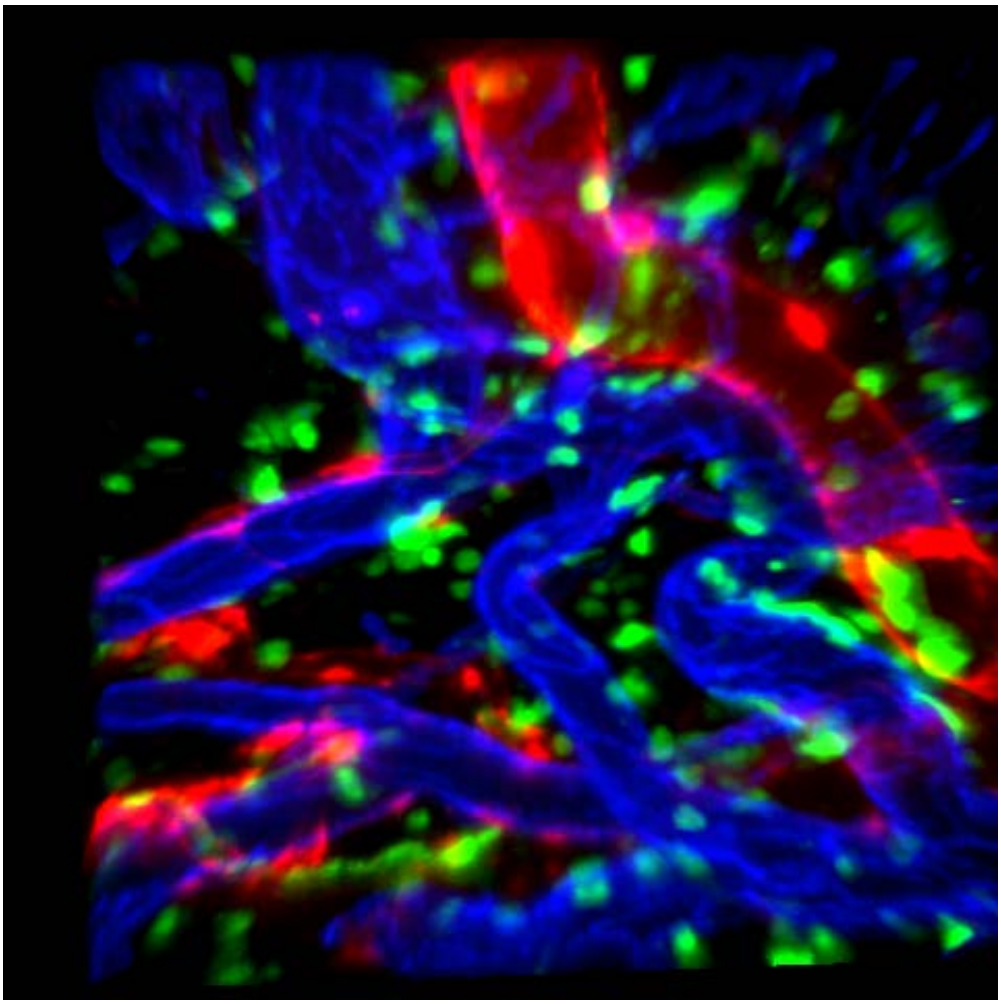
UPPSALA UNIVERSITET

Department of Medical Cell Biology

ANNUAL REPORT

2016



Fastställd av Institutionsstyrelsen 2017

Department of Medical Cell Biology

ANNUAL REPORT

2016

Introduction

First, I would like to commemorate Mats Hjorberg, our senior lecturer in Anatomy, who died tragically and unexpectedly last year. The department remembers Mats with great affection, and is sincerely grateful for his many outstanding achievments in teaching anatomy students. Second, I would like to thank professor Lena Holm who retired in 2016, for the many years of great work at the department. The contributions of Lena in both research and teaching have been highly esteemed by colleagues at the department and by many students. The department welcomes Sebastian Barg as new senior lecturer during 2016. In addition, we have recently recruited two associate senior lecturers, namely Joey Lau Börjesson and Olof Idevall. We also welcome our new HR administrator Carl Lundström.

During the year of 2016 research groups at the department of MCB have obtained several new prestigious research grants. For example, Michael Welsh was awarded a new grant from the Swedish Research Council. New grants were also obtained from,Vinnova/Tillväxtverket (Evelina Vågesjö and Mia Phillipson), the Novo-Nordisk Foundation (Gunilla Westermark) and Hjärnfonden (Sebastian Barg). Xuan Wang was awarded an international VR Post-doc grant. Malou Friederich-Persson was awarded two year funding from the Axel Wenner-Gren Foundation, and Ilkka Pietilä has received funding for a two year post-doc from the Faculty of Medicine.

Professor Nils Welsh organized in 2016 the third Claes Hellerström symposium in Diabetes Research, which attracted some 120 participants. Professor Gunilla Westermark organized the Uppsala Amyloidosis Symposium, which attracted more than 500 international participants. Professor Peter Bergsten was a member of the Organizing committee of the Uppsala Health Summit with theme “Ending Childhood Obesity”. The summit focused on novel solutions to the health problem of obesity epidemic by engaging many sectors of society.

Michael Hultström received a young investigator travel award at the Federation of American Societies of Experimental Biology (FASEB) science research conference on “Renal Hemodynamics and Cardiovascular Function in Health & Disease”. Daniel Espes recieved Swedish Endocrinology Association Best endocrinology dissertation 2016 prize. Furthermore, Gustaf Christoffersson received the EFSD/EASD:s Rising Star Fellowship.

It is also noteworthy that the MCB investigators have eminently communicated their research achievements to the general public via the news media (“tredje uppdraget”). During 2016 Dr. Michael Hultström lectured about heart failure for the Swedish Pensioner’s Society (SPF) on two occations arranged by the Swedish Heart-Lung Foundation with between 40 and 100 attendees per lecture. Mia Phillipson described her research on behavior on immune cells in the body in a video production from Wallenbergstiftelserna. Evelina Vågesjö was featured in the Uppsala University publication Nya Horisonter.

The medical anatomy course KARL, which was headed by Mats Hjortberg, was the winner of the annual KURT contest in 2016, which means that it reached the highest approval by the medical students. Mats Hjortberg was also posthumously awarded the Limbiska Prize of Honor for his teaching of biomedical students. We are also happy to congratulate Johan Kreuger, who was awarded a Pedagogical Rose by the first year medical students in 2016.

As the department has recently expanded by recruiting three new teachers, we anticipate some strain on the economy the upcoming years. Further budget constraints may unfortunately be necessary.

An international KoF17 panel recently evaluated the department, in order to further improve the quality of research and teaching of the department. The feed-back from the panel has not yet been finalized, but the department anticipates that this will lead to considerable future improvements in the organization of research and administration.

The achievements of this department depend heavily on hard and inspired work performed by our teachers, technicians, researchers, post-docs and PhD-students, but also on our staff of co-heads and administrators. Peter Hansell, deputy and assistant head of the department, manages undergraduate teaching with great finesse, and Gunilla Westermark, assistant head of the department, takes a firm responsibility for both our graduate student education, as well as safety and working environment issues. Our financial and human resource administration duties are proficiently handled by Shumin Pan and Carl Lundström, and teaching administration is excellently executed by Björn Åkerblom, Lina Thorvaldson and Erik Sandin. Göran Ståhl helps us all with practical matters and Oleg Dyachok keeps our microscopes in great shape. For all this many sincere thanks, and a special thank you to the PhD students who arranged the delightful Swedish “julgransplundrings party”.

Nils Welsh, Department Head Uppsala 2017-05-25

List of Contents

Organization

Chairman
Nils Welsh

Deputy chairman
Peter Hansell

Vice chairmen
Peter Hansell (Director of undergraduate studies)
Gunilla Westermark (Director of graduate studies)

Department board
(From mid 2014)
Peter Hansell, teacher representative
Mia Phillipson, teacher representative
Leif Jansson, teacher representative
Per-Ola Carlsson, teacher representative
Mats Hjortberg, teacher representative, deputy
Stellan Sandler, teacher representative, deputy
Anders Tengholm, teacher representative, deputy
Gunilla Westermark, teacher representative, deputy
Björn Åkerblom, representative for technical/administrative personnel
Lisbeth Sagulin, representative for technical/administrative personnel, deputy
Lisa Grapensparr, PhD student representative
Fredrik Lyngfalk, student representative
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Carl Lundström, personell administrator, adjunct

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Scientific Reports

Islet vascular physiology and cell therapy

Per-Ola Carlsson, Leif Jansson

The research of the group is mainly focused on the vasculature of the pancreatic islets and its relation to islet endocrine function during normal and diabetic conditions and after transplantation. The endothelial cells, which line all blood vessels, are important not only to distribute nutrients and oxygen to the islets, but our findings show that they also produce mediators which are involved in the regulation of hormone release, cell growth and the blood perfusion through the islets. Furthermore, endothelium-derived substances are likely to modulate the pathogenesis of both type 1 and type 2 diabetes. We have identified a functional reserve of islet endocrine cells. Normally 20-25% of islets are low oxygenated and with low protein biosynthesis, but these cells may be activated upon need during increased functional demands. On the other hand, more islets become downregulated when beta-cell mass is increased. We have also observed that the most blood-perfused islets, having a higher vascular density (Fig. 1), have a superior beta-cell function, proliferation and gene expression. However, these islets are also more prone to cellular death when stressed by hypoxia or cytokines, and they are also more prone to develop amyloid deposits.

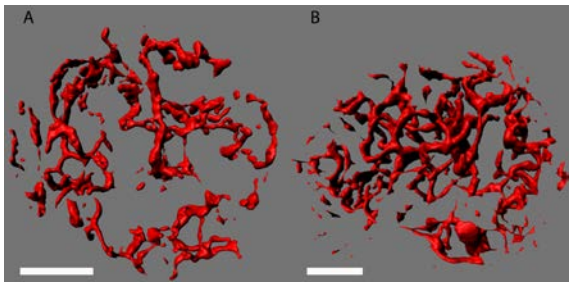


Fig 1. Two-photon confocal images of vascularity in pancreatic islets with low (A) or high (B) blood perfusion (blood perfusion identified by microsphere measurements).

Much of our research within the last years have been devoted to the adaptation of transplanted islets of Langerhans (which contain the insulin-producing beta-cells) to the implantation organ, i.e. the so-called engraftment process, and how this may be affected by different conditions in the recipients. Such transplantations are performed also in humans, but the long-term results are disappointing, probably due to impaired engraftment. Novel strategies to improve engraftment, as well as aspects to prevent cell death and regenerate beta-cells in native and transplanted islets by stem-cell stimuli are based on these findings presently tested by the research group in both experimental and clinical studies (cf. below). Recently, we have focused our translational efforts on the transplantation of insulin-producing cells derived from pluripotent stem cells.

Islet transplantation and beta-cell regenerative medicine (Per-Ola Carlsson)

The overall aim of the research on islet transplantation and beta-cell regenerative medicine is to develop means to intervene with the development of type 1 diabetes mellitus and find treatment strategies to restore glucose homeostasis in patients with type 1 diabetes mellitus using cell therapy. The dual role of the P.I. as experimental and clinical scientist simplifies translational approaches, and the research group is active both at the Department of Medical Cell Biology and the Department of Medical Sciences. Studies are conducted to elucidate the importance of islet endothelial, neural, stromal or their progenitor cells for beta-cell regeneration and function, and to investigate the concept of islet heterogeneity. Other studies investigate the adaptation of pancreatic islets to the implantation organ, i.e. the so called engraftment process, following transplantation (Fig. 2), and develop bioengineering strategies (coating of islets with supporting stem cells, oxygen carriers and growth factors, as well as with use of scaffolds) to improve results of pancreatic islet

transplantation by enhancement of engraftment e.g. by improved revascularization. Human islets are tested in these experimental systems with a focus to produce clinically applicable protocols. We also perform research to develop safe and effective means to generate new human beta-cells by stimulating adult beta-cell proliferation, e.g. by stem cell stimulation, or by stem cell differentiation in vivo. In a collaborative effort with professor Timo Otonkoski at the Biomedicum Stem Cell Centre, University of Helsinki we also focus on how to translate work on insulin-producing cells derived from pluripotent stem cells into an effective clinical therapy for type 1 diabetes. Clinical studies are performed to to improve the results of beta-cell replacement therapy, e.g. by encapsulation of human islets for transplantation in order to avoid immune suppression of the patients. A newly developed oxygenized macrochamber to harbor the islets is tested in a pilot trial with type 1 diabetes patients. The macrodevice protect the islets from immune rejection, whereas oxygen is supplied daily into a refillable oxygen tank. We also develop means for beta-cell imaging by positron emission tomography clinically.

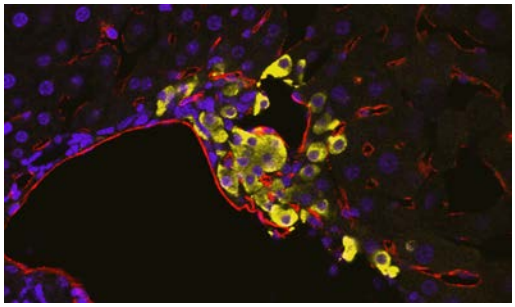
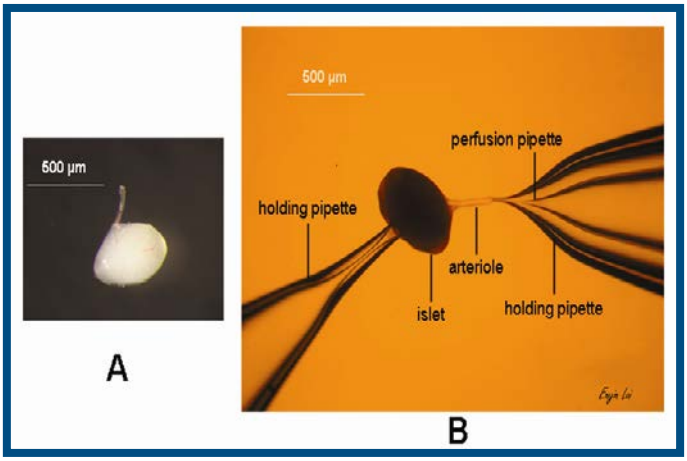


Fig 2. Micrograph showing vascularization of intraportally transplanted islet with disrupted integrity in the wall of a portal vein tributary. Yellow depicts insulin; red CD31 staining for blood vessels and blue DAPI.

