Clin Sci (Lond), 2012. **122**(6): p. 253-70.
142. Zhou, G., et al., Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest, 2001. **108**(8): p. 1167-74.
143. Hardie, D.G. and K. Sakamoto, AMPK: a key sensor of fuel and energy status in skeletal muscle. Physiology (Bethesda), 2006. **21**: p. 48-60.
144. Sargsyan, E., et al., Oleate protects beta-cells from the toxic effect of palmitate by activating pro-survival pathways of the ER stress response. Biochim Biophys Acta, 2016. **1861**(9 Pt A): p. 1151-1160.
145. Maki, M., et al., Structures, functions and molecular evolution of the penta-EF-hand Ca2+-binding proteins. Biochim Biophys Acta, 2002. **1600**(1-2): p. 51-60.
146. Wiederkehr, A. and C.B. Wollheim, Impact of mitochondrial calcium on the coupling of metabolism to insulin secretion in the pancreatic beta-cell. Cell Calcium, 2008. **44**(1): p. 64-76.
147. Solimena, M., et al., Systems biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. Diabetologia, 2018. **61**(3): p. 641-657.
148. Noordeen, N.A., et al., Glucose-induced nuclear shuttling of ChREBP is mediated by sorcin and Ca(2+) ions in pancreatic beta-cells. Diabetes, 2012. **61**(3): p. 574-85.
149. Sakuma, N., et al., Glucose induces calcium-dependent and calcium-independent insulin secretion from the pancreatic beta cell line MIN6. Eur J Endocrinol, 1995. **133**(2): p. 227-34.
150. Lee, S.H., et al., High-throughput screening and bioinformatic analysis to ascertain compounds that prevent saturated fatty acid-induced beta-cell apoptosis. Biochem Pharmacol, 2017. **138**: p. 140-149.
151. Weyer, C., et al., A high fasting plasma insulin concentration predicts type 2 diabetes independent of insulin resistance: evidence for a pathogenic role of relative hyperinsulinemia. Diabetes, 2000. **49**(12): p. 2094-101.
152. Le Stunff, C. and P. Bougneres, Early changes in postprandial insulin secretion, not in insulin sensitivity, characterize juvenile obesity. Diabetes, 1994. **43**(5): p. 696-702.
153. Li, Y.V., Zinc and insulin in pancreatic beta-cells. Endocrine, 2014. **45**(2): p. 178-89.

154. Howell, S.L., et al., Role of zinc and calcium in the formation and storage of insulin in the pancreatic beta-cell. Cell Tissue Res, 1978. **188**(1): p. 107-18.
155. Myers, S.A., Zinc transporters and zinc signaling: new insights into their role in type 2 diabetes. Int J Endocrinol, 2015. **2015**: p. 167503.
156. Yang, L., et al., Polymorphisms in metallothionein-1 and -2 genes associated with the risk of type 2 diabetes mellitus and its complications. Am J Physiol Endocrinol Metab, 2008. **294**(5): p. E987-92.
157. Chen, H., et al., Overexpression of metallothionein in pancreatic beta-cells reduces streptozotocin-induced DNA damage and diabetes. Diabetes, 2001. **50**(9): p. 2040-6.
158. Nygaard, S.B., et al., Exogenous metallothionein potentiates the insulin response at normal glucose concentrations in INS-1E beta-cells without disturbing intracellular ZnT8 expression. Basic Clin Pharmacol Toxicol, 2015. **116**(2): p. 173-7.
159. Park, S.G., P. Schimmel, and S. Kim, Aminoacyl tRNA synthetases and their connections to disease. Proc Natl Acad Sci U S A, 2008. **105**(32): p. 11043-9.
160. Cnop, M., et al., Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. Diabetes, 2001. **50**(8): p. 1771-7.
161. Sol, E.M., et al., Glucolipotoxicity in INS-1E cells is counteracted by carnitine palmitoyltransferase 1 over-expression. Biochem Biophys Res Commun, 2008. **375**(4): p. 517-21.
162. Hellemans, K., et al., Peroxisome proliferator-activated receptor alpha-retinoid X receptor agonists induce beta-cell protection against palmitate toxicity. FEBS J, 2007. **274**(23): p. 6094-105.
163. Sarkar, S.A., et al., Expression and regulation of chemokines in murine and human type 1 diabetes. Diabetes, 2012. **61**(2): p. 436-46.
164. Collier, J.J., et al., Pancreatic islet inflammation: an emerging role for chemokines. J Mol Endocrinol, 2017. **59**(1): p. R33-R46.
165. Lee, Y.S., et al., The fractalkine/CX3CR1 system regulates beta cell function and insulin secretion. Cell, 2013. **153**(2): p. 413-25.
166. Zweemer, A.J., et al., Bias in chemokine receptor signalling. Trends Immunol, 2014. **35**(6): p. 243-52.
167. Bishop-Bailey, D., et al., Lipid-metabolizing CYPs in the regulation and dysregulation of metabolism. Annu Rev Nutr, 2014. **34**: p. 261-79.
168. Miura, Y., The biological significance of omega-oxidation of fatty acids. Proc Jpn Acad Ser B Phys Biol Sci, 2013. **89**(8): p. 370-82.
169. Ferdinandusse, S., et al., Identification of the peroxisomal beta-oxidation enzymes involved in the degradation of long-chain dicarboxylic acids. J Lipid Res, 2004. **45**(6): p. 1104-11.
170. Wanders, R.J., J. Komen, and S. Kemp, Fatty acid omega-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. FEBS J, 2011. **278**(2): p. 182-94.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1476

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-355090

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2018