Pancreatic islet blood flow and endocrine function (Leif Jansson)

The overall purpose is to functionally evaluate the vascular system and especially map blood flow regulatory mechanisms in the islets of Langerhans during normal conditions and during various degrees of glucose intolerance and overt diabetes**.** An important rational behind these experiments is the diabetes-induced endothelial dysfunction, which also affects the islet vasculature. This detailed knowledge can then be applied to facilitate targeted delivery of substances to the islets by selectively increasing the blood flow to the islets. This is initially performed in experimental animals, but we intend to transfer our results also to humans. Substances of interest for facilitated delivery include immunomodulatory substances, substances stimulating regeneration of beta-cells, contrast agents to increase visibility of islets during imaging procedures for easier quantification of islet mass, substances affecting islet endothelial cells to ameliorate the endothelial dysfunction seen in diabetes, which is generalized but also specifically affects the islets.



In this context we will also study islet endothelial cells (EC) (from different animal models and human islets both in vivo and in vitro.

EC are isolated aand cultured under flow with various additions to study the expression of different intracellular and plasma membrane proteins. This will enable us to *e.g.* identify changes in endothelial function caused by hyperglycemia, hyperlipidemia and other conditions associated with

impaired glucose tolerance (IGT). It will also identify endothelial markers, which can be used to further improve selective delivery of substances to the islet vasculature. Changes in endothelial function can then be further evaluated with our palette of in-vivo techniques.
On the basis of our findings on normal islet blood flow regulation we will continue our studies to evaluate disturbances occurring during IGT and type 2 diabetes. We have previously observed that these conditions are invariably associated with an increased islet blood flow. Thus, in this context we would be interested to evaluate mechanisms by which to decrease islet blood flow. However, the mechanisms underlying this are as yet largely unknown. We now aim to further clarify these mechanisms and to evaluate to what extent we can normalize islet blood flow. In relation to this, we also plan to investigate if normalization of islet blood flow can ameliorate IGT. We also aim to study, by imaging techniques, if the results on blood flow regulation obtained in rodents are applicable also to humans.
In summary, the general aim is to advance and use our knowledge on islet blood flow regulation to develop techniques to affect islet endocrine function by modulation of islet blood flow. Thereby we will, in a longer perspective, be able to more selectively target drugs to the islets, and facilitate imaging of the islets to obtain improved determinations of beta-cell mass during T1D and T2D.

Members of the group

Per-Ola Carlsson, MD, professor, Senior Consultant in Endocrinology and Diabetology

Leif Jansson, MD, professor

Arne Andersson, MD, professor em.

Joey Lau, assistant professor

Monica Sandberg, asssistant professor

Sara Bohman, lecturer

José Caballero, MD, post-doc

Xiang Gao, post-doc

Daniel Espes, MD, post-doc

Carl Johan Drott, MD, PhD student

Hanna Liljebäck, MD, PhD student

Liza Grapensparr, PhD student

Sara Ullsten, PhD student

Louise Magnusson, PhD student

Zhanchun Li, laboratory engineer

My Quach, laboratory engineer

Lisbeth Sagulin, laboratory engineer

Petra Franzen, laboratory engineer

Birgitta Bodin, laboratory technician

Rebecca Hilmius, research nurse

Karin Kjellström, research nurse

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Dissertations

Espes, D.(2016).Engraftment of Pancreatic Islets in Alternative Transplantation Sites and the Feasibility of *in vivo* Monitoring of Native and Transplanted Beta-Cell Mass.

Agencies that support the work

The Juvenile Diabetes Research Foundation USA

The Swedish Research Council

The Swedish Diabetes Association

The Torsten Söderberg Foundation

The Diabetes Wellness Foundation

The Swedish Juvenile Diabetes Fund

The Novo Nordisk Foundation

The Olle Engkvist Byggmästare Foundation

The Uppsala-Örebro Regional Research Foundation

The Gunvor & Josef Ane’rs Foundation

The Thuring Foundation

Svenska Sällskapet för Medicinsk Forskning

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Strategic Funding, StemTherapy

The Family Ernfors Foundation

Goljes Memorial Fund

Islet function in childhood obesity and type 2 diabetes mellitus

Peter Bergsten

Background

The prevalence of persons with metabolic disease including type 2 diabetes mellitus (T2DM) is expected to rise from 3% in 2000 to almost 5% in 2030. Since obesity is strongly linked with T2DM, the increasing prevalence of over-weight and obesity especially among children, reaching 20% in Sweden, is of particular concern. The rise in obesity has a multi-factorial background, where both genetic and environmental factors contribute. In obese children elevated levels of both insulin and glucagon are observed at fasting as well as during oral glucose tolerance test (OGTT). High levels of glucagon elevate glucose leves, which in turn elevate insulin levels. This bi-hormonal perturbance may therefore be an important factor precipitating development of metabolic disease including T2DM in the obese children.

Aim

The overall aim was to normalize the high insulin and glucagon levels seen in obese children. The aim was addressed by work *in vivo* from the obese and lean children and *in vitro* from the islets of Langerhans and beta-cell lines.

In vitro, we wanted to delineate mechanisms by which free fatty acid palmitate affect insulin and glucagon secretion.

In vivo, we wanted to describe how insulin and glucagon levels associate to glucose intolerance in obese children.

Elevated palmitate concentrations and hyperinsulinemia

We have shown that free fatty acid levels were elevated in young obese children and that free fatty acid palmitate in isolated islets and beta-cell lines induced insulin hypersecretion (Staaf et al 2016). When isolated islets were exposed to prolonged elevated palmitate levels, as observed in obese subjects and T2DM, insulin secretion is impaired (Fig 1). However, this impaired insulin sceretion was preceded by islet insulin hypersecretion. Thus, it appeared that before palmitate-induced impairment of insulin secretion and loss of beta-cell mass occurred, enhanced insulin secretion was observed.

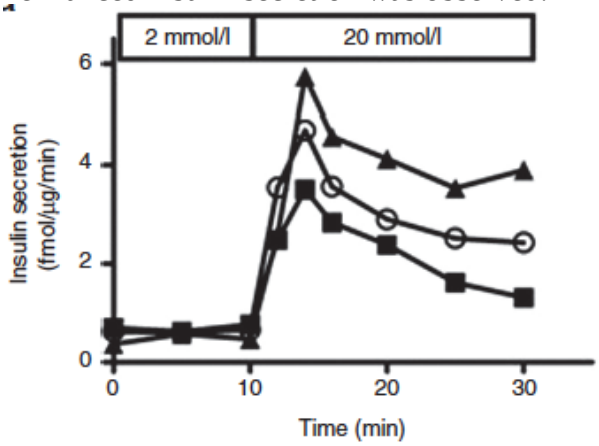

Figure 1: Glucose-stimulated insulin secretion from isolated human islets exposed to 0.5 mM palmitate for 0 (open circles), 2 (closed triangles), or 7 (closed squares) days (Staaf et al, *Ped Res*, 2016).

In young obese and lean children belonging to the "Uppsala Longitudinal Study of Childhood Obesity" (ULSCO) (Forslund et al 2014), we investigated if the observed palmitate-induced alterations in insulin secretory patterns were evident also *in vivo*. Obese children are referred to the Uppsala University Children's Hospital, where they are examined and treated. Both the obese children and lean controls are enrolled in the ULSCO cohort. When their insulin secretory response to glucose was measured by oral glucose tolerance test (OGTT), insulin levels at fasting and 30 min of OGTT were accentuated in obese children with elevated palmitate levels but attenuated in obese adolescents with elevated palmitate levels (Fig 2). Indeed, secretory levels in the adolescents were similar to those observed in lean controls. Based on the findings in the isolated islets and the fact that some of these adolescents progressed to overt T2DM, we hypothesized that this "normalization" reflects impaired beta-cell function in the older obese individuals and that insulin hypersecretion observed in isolated human islets (Fig 1) and obese children (Fig 2) is an etiological factor in the development of obesity precipitating overt T2DM in susceptible individuals.

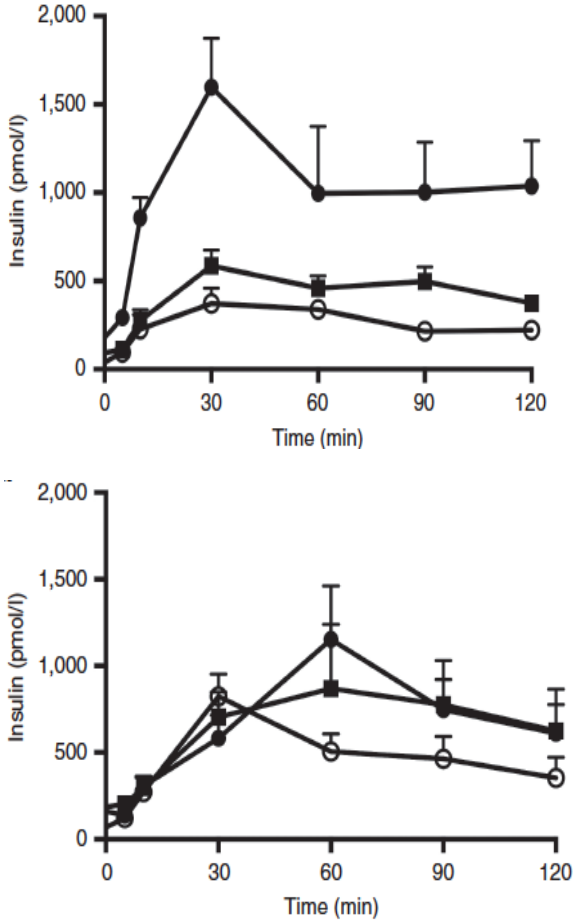

Figure 2: Oral glucose tolerance test in obese pre-pubertal (top panel) and pubertal (bottom panel) children with high palmitate (closed circles), low palmitate (closed squares) and lean controls (open circles) (Staaf et al, *Ped Res*, 2016).

Attenuation of insulin hypersecretion

In isolated islets approaches to attenuate beta-cell hypersecretion were conducted to defining underlying causes for the observed accentuated secretory activity in insulin-producing beta-

cells using translational approach. Isolated human islets are exposed to compounds known to affect insulin secretion and their effects on insulin hypersecretion determined. These approaches identified G-protein coupled receptor FFAR1, which has palmitate as ligand, which was investigated for its effects on insulin hypersecretion (Kristinsson et al, *BBA*, 2015).

Combined hyperinsulinemia and hyperglucagonemia

Accentuated glucagon and insulin secretion was observed in obese children with high circulating palmitate concentrations (Manell et al, 2016). We investigated how elevated levels of glucagon correlated with glucose tolerance in children with obesity. Whereas obese children with normal and impaired glucose tolerance had moderately elevated isnul and glucagon levels at fasting and duing OGTT, children with T2DM had elvated levels (Fig 3).

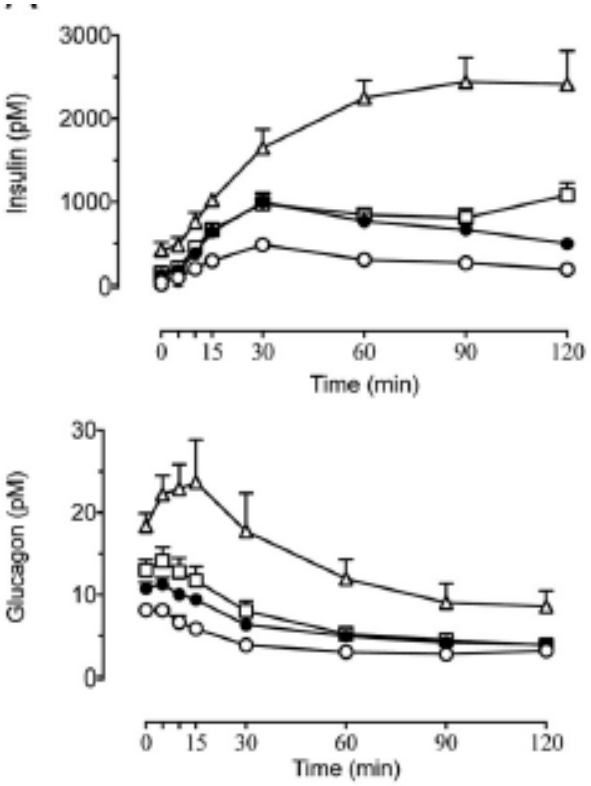

Figure 3. Plasma levels of insulin (top panel)and glucagon (bototm panle) during an OGTT in lean adolescnsts (opne circles), obese adolescsnt with NGT (closed circles), impaired gluceo toleand (squares and type 2 diabetes /traingles)

Also, in isolated human islets mechanisms for glucagon hypersecretion were investigated.

Significance

The results of the project are expected to identify novel principles of normalizing hypersecreting beta- and alpha-cells. These principles will be evaluated in the young obese individuals as intervention strategies, which are critical since the window of opportunity to preventing impaired beta- and alpha-cell function and apoptosis in juvenile obesity appears to be limited.

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Swedish Diabetes Association

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Physiology of pancreatic islet hormone secretion

Anders Tengholm

The research in our group aims at clarifying the mechanisms regulating the release of insulin, glucagon and other hormones from the islets of Langerhans. Insufficient secretion of blood-glucose-lowering insulin and dysregulated secretion of blood-glucose-elevating glucagon are

hallmarks of diabetes. Elucidation of the mechanisms underlying islet hormone secretion and the malfunctions causing diabetes is expected to provide new strategies for treatment of the disease. By combining biochemical and molecular biological techniques with fluorescent cell signalling biosensors and live cell imaging methods, we study the spatio-temporal dynamics of signalling processes regulating secretion in single cells and intact mouse and human pancreatic islets. At present we are focusing on the following issues.

ATP, Ca^{2+} and cAMP signalling in β-cell stimulus-secretion coupling

Insulin is released from β-cells in response to glucose, other nutrients, hormones and neural factors. The hormone is released in pulses with the kinetics determined by a complex interplay between second messengers and signalling proteins beneath the β-cell plasma membrane. Glucose stimulation of β-cells results in uptake and metabolism of the sugar, elevation of the intracellular ATP/ADP ratio, closure of ATP-sensitive K^+ channels in the plasma membrane, depolarization and voltage-dependent Ca^{2+} influx, which triggers exocytosis of insulin secretory granules. The exocytosis response is amplified by the messenger cAMP, which is generated in β-cells during glucose stimulation as well as upon glucagon and incretin hormone receptor activation. Our lab has discovered that glucose triggers coordinated oscillations of Ca^{2+} and cAMP in β-cells, and that this response is important for pulsatile insulin secretion. During the past year we have also found that impaired glucose-induced cAMP formation contributes to defective insulin secretion from islets cultured under diabetes-like conditions with elevated concentrations of the free fatty acid palmitate (Fig. 1).

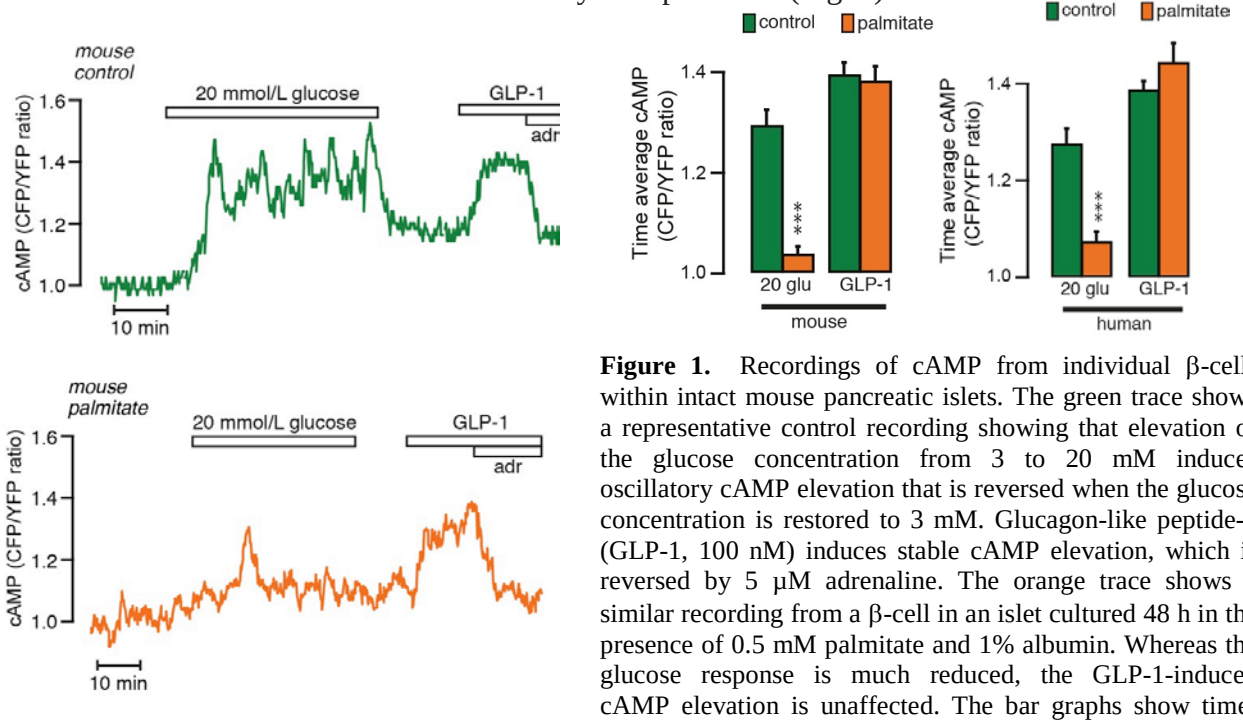

Figure 1. Recordings of cAMP from individual β-cells within intact mouse pancreatic islets. The green trace shows a representative control recording showing that elevation of the glucose concentration from 3 to 20 mM induces oscillatory cAMP elevation that is reversed when the glucose concentration is restored to 3 mM. Glucagon-like peptide-1 (GLP-1, 100 nM) induces stable cAMP elevation, which is reversed by 5 µM adrenaline. The orange trace shows a similar recording from a β-cell in an islet cultured 48 h in the presence of 0.5 mM palmitate and 1% albumin. Whereas the glucose response is much reduced, the GLP-1-induced cAMP elevation is unaffected. The bar graphs show time-average cAMP elevations ± s.e.m. in response to 20 mM glucose and GLP-1 in mouse and human β-cells. From Tian et al, Diabetes 64:904-15, 2015.

Using various cell signalling biosensors we aim to clarify the mechanisms underlying the generation of cAMP oscillations and how the cAMP effectors protein kinase A and Epac, a guanine nucleotide exchange factor for Rap GTPases, are involved in the regulation of insulin secretion. We have found that protein kinase A, in addition to potentiating exocytosis in response to cAMP-elevating hormones, is important for proper initiation of insulin secretion by glucose. Moreover, recent work from the lab has demonstrated that cAMP and Ca^{2+} signals trigger translocation of Epac to the β-cell plasma membrane. The downstream effects as well as functional importance of these signalling steps are currently under investigation.

Autocrine feedback signalling in β-cells

Exocytosis of insulin granules not only results in the release of insulin, but also of several other granule constituents, which affect β-cell function in an autocrine manner. Activation of insulin receptors leads to PI3-kinase-mediated formation of the phospholipid PtdIns(3,4,5)P_3. Using fluorescent reporters we have demonstrated that glucose stimulation of β-cells results in pronounced PtdIns(3,4,5)P_3 oscillations in the plasma membrane that reflect pulsatile insulin secretion and the associated autocrine insulin receptor activation. Although insulin has been found to exert positive feedback on insulin biosynthesis and β-cell proliferation, it is less clear whether insulin acutely stimulates or inhibits insulin secretion.

Insulin is stored in a crystalline complex with Zn^{2+} and this ion is co-released with insulin and exerts feedback effects at multiple levels. The granules also contain ATP and we recently discovered that ATP co-released with insulin activates purinergic $P2Y_1$-receptors, which results in phospholipase C activation and short-lived (<10 s), local increases of diacylglycerol (DAG) in the plasma membrane (Fig 2). These DAG spikes results in rapid recruitment and activation of several protein kinase C isoforms. Using various optical single-cell assays we are currently investigating how insulin, Zn^{2+} and ATP affect signalling and secretion in β-cells.

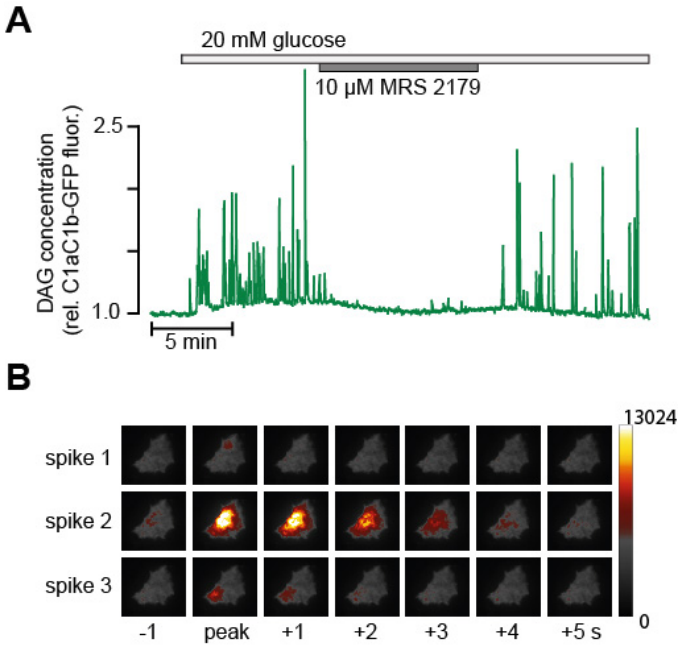

Figure 2. (A) Glucose stimulation of a mouse β-cell triggers pronounced DAG spiking in the plasma membrane that is monitored with a fluorescent DAG reporter. The response is reversibly inhibited when the autocrine action of ATP is blocked with the purinergic receptor antagonist MRS2179.

(B) The DAG spikes are typically spatially confined. Each row shows a sequence of pseudo-colored 14-bit images starting 1 s before the appearance of a DAG spike and displays the DAG reporter fluorescence every second during the following 6 seconds.

Mechanisms controlling the release of glucagon, somatostatin and pancreatic polypeptide

In diabetes there is not only an impaired secretion of insulin, but poor regulation of blood-glucose elevating glucagon contributes to the hyperglycemia underlying diabetes complications. Pancreatic polypeptide is another islet hormone of potential importance for blood glucose regulation by effects on gastric emptying. The fourth islet hormone, somatostatin, is a potent inhibitor of the release of the other hormones and probably has a paracrine function. Other paracrine events in the islets involve insulin-promoted inhibition of glucagon secretion and glucagon-potentiated insulin secretion. Like for insulin, the secretion of glucagon and somatostatin is pulsatile. Our lab has demonstrated that the glucagon pulses are in opposite phase to insulin and somatostatin, which has important implications for the understanding of the action of insulin and glucagon on glucose production in the liver. Glucose inhibits glucagon secretion and stimulates somatostatin secretion but consensus is lacking regarding the underlying mechanisms. Like in β-cells, glucose metabolism plays a key role and Ca^{2+} is the main trigger of exocytosis in both glucagon-releasing α-cells and somatostatin-releasing δ-cells. Fig. 3 illustrates our present working model for glucose regulation of glucagon secretion.

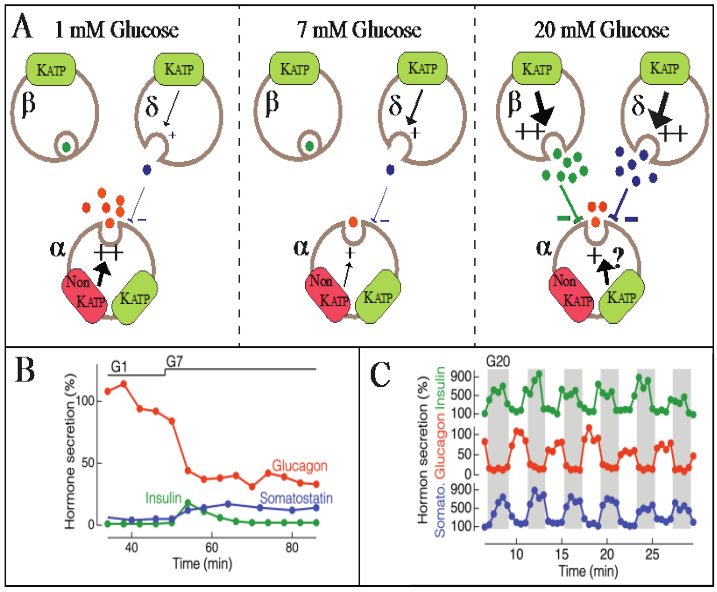

Figure 3. Model for glucose regulation of glucagon release. (A) In the 1-7 mM range (G1, G7), glucose controls glucagon release via an intrinsic non-K_{ATP} channel-dependent mechanism in α-cells and paracrine release of somatostatin from δ-cells has only a tonic inhibitory effect. (B) The graph showing glucose inhibition of glucagon secretion is expressed in percent of stimulated secretion at 1 mM glucose. To get an impression of the relative magnitudes of the corresponding insulin and somatostatin responses, their secretion are expressed in percent of stimulated secretion in response to 0.5 mM tolbutamide. (A, C) At 20 mM glucose (G20) the K_{ATP}-independent mechanism no longer stimulates glucagon secretion and the pulsatility is generated via paracrine release of inhibitory factors from β- and δ-cells. The question mark indicates that a stimulatory effect of high glucose in the α-cell is not necessarily K_{ATP} channel-dependent. Hormone secretion data have been recalculated as percentage of estimated secretion at 1 mM glucose (From Gylfe Diabetes 62:1391-1393, 2013.

We are currently investigating intracellular ATP, Ca^{2+} and cAMP signalling in relation to hormone release from the different islet cells as well as the importance of paracrine intercellular communication for generating the different secretory patterns. Fig. 4 shows Ca^{2+} recordings from α- and β-cells within the same islet. This experiment illustrates that α-cells show Ca^{2+} oscillations at 3 mM glucose, which is too low to activate the β-cells. Elevation of the glucose concentration to 20 mM causes a temporary interruption of Ca^{2+} signaling in the α-cells and induces well-synchronized Ca^{2+} oscillations in β-cells. α-cell Ca^{2+} signaling is subsequently restored with Ca^{2+} oscillations that are synchronized not only among different α-cells but also between α- and β-cells. These findings are unexpected in light of the suppressed glucagon secretion and the anti-phase pulses of glucagon and insulin (Fig. 3), and indicate that glucagon release may be controlled by somatostatin in a Ca^{2+}-independent manner.

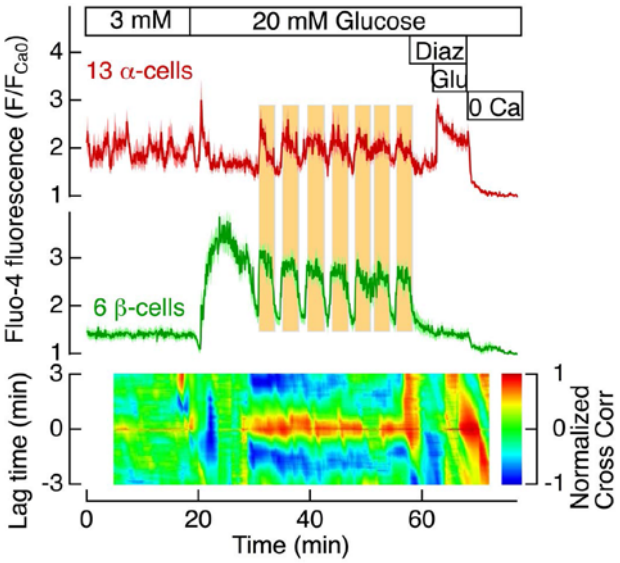

Figure 4. The graphs show average cytoplasmic Ca^{2+} concentrations (red for α- and green from β-cells ± s.e.m. (pink for α- and light green for β-cells) for all 13 α-cells and 6 β-cells in a single mouse islet loaded with the fluorescent Ca^{2+} indicator fluo-4 and with red fluorescent protein expression in the α-cells. The vertical yellow background areas are aligned to glucose-induced peaks of the Ca^{2+} oscillations in the β-cells. The coloured area in the bottom panel shows a two-dimensional cross-correlogram with time on the x axis and the lag time of the correlation on the y axis and the normalized cross-correlation amplitude coded in color. From Li et al, FASEB J 29:3379-88, 2015.

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Agencies that support the work

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The Swedish Diabetes Association

Novo Nordisk Foundation

Family Ernfors Foundation

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Cellular architecture and organelle communication

Olof Idevall-Hagren

The architecture of the prototypic mammalian cell has been the focus of intense study since the early days of microscopy. With the development of electron microscopy for the biological sciences in the 1950's came the detailed characterization of most cellular organelles, like the endoplasmic reticulum (ER), mitochondria, the Golgi apparatus and secretory vesicles. More recently, using live cell imaging techniques, it has been found that these organelles are highly dynamic structures that constantly reform, reshape and redistribute within the cell. Many organelles also seem to communicate through direct contacts, formed by protein and lipid complexes. At these sites, information flow between the organelles in the form of lipids, ions and proteins help control the specific organelle function. Using high-resolution fluorescence microscopy together with genetically encoded biosensors and molecular tools we study and manipulate these cellular structures in order to better understand their function.

Organelle dynamics in β-cells

Malfunctioning β-cells strongly contributes to diabetes development and progression by failing to secrete sufficient amounts of insulin. Insulin is produced in the ER and stored in granules awaiting metabolically generated release signals. The failing β-cells exhibit defects in both insulin production and secretion, indicating impaired functions of both the ER and the mitochondria. Indeed, β-cells from diabetics exhibit both reduced protein synthesis capacity and mitochondrial metabolism. Organelles like the ER and mitochondria have in recent years been shown to be highly dynamic and to form dynamic contacts with eachother through which ions and lipids can be exchanged. Determining the molecular composition of these organelle tethers has proven challenging due to their transient nature and small sizes. In recent work we show that the ER is anchored to the plasma membrane via protein-lipid interactions mediated by the E-Syt protein family (Figure 1). Genetic ablation of these contacts results in massive rearrangement of the ER. We also found that these contacts form in response to changes in the cytoplasmic Ca^{2+} concentration. In β-cells stimulated with glucose to secrete insulin, these contacts form in a Ca^{2+}-dependent manner that coincide in space and time with secretion, indicating a direct involvement of the ER in the regulation of insulin secretion (Figure 2). Current work aims at: 1) characterizing the role of E-Syts in β-cell function, 2) identifying other proteins that accumulate at these membrane contact sites, 3) investgating wether these structures are altered in diabetic β-cells. Hopefully this can help us better understand how altered ER function contributes to the progression of diabetes.

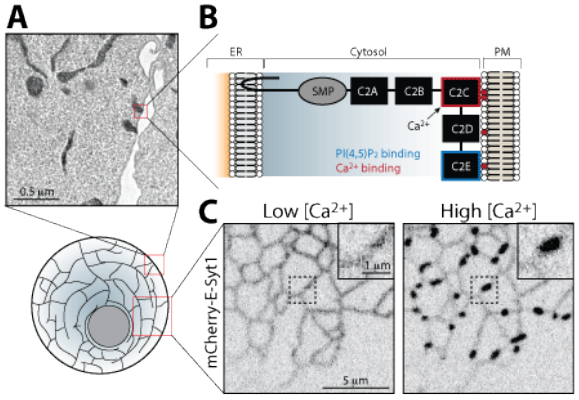

***Figure 1**: **A**. Electron micrograph of a fibroblast where the ER is stained black. Notice how parts of the ER are in close proximity of the cell periphery (red box). **B**. Schematic illustration depicting Extended-Synaptotagmin-1 (E-Syt1), an ER-anchored protein that also binds the plasma membrane by interactions with specific lipids. C. Confocal microscopy images of a very flat cell expressing fluorescence-tagged E-Syt1 under conditions where the cytoplasmic Ca^{2+}concentration is low or high. Notice how the molecules aggregate at the plasma membrane when the Ca^{2+} concentration increases.*

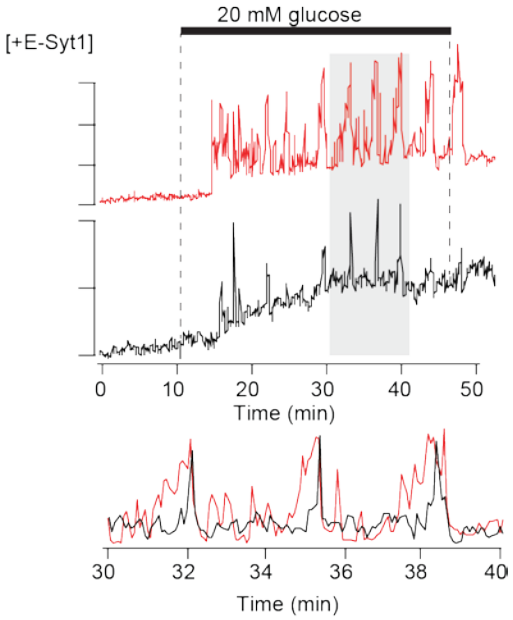

***Figure 2**: Figure shows glucose insduced oscillations of the cytosolic Ca^{2+} concentration (red) and the corresponding recruitment of the ER-localized E-Syts (back) to the plasma membrane. The gray area is shown below on an expanded time axis. Notice the close association between changes in Ca^{2+} and binding of E-Syts to the plasm amembrane.*

Optogenetic tool development and implementation

Optogenetics is the modification and use of light-regulated proteins, typically isolated from plants or bacteria, to enable control of cellular processes by illumination. Expression of optogenetic tools has for example enabled light-dependent control of neurotransmitter release, insulin release, cell migration and transcription. We have previously developed tools that allows light-dependent recruitment of lipid synthesizing and degrading enzymes to the plasma membrane, leading to the discovery that rapid changes in lipid levels can polarize cells and is sufficient to induce e.g. directed cell migration. We have also used these tools to demonstrate that the ER is physically tehtered to the plasma membrane through the interaction of the ER-localized E-Syts with the anionic plasma membrane lipid PI(4,5)P_2. This lipid regulates a plethora of other functions of importance for β-cell function, including ion fluxes and exocytosis, but to what extent the lipid actually control any of these processes under physiological settings is unknown. We are currently using adenoviral versions of

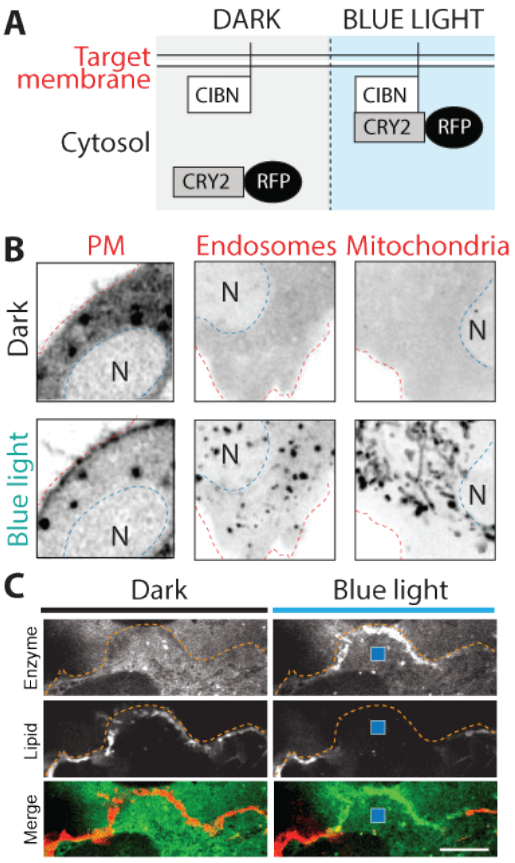

***Figure 3: A,B.** Drawing showing the principle of light-induced hetero-dimerization. One part of the optogenetic module (CIBN) can be anchored to any cellular membrane (target membrane) whereas the other part (CRY2) can be fused to a protein of interest (here a Red Fluorescent Protein). Blue-light illumination promotes the interaction between CIBN and CRY2 and causes redistribution of the protein of interest to the target membrane. **C.** Focal blue-light illumination (blue square) allows recruitment of a lipid-degrading enzyme (green) to a restricted part of the plasma membrane, resulting in corresponding loss of a specific lipid (red).*

these optogenetic tools togehter with measurments of Ca^{2+} concentration changes and insulin secretion to malipulate $PI(4,5)P_2$ levels in intact mouse and human β-cells. We will also further develop these optogenetic tools to allow formation of inducible contacts between various cellular organelles and to alter organelle distribution within cells in order to determine how organelle tethers and distribution affects β-cell function. Since optogenetics is a non-invasive technique, we also work on adapting it to *in vivo* settings. Additionally, we are developing, togehther with a group at Zhejiang University, a light-array that will enable high-throughput optogenetic manipulations.

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Members of the group

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Agencies that support the work

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The Malin and Lennart Philipson foundation

Novo Nordisk foundation

STINT

Magnus Bergwalls stiftelse

Åke Wibergs stiftelse

Uppsala University

Mechanisms of regulated exocytosis

Sebastian Barg

Exocytosis is fundamental to every cell and crucial to intracellular transport, protein sorting, and cell-to-cell communication. In both neurons and endocrine cells, exocytosis leads to the release of neurotransmitters and hormones, and defects in this process can underlie disease, such as type-2 diabetes. In our lab we are interested in the cell biology of insulin secretion, with a focus on the life-cycle of insulin-containing secretory granules. We study exocytosis in pancreatic ß-cells using advanced light microscopy (TIRF, super-resolution and single molecule imaging) in combination with electrophysiology. Both methods are sensitive enough to observe single granules and even individual protein molecules in a living cell

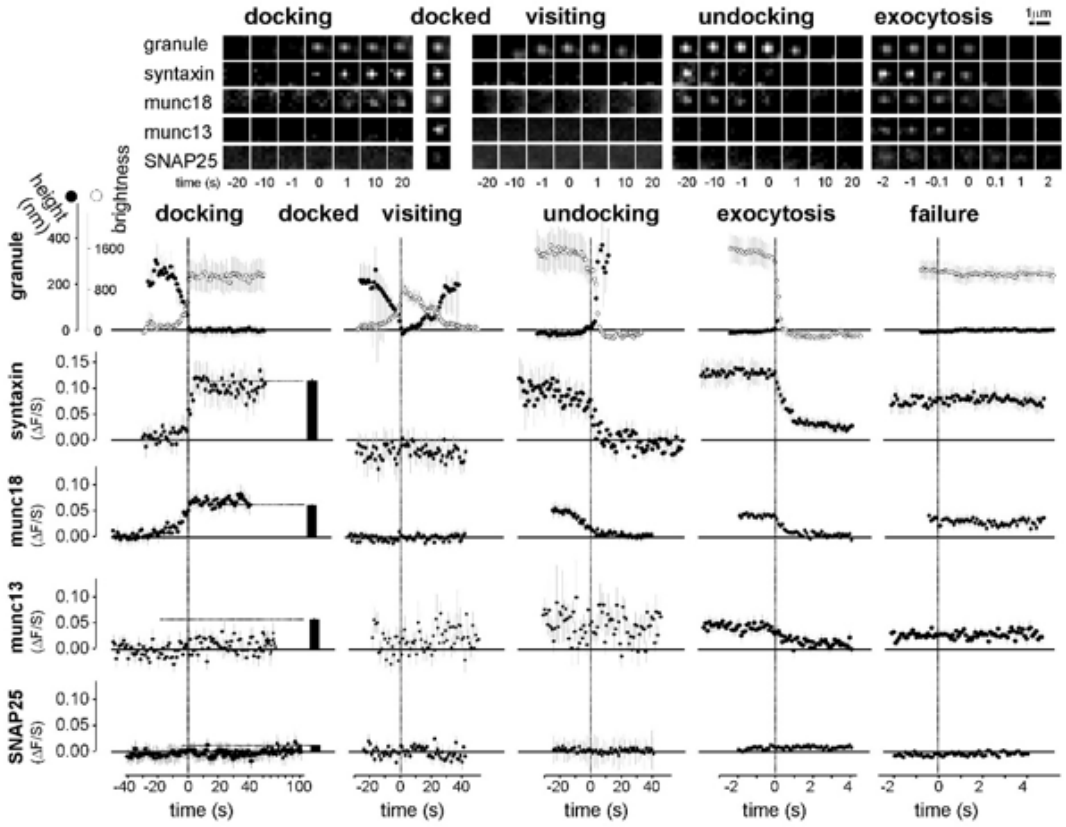
Molecular architecture of the insulin granule release site

Every ß-cell contains thousands of secretory granules that store insulin. When blood glucose is elevated, these granules undergo regulated exocytosis and release the hormone into the blood stream. Before this can happen, granules have to reach the plasma membrane, where they “dock” and then assemble the exocytosis machinery. When insulin is released, these steps quickly become limiting for how much insulin is released.

The docking process is not understood in molecular terms, but many of the proteins involved have been identified. One hypothesis that we are currently testing is that some of these proteins (including t-SNAREs) pre-assemble at small hotspots in the plasma membrane. These hotspots, perhaps related to lipid rafts, may then recruit granules and act as “launching pads” for exocytosis. There is evidence that this docking step is impaired in type-2 diabetes, and the most important “diabetes gene” affects expression of a protein involved in granule docking. How do cells compartmentalize their plasma membrane to organize such sites? Which proteins are recruited to these hotspots, when, and at how many copies? And how are docking sites regulated and what distinguishes release-ready granules from those that are merely docked?

The three SNARE proteins syntaxin, SNAP25 and synaptobrevin are central to membrane fusion during exocytosis. Since two of these, the t-SNAREs syntaxin and SNAP-25 inhabit the plasma membrane, one expects them to collect at the exocytic site before a vesicle or granule can fuse there. Indeed, we found t-SNAREs cluster near docked granules and quantitative image analysis shows association of GFP-labeled syntaxin and SNAP25 with granules in live Ins1- or PC12-cells. The interaction depends on the N-terminal Habc domain of syntaxin, rather than formation of a SNARE complex. Up to 70 molecules of syntaxin are recruited to the granule site during docking, and lost during undocking and exocytosis. We show further that these protein clusters assemble at the release site when a granule arrives from the deeper

cytosol to dock at the plasma membrane. These measurements show that insulin granule docking coincides with rapid *de novo* formation of syntaxin1/munc18 clusters at the nascent docking site, which stabilizes the docked state. Interfering with this clustering prevents docking. By systematically deterimining when certain proteins arrive at the docking site, in relation to the granule docking event, we have reconstructed the sequence of how the release machinery assembles. Importantly, protein recruitment also correlates with the granules' ability to exocytose. For example, recruitment of L-type Ca^{2+}-channels to the release site dramatically increases the probability of a granule to undergo exocytosis, which we could show by imaging both the channel itself and the resulting localized Ca^{2+}-influx. Interestingly, this architecture was disturbed in islets from human donors with type-2 diabetes.



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Members of the group

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Agencies that support the work

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Swedish Research Council (Vetenskapsrådet)

Swedish Society for Medical Research

Barndiabetesfonden

Novo Nordisk Foundation

European Foundation for the Study of Diabetes/MSD

European Foundation for the Study of Diabetes/BI

The Carl Tryggers Foundation

The Göran Gustafsson Foundation

Family Ernfors Foundation

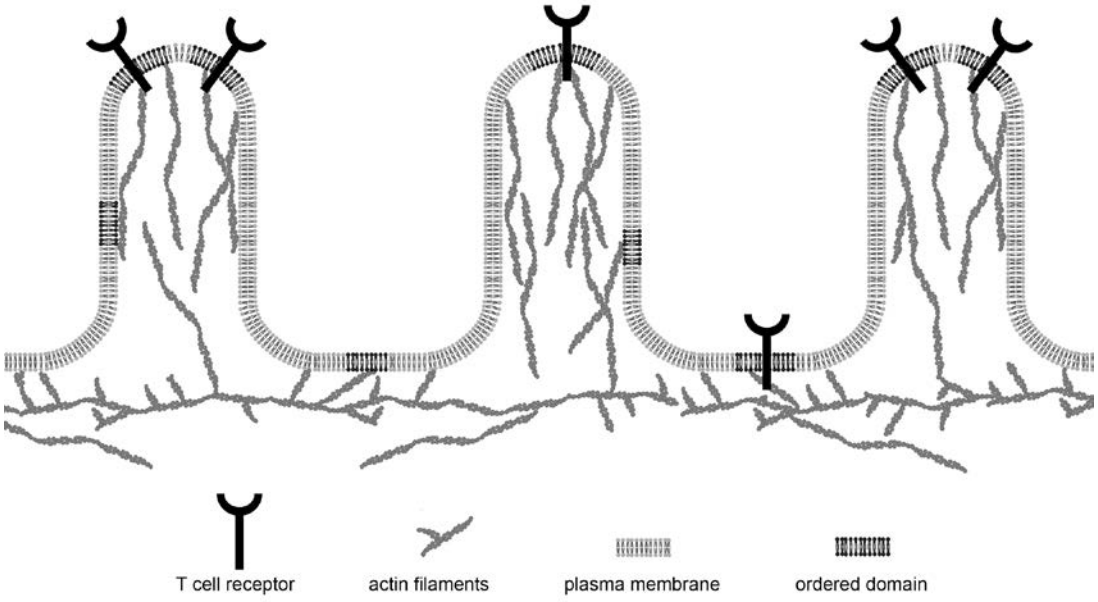
OE och Edla Johanssons stiftelse

PO Zetterlings stiftelse

The functional organisation of the T cell plasma membrane

Ingela Parmryd

The plasma membrane of eukaryotic cells contains nanodomains, commonly referred to as lipid rafts, where the lipids are more tightly packed (ordered) than in the rest of the plasma membrane. Domains of different order help segregating molecules and are important for processes like membrane tubulation, signal transduction and infection. In model membranes lateral lipid-lipid interactions are sufficient for the formation of ordered domains. However, we have recently demonstrated that lipid rafts form when actin filaments are pinned to the plasma membrane via phosphoinositides and when extracellularly exposed receptors are pinned by antibodies (Dinic et al., 2013), suggesting that the mechanism for lipid raft formation is lipid-protein interactions. We have shown that T cell signalling is initiated upon lipid raft aggregation that can be triggered by cold stress and changes in the plasma membrane lipid composition. We have recently shown that the T cell receptor in resting T cells resides in lipid rafts that are brought together upon receptor engagement (Dinic et al., 2015). We are now investigating what is triggering the formation of lipid rafts in more detail and how membrane order affects the molecular clustering that accompanies T cell signalling using techniques like superresolution microscopy and fluorescence correlation spectroscopy.



The

Localisation of the T cell receptor. The TCR is found in ordered plasma membrane domains that form where actin filaments are pinned to the plasma membrane. To scan the environment TCR-containing ordered domains at the tip of membrane protrusions positions the receptor at an ideal position. From Dinic et al., 2015.

The cell surface is neither flat nor smooth but surface topography is ignored in current models of the plasma membrane. Using high resolution topographical maps of live cells, we and our collaborators have demonstrated that apparent topographical trapping is easily mistaken for

elaborate membrane model features like hop diffusion and transient anchorage. Even binding could be the result of apparent topographical trapping when single particle tracks are interpreted in 2D although the molecules are moving in 3D. We are together with collaborators at CBA at UU developing a method to analyse diffusion coefficients on non-flat surfaces and in collaboration with the ALM facility at SciLifeLab address how membrane topography can be accounted for in superresolution single molecule localisation microscopy of membrane molecules.

γ□δ□ T cells is a T cell subset that recognise and kill cancer cells that accumulate high levels of phosphoantigens, small organic compounds with phosphate groups. There is a positive correlation between the γ□δ□ T cell number and cancer cell death making γ□δ□ T cells appealing candidates for immunotherapy and it seems as if colon cancer patients have lower numbers of circulating γ□δ□ T cells than healthy individuals. Together with collaborators at the UAS we address how the prevalence of γ□δ□ T cells in colon cancer patients at the four different cancer stages varies and characterise the γ□δ□ T cells regarding differentiation status, tumour homing, proliferation and cytotoxicity. Together with collaborators at SU we have found that media from erythrocytes infected with *P. falciparum* can stimulate γ□δ□ T cell prolifieration suggesting that phosphoantigens both are produced in and secreted from these cells. We now address at which parasite stage this production occurs and how the plasma membrane order vary with the γ□δ□ T cell differentiation stage.

We develop image analysis software to get quantitative and objective answers to our questions. We have developed and patented the method RBNCC (replicate based noise corrected correlation) where image noise, which is unavoidable and leads to the underestimation of the underlying correlation, can be eliminated from correlation measurements. We have performed detailed studies on coefficients developed for use in colocalisation analyses revealing that several are not fit for their purpose. We advocate that coloocalisation analysis should be divided into the two subgroups co-occurrence and correlation (Adler & Parmryd, 2013) and that only pixels where both fluorophores are present should be included in correlation analyses (Adler & Parmryd, 2014). We now investigate how deconvolution affects correlation analysis and how membrane topography can be accounted for in colocalisation analysis of membrane molecules.

Members of the group

Ingela Parmryd, associate professor

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Agencies that support the work

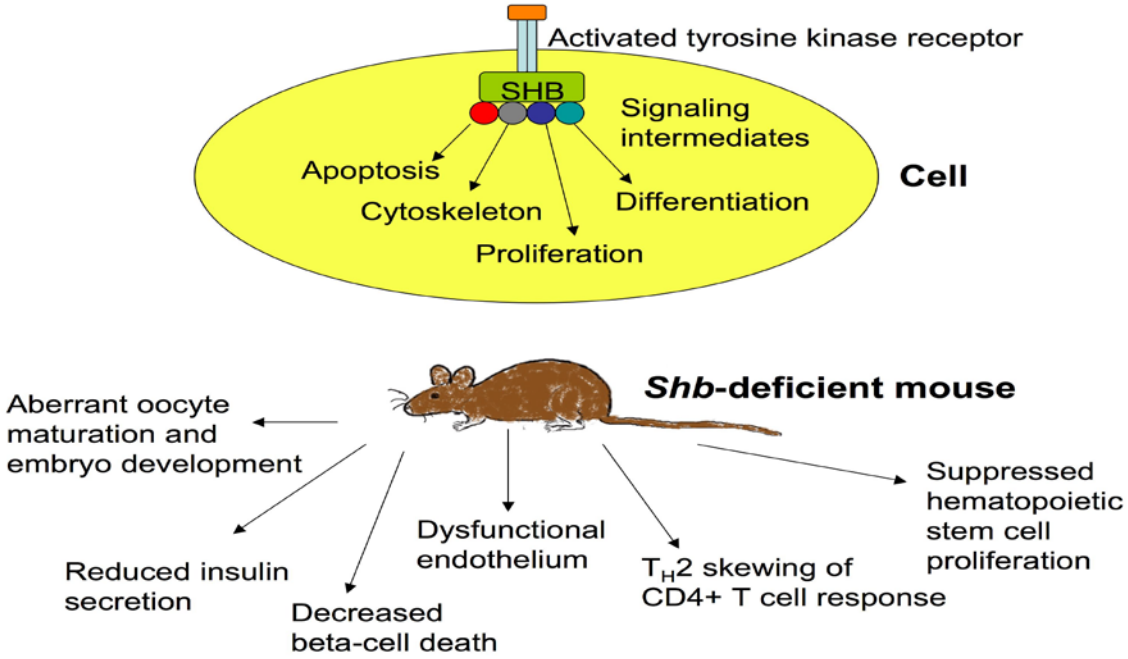
The Swedish Research Council

AFA Insurance

Importance of Shb-dependent signaling for angiogenesis and hematopoiesis

Michael Welsh

Shb is an SH2-domain adapter protein operating downstream of tyrosine kinase receptors such as VEGFR-2, FGFR-1, PDGF-receptors and the T cell receptor. The effects of Shb are pleiotropic and context dependent. We have recently generated a *Shb*-knockout mouse to assess the physiological relevance of Shb in vivo.



We observe impaired glucose homeostasis due to insufficient insulin secretion in Shb-deficient mice. In addition, the β-cells exhibit reduced stress sensitivity. These effects appear to be a consequence of constitutive FAK activation.

Shb-knockout mice display reproductive abnormalities with a transmission ratio distortion of the knockout allele related to female reproduction. Consequently, oocyte maturation is impaired in the absence of Shb and this relates to abnormal signaling via the ERK-RSK-S6 pathway. In addition to aberrant oocyte maturation, *Shb*-knockout embryos are morphologically abnormal and do not implant well.

Shb-knockout mice also display reduced angiogenesis and this causes diminished tumor expansion (subcutaneously injected tumor cells or inheritable RIP-Tag insulinomas). *Shb* deficient endothelial cells have abnormal cytoskeleton and adherens junctions that may contribute to deficient angiogenesis. In addition, *Shb*-knockout vascular physiology shows signs of compensatory mechanisms (increased blood flow velocity and an increased frequency of intermediately sized arterioles as determined by micro-CT) to counteract the adverse effects of the endothelial dysfunction. Although vascular performance under normal conditions appears relatively unaffected by the absence of *Shb*, recovery after ischemia was found to be impaired in both the cremaster and hindlimb muscles, which was primarily dependent on *Shb* deficiency in the vasculature and not in myeloid cells. Multiple signalling abnormalities in *Shb* knockout endothelial cells were noted, included elevated basal and reduced VEGF-stimulated FAK, ERK, Akt and Rac1 activities. The absence of *Shb* increases melanoma metastasis and the studies aim at identifying mechanisms explaining this finding. We have obtained the *Shb/loxP* mouse that conditionally deletes *Shb* in endothelial cells, providing additional means to analyse metastasis further. The cellular processes responsible for Shb-dependent signalling in endothelial cells are poorly understood but we are developing means to analyse the dynamics of SHB/VEGFR2, SHB/FAK and VEGFR2/FAK interactions in endothelial cells by TIRF (total internal reflection fluorescence) microscopy, which will aid us in improving the understanding of this process.

The absence of Shb exerts effects on hematopoiesis and peripheral T lymphocyte function. CD4+ T lymphocytes show a Th2 skewing of their response to stimulation in the absence of Shb and this could be of relevance for understanding allergic responses. *Shb* knockout hematopoietic stem cells show lower rates of proliferation due to elevated FAK signalling. Development of BCR-ABL1-induced leukemia was accelerated in the absence of Shb, again due to elevated FAK activity. Further studies will be conducted in order obtain a better understanding of Shb in leukemia.

Our current research effort is mainly focussed on investigating:

A) The relevance of vascular dysfunction as a consequence of Shb deficiency in endothelial cells for tumor metastasis

B) The development of leukemia in relation to Shb deficiency

C) Understanding the dynamics of SHB-VEGFR2-FAK interaction in endothelial cells using TIRF microscopy

Members of the group

Michael Welsh - Professor

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Maria Jamalpour – PhD-student

Xiujuan Li- Post-doc

Ilkka Pietilä- Post-doc

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Agencies that support the work

The Swedish Cancer Foundation

The Swedish Research Council

Stiftelsen Familjen Ernfors fond

EXODIAB

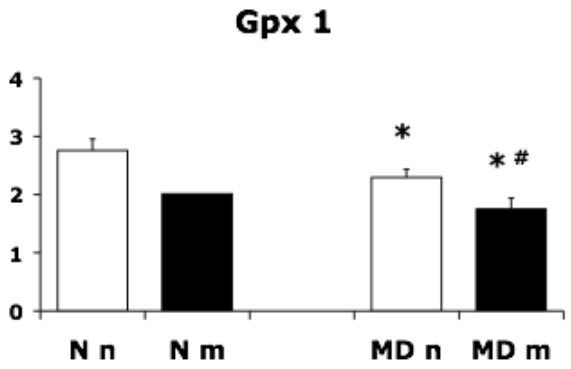
Complications in pregnancy

Ulf Eriksson, Parri Wentzel

We are studying different types of pregnancy complications, resulting in disturbed embryo-fetal development as a consequence of altered maternal metabolism (caused by diabetes, obesity, or ethanol intake). Our short-term aims are to clarify and understand the mechanisms and patterns of dysmorphogenesis; the long-term aim is to prevent the maternal and fetal damage. We work with animal models *in vivo*, and *in vitro* culture of whole embryos, embryonic tissues and embryonic cells.

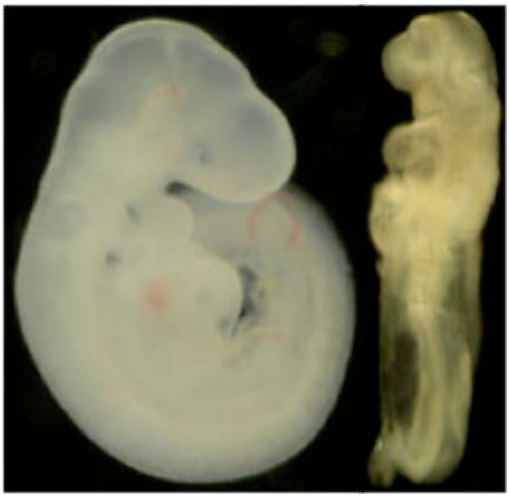
Diabetes in the pregnant women is associated with an increased risk for malformations in the offspring and preeclampsia in the mother. We have studied the mechanisms behind the disturbed development of the offspring in animal models, embryo culture, as well as by *in vitro* culture of embryonic tissues and cells. In earlier work, we reported the occurrence of oxidative stress in embryos exposed to a diabetic environment. We have been able to block the diabetes-induced damage to the embryo and fetus by several agents, such as arachidonic acid, inositol, N-acetylcysteine, BHT, vitamin E and C, and folic acid. We have also started to investigate the importance of genetic predisposition for the development of malformations, a project, which is currently yielding data regarding the importance of the maternal and fetal genomes and epigenomes for the development of fetal dysmorphogenesis in diabetic pregnancy.

We have identified one gene, Glutathione Peroxidase-1, which is underexpressed in malformed offspring of diabetic rats (compared with non-malformed offspring of same litter), and its gene product, the antioxidative enzyme Gpx-1, is less distributed in the embryonic tissues, and its enzymatic activity markedly decreased. These findings can be related to the enhanced oxidative stress involved in the embryo-fetal dysmorphogenesis of diabetic pregnancy.



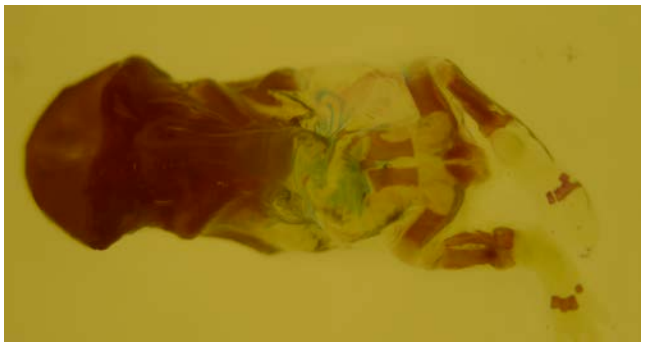
Decreased expression of Gpx-1 in malformed compared to non-malformed offspring of diabetic rats.

Recently we have found evidence for a new teratological pathway in diabetic pregnancy, activation of the receptor for advanced glycation end products (RAGE). We will persue this line of research by identifying the ligand(s) causing the RAGE activation, and by investigating the possible therapeutical effects of blocking the RAGE response in embryos exposed to a diabetic environment.



Obesity in the pregnant woman is associated with increased risk for congenital malformations, in particular the risk for neural tube defects and cardiac malformations been found to be increased. We are currently involved in creating an animal model for this type of pregnancy, as well as attempting to affect embryonic development *in vitro* by subjecting the embryos from control rats to serum from either control or high-fat diet rats in whole embryo culture for 48 hours. We found increased incidence of growth retardation and malformations in the embryos cultured in serum from hig-fat diet rats.

Rat fetus lacking tail, from obese mother



Intake of ethanol during pregnancy can harm the offspring; the risk increases with increased consumption. We have studied this situation, and attempted to alter the maternal defense against free oxygen radicals *in vivo* and *in vitro*, in order to diminish the ethanol-induced damage. We are studying possible biomarkers for maternal ethanol intake, by investigating embryonic tissues exposed to ethanol.

We are currently conducting a collaborative study on the dietary habits during pregnancy of women who have given birth to a child with Attention-Deficit/Hyperactivity Disorder.

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Publications 2014-

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Agencies that support the work

The Novo Nordisk Foundation

The Swedish Diabetes Association

Stiftelsen Familjen Ernfors fond

Pathogenesis of type 1 Diabetes Mellitus

Stellan Sandler

The prevailing view is that an autoimmune reaction selectively destroys the insulin-producing β-cells in the pancreas in type 1 diabetes (T1DM). The aim of this project is to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in this disease. We postulate that after certain types of damage β-cell function can be restored (Fig. 1). Furthermore, we believe that the β-cell is not a passive victim during a situation of potentially harmful exposure, but depending on gene expression and functional activity of the β-cell, the outcome can be affected. The aims of the present research projects are to investigate cellular and molecular mechanisms involved in pancreatic β-cell damage and repair in T1DM.

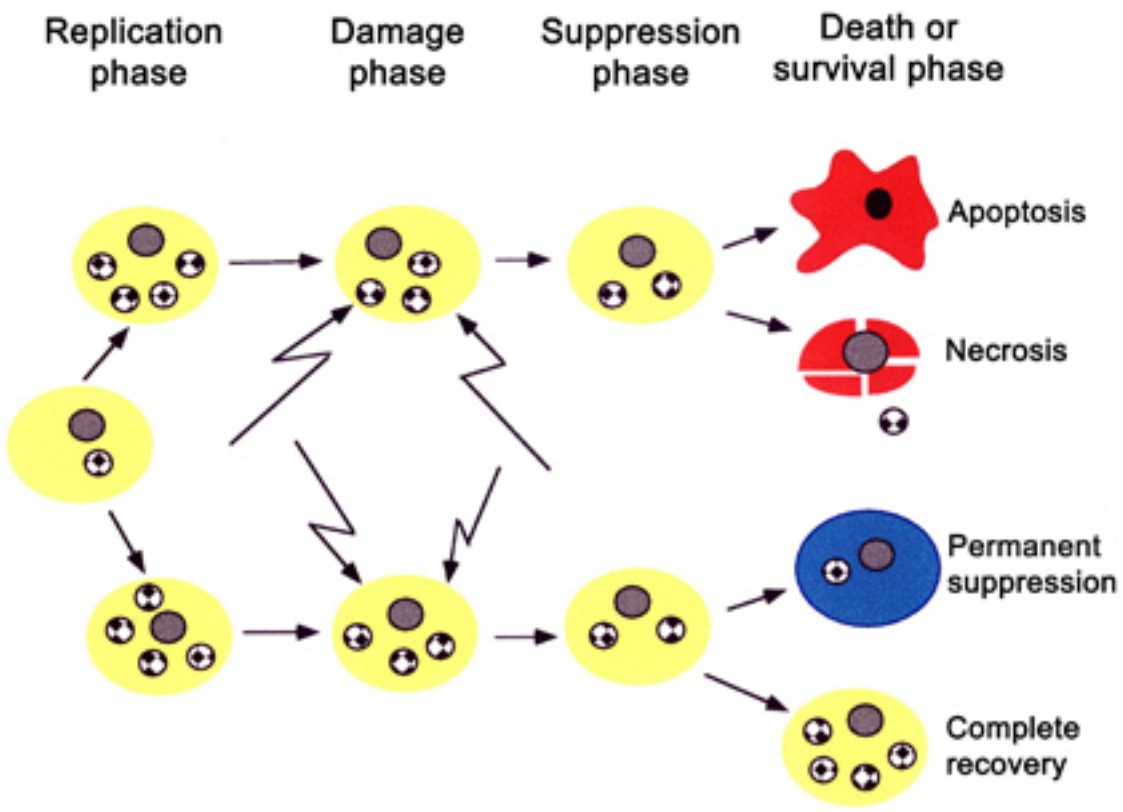

Fig. 1. Schematic view of the β-cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β-cell replication is increased, but later it becomes restricted. After birth β-cells acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate stimuli. At one or several occasions in life, β-cells in some individuals are subject to damage (irregular arrows) which will lead to suppressed β-cell function and a reduction in insulin secretion. Depending on the genetic predisposition an autoimmune reaction will be launched which in certain individuals will cause extensive cell death leading to type 1 diabetes. In other individuals β-cells will survive, but their secretory function is impaired, which may have consequences for the glucose homeostasis. In some other individuals the β-cells may completely recover and the glucose tolerance will only be transiently disturbed. The latter outcome is most likely also dependent on genes regulating β-cell resistance to damage and β-cell repair.

Topics that are currently being investigated

A. Characterization of the regulatory T cell response in diabetic mice

B. Exploration of the bank vole as an animal model for human diabetes

C. Antiviral intervention in NOD mice

Example of findings and hypopthesis

Role of regulatory T-cells (T_{reg}) in T1DM (cf. topic A)

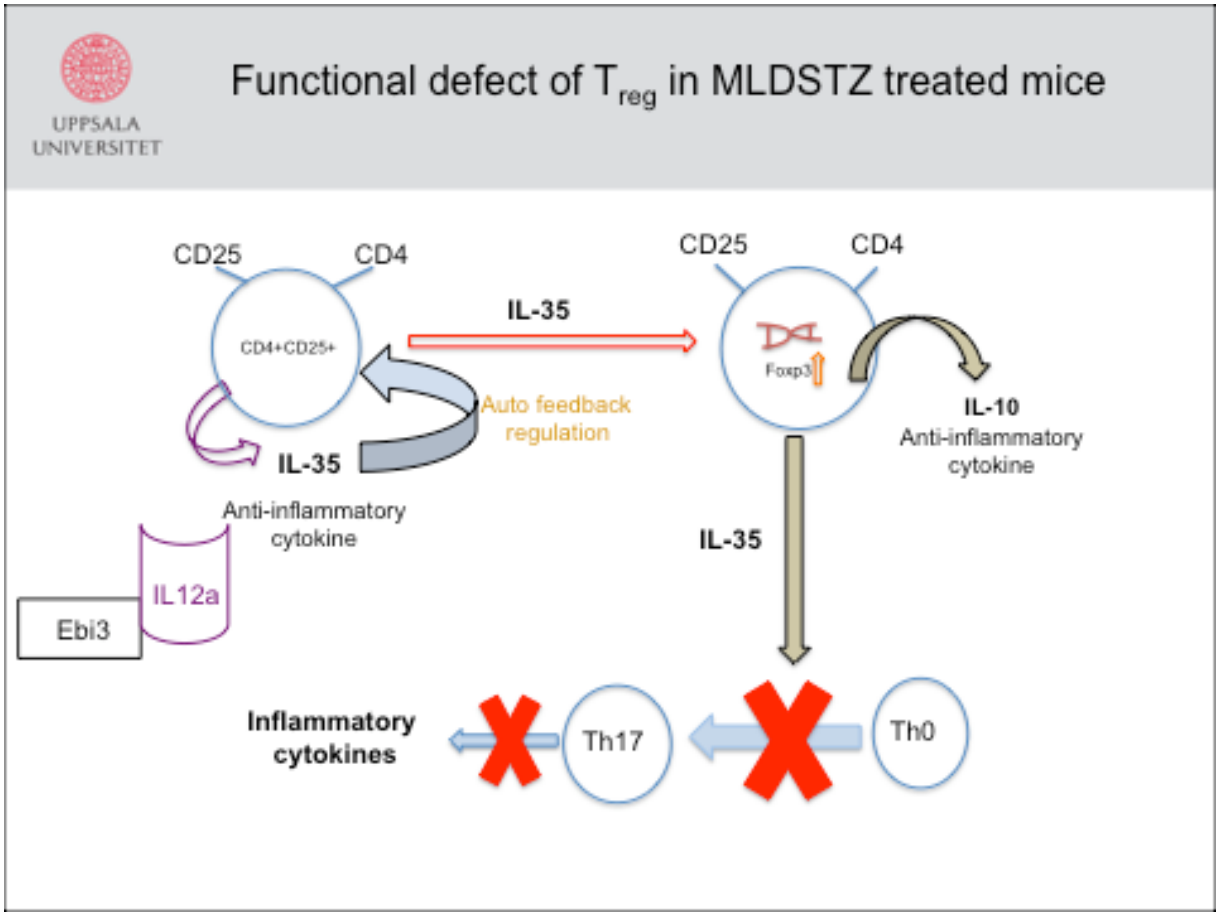

Fig. 2. Based of a number of different experiments we propse that Tregs in the multiple low dose streptozotocin (MLDSTZ) model of T1DM are functionally impaired, since a key cytokine (IL-35) is not being up-regulated in response to the proinflammatory environment induced by MLDSTZ.

Currently we are investigating the role of IL-35 in human T1DM. For instance we have found a correlation between remaining C-peptide levels in T1DM and IL-35. PhD student was awarded the Young Investigator Award from the Scandinavian Society for the Study of Diabetes in April 2016 for best published article year 2015 (reference #15 below).

Pancreatic islet in a diabetic bank vole

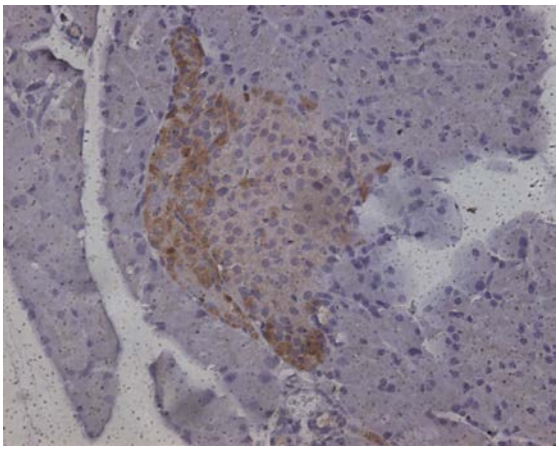

*Fig. 3. Pancreatic islet of a female colonized bank vole 18 weeks of age. The bank vole was hyperglycemic (17.4 mM) 120 min after the IPGTT and serum insulin was elevated (2.34 mg/ml). The section was IHC stained with an Ljungan virus-specific antibody (brown colour) showing strong staining in some areas and weaker staining in the remaining area of the islet. Magnification 400X (*cf Aim D above)

Significance

The aims of the present research projects are to investigate cellular and molecular mechanisms related to pancreatic β-cell damage and repair in T1DM, and in some cases probably also in T2DM. It is anticipated that a deeper knowledge of these issues will lead to new strategies for intervention in the autoimmune β-cell destructive processes, as well as novel methods to enhance β-cell resistance against direct cytotoxic damage. We hope that by studying cell signaling and the mechanisms leading to β-cell death, it will be able possible to elucidate which factors that are crucial for β-cell survival and possibly indentify candidate genes/proteins conferring β-cell susceptibility or resistance to destruction in T1DM.

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Alnalysis of pancreatic islet morphology of diabetic bank voles revealed alterations seen in type 2 diabetes.

Submiitted

Members of the research group

The following colleagues are engaged in the projects described above:

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Agencies that have supported the work

The Swedish Research Council

The European Foundation for the Study of Diabetes

The Swedish Diabetes Association

Barndiabetesfonden

Stiftelsen Familjen Ernfors fond

Role of tyrosine kinases in β-cell apoptosis and diabetes

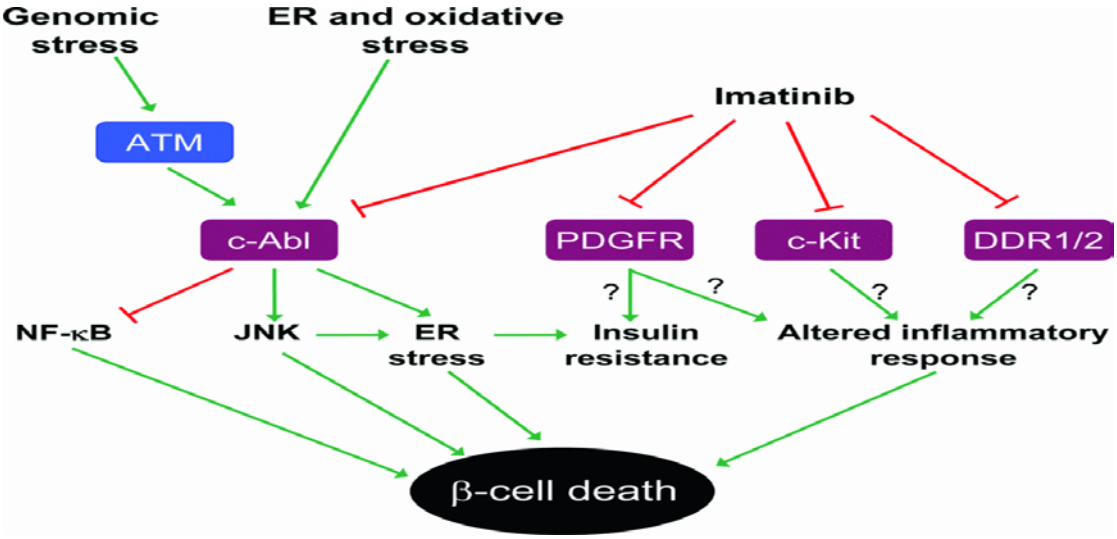
Nils Welsh

It has recently been observed that patients suffering from both leukemia and diabetes were cured from not only leukemia, but also diabetes, when treated with the tyrosine kinase inhibitor Imatinib. (Veneri et al., N Engl J Med. 2005 352:1049-1050). An anti-diabetic action of Imatinib in Type 2 diabetes is further supported by our recent observation that Imatinib

counteracts high-fat diet induced insulin resistance and hyperglycemia in rats (Hägerkvist et al., Clinical Science, (Lond). 2008 114(1):65-71). Moreover, in a study from 2009, Imatinib was also observed to induce remission of diabetes in db/db mice, possibly via decreasing insulin resistance and increasing the beta-cell mass (Han et al., Diabetes. 2009 58(2):329-3). Thus, in both animal models and in Type 2 diabetes patients Imatinib seems to improve glycemic control, possibly via an insulin sensitizing effect.

Imatinib appears to prevent and reverse not only Type 2 diabetes, but also diabetes of animal models with a Type 1 diabetes resembling disease. We have shown that Imatinib protects against beta-cell death in vitro and prevents diabetes in NOD mice and in streptozotocin-diabetic mice, both models for human beta-cell destruction and Type 1 diabetes (Hagerkvist et al., FASEB J. 2007 Feb;21(2):618-28, Hagerkvist et al., Cell Biol Int. 2006 30(12):1013-7). More recently, it has been observed by others that both Imatinib and Sunitinib not only prevented, but also reversed new-onset diabetes in NOD mice (Louvet et al., Proc Natl Acad Sci U S A. 2008 105(48):18895-900). Thus, there exists proof-of-principle in animal models for an anti-diabetic effect of Imatinib and similar tyrosine kinase inhibitors, and that a limited treatment period will not only reverse diabetes, but also mediate long-term protection against re-precipitation of the disease. This has led us (Mokhtari and Welsh, Clin Sci (Lond). 2009 118(4):241-7) and other investigators to propose clinical trials in which Imatinib is given to new-onset Type 1 diabetes patients.

The work by others and us indicates that Imatinib counteracts diabetes via different molecular mechanisms (Figure 1).



It appears that the four known targets of Imatinib, c-Abl, PDGFR, c-Kit and DDR1/2, may all play a role in the pathogenesis of diabetes. C-Abl is a proapoptotic tyrosine kinase that promotes beta-cell death when activated. Improper activation of the PDGF receptor has also been reported to occur in diabetes, and this may lead to increased insulin resistance of peripheral tissues. Activation of c-Kit and DDR1/2 is known to affect innate immunity, a component of the immune system that promotes inflammation and beta-cell dysfunction. Thus, it is conceivable that Imatinib, by targeting several pathways simultaneously, mediates a

Figure 1 Possible mechanisms for the anti-diabetic effects of imatinib
Imatinib is known to inhibit the tyrosine kinases c-Abl, PDGFR, c-Kit and DDR1/2. Most likely, imatinib-induced protection against diabetes is mediated not by one single pathway, but via different molecular mechanisms. β-Cell survival is promoted by inhibition of c-Abl, which leads to decreased activation of the pro-apoptotic MAPK JNK and increased activation of the anti-apoptotic transcription factor NF-κB. c-Abl inhibition might also lead to a dampened ER-stress response, via JNK or other pathways. Inhibition of PDGFR could contribute to decreasing peripheral insulin resistance and inflammatory processes, thereby promoting β-cell survival. Moreover, inhibition of c-Kit and DDR1/2 might also add to the anti-diabetic effects of imatinib, possibly by interfering with inflammatory responses.

stronger antidiabetic effect than other drugs that affect only one particular pathway.

It is the aim of this project to elucidate closer the mechanisms by which tyrosine kinases control beta-cell death and function. We are currently investigating Imatinib-mediated control of AMPK, NF-kappaB, JNK, p38, PI3-kinase, SHIP2, PTEN, FAK, IRS1/2, beta-catenin, AKT and ERK signaling events. For this purpose insulin producing cells, either at basal conditions or under stress, are analyzed by immunoprecipitation, immunoblotting, confocal microscopy, real-time PCR, microarray analysis, flow cytometry and gel shift analysis. Cells are also genetically manipulated by lentiviral vectors to achieve up-or down-regulation of specific gene products. Signaling events will be correlated to beta-cell survival and function, as assessed by analysis of insulin production and apoptotic events. This will hopefully lead to a better understanding of the molecular events by which Imatinib protects against diabetes. Such improved knowledge may pave the way for a novel and improved treatment of diabetes.

Members of the group

Camilla Krishanovskii - Post-doc

Kyril Turpaev - Post-doc

Xuan Wang – Post-doc

Andris Elksnis – PhD student

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Agencies that support the work

The Swedish Diabetes Association

Stiftelsen Familjen Ernfors Fond

Diabetes Wellness

Barndiabetesfonden

EXODIAB

Renal Physiology

A. Erik Persson

The renal control of excretion is essential for fluid balance and blood pressure. One factor of great importance in regulation of fluid excretion is the tubuloglomerular feedback (TGF) control mechanism in the juxtaglomerular apparatus (Fig1). The macula densa cells in the distal part of the nephron senses the fluid flow rate. This information is used to activate the extraglomerular mesangial cells that modulate the response via influences from both hormones

and fluid volume balance factors. Activation of the TGF mechanism finally leads to a contraction of the afferent arteriole. Renal renin release from the granular cells of the juxtaglomerular apparatus is controlled via the same mechanism.

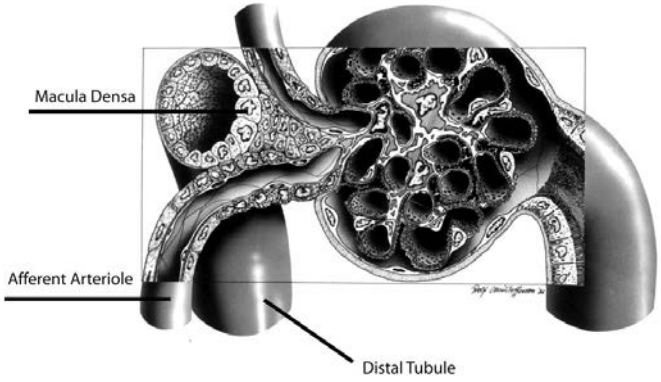

Figure 1. Schematic illustration of the juxtaglomerular apparatus (JGA) with the macula densa cells in wall of the distal tubule and the glomerular arterioles.

Our group studies how hormones and other factors, e.g. nerves and NO, influence the overall function of the TGF mechanism and renin release using micropuncture techniques. We also employ isolated perfused tubule and arteriole techniques using fluorophores and digital imaging methods to determine calcium, chloride and NO in the macula densa cells and in the arteriolar smooth muscle cells. NO is also measured via microelectrodes. These techniques are used to investigate the sensing step in the TGF, the modulation step in the mesangial cells and the calcium release and contractile response of the arterioles. The juxtamedullary nephron preparation is used to visualise afferent arteriolar endothelial cells to measure calcium and NO. Our studies aim at understanding how the TGF mechanism and renin release operates, the effect of renal oxidative stress, NO and nerves on kidney function and to find the mechanism responsible for development of arterial hypertension.

Arterial hypertension is one of the most important health problems in the Western world and an important risk factor for cardio-vascular disease (CVD) and stroke. Unfortunately, these risk factors are only partly reduced during treatment with the existing drugs. Patients with treatment for hypertension have a reduced risk for stroke of about 50 % but still a 5 times higher risk than those without hypertension. The risk for CVD is only reduced 15 % with treatment and there is a 6-7 times higher risk for CVD compared to individuals without increase in blood pressure. Therefore it is important to further investigate how hypertension develops and find new and effective principles to prevent and treat the disease. Reduction of renal oxidative stress may increase nitric oxide (NO) bioavailability and thereby play an important role in preventing and/or treating CVD. To investigate the potential roles of oxidative stress and NO-deficiency in the development of CVD. Treatment modalities that reduce oxidative stress and/or increase NO-bioavailability will be assessed in both experimental models and clinical trials in order to find new and more efficient ways to treat or prevent CVD.

We have advanced equipment for investigating renal and cardiovascular function, and imaging systems for measuring oxidative stress and NO production (in vivo and in vitro). In collaborations with physicians at different hospitals we have clinical trials to investigate the potential role of oxidative stress and NO-deficiency in CVD. In our experimental and clinical studies we aim to further investigate the link between renal and cardiovascular dysfunction, and to explore the potential benefits from reducing oxidative stress (e.g. antioxidant, nitroxide, low-sodium treatment) or increasing NO production (e.g. L-arginine or nitrate supplementation). The juxtaglomerular apparatus is a critical regulator of glomerular filtration rate, fluid excretion and renin release, factors that determine blood pressure. We believe that treatment strategies aiming to reduce oxidative stress and/or increase NO-bioavailability could be of great value in the future to treat hypertension to prevent stroke and cardio-vascular disease.

Members of the group

A. Erik Persson - Professor emeritus

Mattias Carlström - Researcher

Andreas Patzak - Guest researcher

Suênia Sampaio-Guest resercher

Gau Xian – Post doc

Ammar Farman - Graduate student

Peter Flacker- Graduate student

Zheng Bing Zhuge-Graduate student

Josiane de Campos Cruz-post doc

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Agencies that support the work

The Swedish Research Council

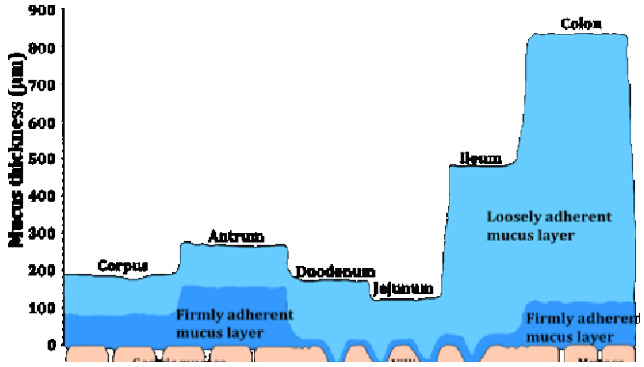
STINT

Gastro-intestinal protection mechanisms studied in vivo

Lena Holm

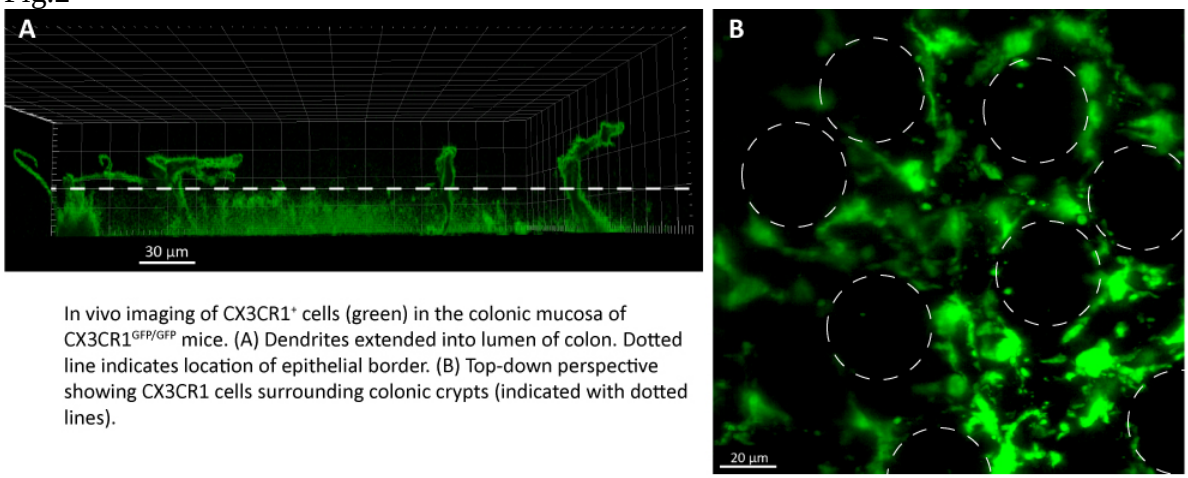
During homeostasis, the colonic mucus successfully separates the vast luminal microbiota from the single epithelial cell layer and resident immune cells of the mucosa. When this barrier fails, colitis is established. Our research focuses on **the interplay between the commensal microbiota, administered probiotics and the colonic mucosal barrier in health and during colitis**, with special emphasis on the underlying mechanisms of colitis induction and probiotic protection. We have developed an animal model allowing direct access to the colonic mucosa with **intravital microscopy**, and the majority of our experiments include *in vivo* studies of the mucus layers (Fig. 1), epithelium, immune cells and blood flow.

Fig 1. The mucus layers in different parts of the GI tract



During the last years we have extensively increased our possibilities to perform high-resolution longitudinal *in vivo* studies of interactions of the microbiota/probiotics/mucus with the epithelium and immune cells in real-time by adapting our *in vivo* model to high-speed confocal microscopy available in our lab (Fig.2). Furthermore, with a recently installed Laser Speckle Contrast Analysis setup, blood flow of colitic and healthy parts of the colon will be performed.

Fig.2



In vivo imaging of CX3CR1^{+} cells (green) in the colonic mucosa of CX3CR1$^{GFP/GFP}$ mice. (A) Dendrites extended into lumen of colon. Dotted line indicates location of epithelial border. (B) Top-down perspective showing CX3CR1 cells surrounding colonic crypts (indicated with dotted lines).

The influence of pre- and pro-biotics on mucus dynamics, bacterial composition, inflammatory variables and epithelial tight junctions are studied to elucidate the mechanisms behind their protective effects (Fig. 3). We have shown that pretreatment with **probiotics** (*L. reuteri*) prevent DSS-induced colitis in rats and mice. Up regulation of P-selectin in the colonic venules was prevented by probiotic therapy, and in vivo fluorescence microscopy confirmed these results by showing decreased leukocyte rolling and adhesion to endothelial cells, as well as decreased platelet-endothelial cell interactions. There are no intestinal *in vitro* culture systems that replicate the complexity of the secreted mucus barrier. However, our *in vivo* model uniquely enables reliable measurements of thickness and permeability of the mucus barrier. Using this model we have demonstrated that the adherent gastric and colonic mucus gel in vivo can be divided in two layers, a firmly and a loosely adherent layer (Fig 1). The firmly adherent mucus layer acts as a barrier towards hydrochloric acid in the stomach and luminal bacteria in the colon. In addition to the barrier function of the firm mucus resulting in significantly lower number of bacteria than in the loosely adherent mucus (1/10), we found that the composition of the microbiota differed substantially between the two layers. The difference in bacterial numbers and composition was completely eradicated in DSS-induced colitic rats, where high levels of translocated bacteria were found in the mesenteric lymph nodes. Interestingly, pretreatment with *L. reuteri* (cocktail of 4 strains) prevented bacterial translocation and colonic inflammation but did not influence on the distorted mucus microbiota. Furthermore, we showed that each of two different *L. reuteri* strains protected mice against DSS-induced colitis. Mechanisms behind this protection involved increased firmly adherent mucus thickness and tight junction expression. We have also constructed luminescent and fluorescent *L. reuteri*

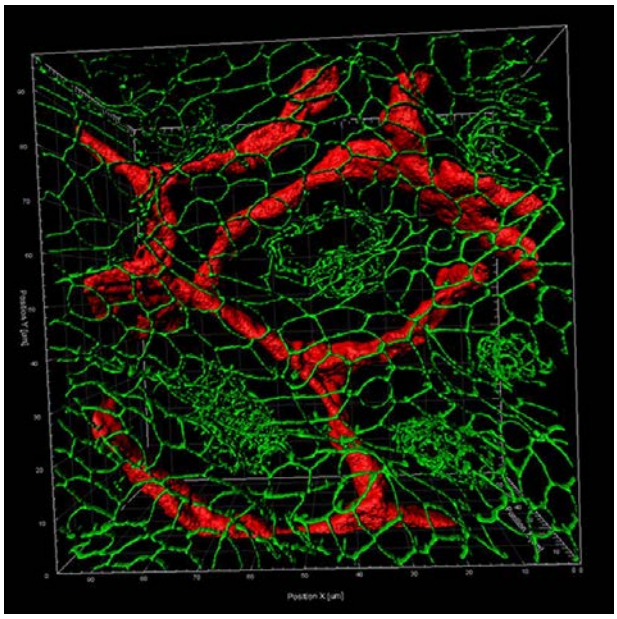


Fig 3. 3D-visualization of capillaries (stained with CD31: red) surrounding colonic crypts just below the luminal epithelial layer (tight junctions stained with ZO-1: green).

R2LC, which will provide useful tools for future *in vivo* studies of interactions between the bacteria and the host.

We have shown that dietary nitrate induces potent protection against NSAID induced upper GI inflammation. Bacteria in the oral cavity reduce nitrate to nitrite, which is further reduced to nitric oxide, NO, in the acidic stomach. NO strengthen the mucosal barrier by increasing mucus thickness and blood flow. We have, however, also shown protection by dietary nitrate even further down in the intestine where luminal NO is not increased. Leukocyte recruitment in response to proinflammatory chemokine and NSAID was decreased. Despite attenuation of the acute immune response, the overall ability to clear a bacterial infection was not suppressed.

Members of the group

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Shokoufeh Karimi, PhD student*

Haoyu Liu, PhD, post doc

Richard Shore, PhD student**

Tomas Waldén, PhD, post doc

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Agencies that support the work

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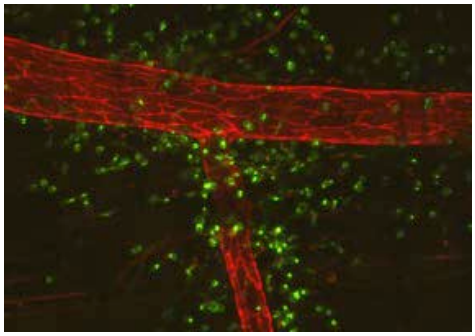
BioGaia AB

Nanna Swartz foundation

Targeting leukocytes in health and disease

Mia Phillipson

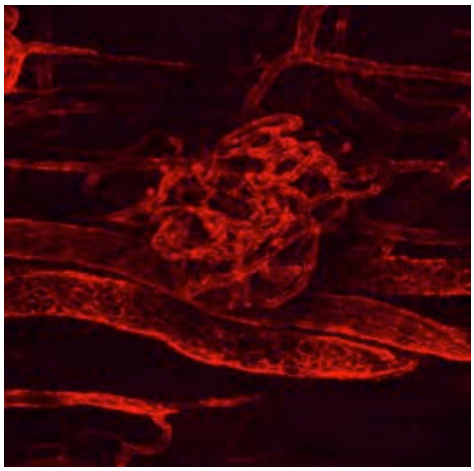
The capacity of circulating leukocytes to arrest on the surface of inflamed endothelium, transmigrate, and penetrate into the underlying tissue are key steps in response to infections as well as other inflammatory processes. The importance of recruited as well as tissue resident leukocytes also during homeostasis, angiogenesis and tumor growth is increasingly being acknowledged. Expanding the knowledge of the mechanisms that regulate the recruitment and actions of leukocytes is very important to be able to control and eventually limit inflammatory response, tumor growth and tissue damage.



A venule (anti-CD31, red) with emigrated neutrophils (anti-Gr1, green).

The overall aim of the research conducted in my laboratory is to uncover novel roles of leukocytes and to find means to regulate their specific functions in settings spanning from organ development to tissue healing, angiogenesis and inflammation. By employing state of the art techniques for studies of leukocyte trafficking and interactions *in vivo* (high speed confocal microscopy), we are delineating how leukocytes are recruited to sites of inflammation or hypoxia as well as their effector functions in tissue. The signals and chemokines initiating

leukocyte recruitment as well as the adhesion molecules involved in the different steps of the leukocyte recruitment cascade are being investigated. We have established a new step in the leukocyte recruitment cascade, intravascular crawling, and study how chemokines are transported into the inflamed venules. We recently found that chemokines sequestered on endothelial heparan sulphate direct crawling leukocytes towards optimal sites for transmigration (Massena et al., Blood, 2010). We also investigate how the intestine can withstand the constant pressure of the commensal bacterial flora without developing inflammation, and are presently mapping the role, behaviour and interactions of different intestinal leukocytes during homeostasis as well as colitis.



The reestablished glomeruli-like islet vasculature surrounded by muscle blood vessels two weeks after transplantation to striated mouse muscle.

In addition, we recently identified a clinically relevant and attractive approach of curing type 1 diabetes, since islets transplanted to muscle became fully revascularized and therefore functioned better compared to islets implanted in the liver (Christoffersson et al., Diabetes, 2010), the organ traditionally used for islet transplantation. Means to limit the immunosupressing therapies required following allogeneic islet transplantations have also been investigated (Vågesjö et al. Cell Transplatation, 2015). A specific neutrophil subtype with pro-angiogenic features was recently demonstrated in the circulation, and was found to be recruited to sites of hypoxia by Vascular Endothelail Growth Factor A (VEGF-A) (Christoffersson et al., Blood, 2012, Massena et al., Blood, 2015). We currently aim to accelerate angiogensis and woud healing by affecting the microenvironment to induce specific leukocytes recruitment as well as phenotype shifts of tissue resident leukocytes.

Members of the group

Mia Phillipson - Professor

David Ahl – PhD student

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Antoine Giraud – Research Engineer

Carmen Herrera Hidalgo – PhD student

Jalal Lomei – PhD student

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John Sedin – Post Doc

Cédric Seigneiz – Post Doc

Hava Lofton Tomenius - Reseracher

Evelina Vågesjö – Post Doc

Tomas Waldén – Post Doc

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2. C Seignez, **M Phillipson.** The multitasking neutrophils and their involvement in angiogenesis. *Current Opinion in Hematology* (Woltwers Kluwer Health), 24:3-8, 2017.

3. J Kreuger, **M Phillipson**. Targeting vascular and leukocyte communication in angiogenesis, inflammation and fibrosis. *Nature Reviews Drug Discovery*, 15:125-42, 2016.
4. E Vågesjö, L Jansson **M Phillipson**. Ongoing research in the diabetes field, Chapter in ***Handbok för nybörjare, Typ 1 Diabetes, Det är ju bara en spruta***, Dr Eva Ludvigsen, Springer, ISBN: 978-9187595240, 2016
5. **M Phillipson,** L Claesson-Welsh, M Welsh. VEGFA-induced vascular permeability and leukocyte extravasation. ***Endothelial Signaling in Development and Disease***, Drs Mirko HH Schmidt and Stefan Liebner, Springer, ISBN: 978-1-4939-2907-8, 2015.

Dissertations

Vågesjö, E.(2016).Exploring immune cell functions and ways to make use of them.

Agencies that support the work

Knut and Alice Wallenberg foundation

Ragnar Söderberg Foundation

Swedish Foundation for Strategic Research

Swedish Research Council

The Diabetes Wellness Foundation

The Ernfors family foundation

The Novo Nordic Foundation

The Swedish Diabetes Foundation

Vinnova

The research group also takes part of the strategic funding for Diabetes (*Excellence of diabetes research in Sweden; Exodiab*) shared between Lund and Uppsala Universities

Renal physiology and kidney disease

Fredrik Palm

Diabetic Nephropathy

Diabetes mellitus is the most common cause for end-stage renal disease. The exact mechanisms mediating diabetes-induced kidney damage (diabetic nephropathy) are largely unknown despite intense research. The aim of this research program is to study effects of diabetes on renal metabolism and microcirculation in relation to functional changes. The ultimate goal is to find new treatment strategies to avoid the development of kidney dysfunction during diabetes.

We were the first laboratory to report kidney hypoxia in diabetes (Palm et al., Diabetoligia 2003, 46(8):1153-1160) and this finding has recently been confirmed in diabetic patients with established nephropathy (Wang et al., J Magnet Res Imag 2011, 33(3):655-660). Since then,

our work has focused on identifying the mechanisms resulting in the diabetes-induced kidney hypoxia. So far, we have identified several contributing mechanisms, including increased oxidative stress, altered red-ox balance, increased renal oxygen consumption and increased tubular electrolyte transport work due to both increased glomerular filtration, but also increased glucose transport in the proximal tubule. Recently we have made a very significant observation in rats treated with the mitochondrial uncoupler dinitrophenol for up to four weeks. These otherwise healthy rats displayed excessive oxygen utilization, due to the uncoupled mitochondria, and developed pronounced kidney hypoxia. Interestingly, these rats also displayed 50% increased urinary protein excretion, tubulointerstitial damage and infiltration of immune cells. Therefrore, we are the first to show that increased oxygen utilization is enough to cause kidney hypoxia and nephropathy. This is a majopr breakthough sine previous studies always have been associated with confounding factors, such as hyperglycemia, increased oxidative stress and altered tubular transport.

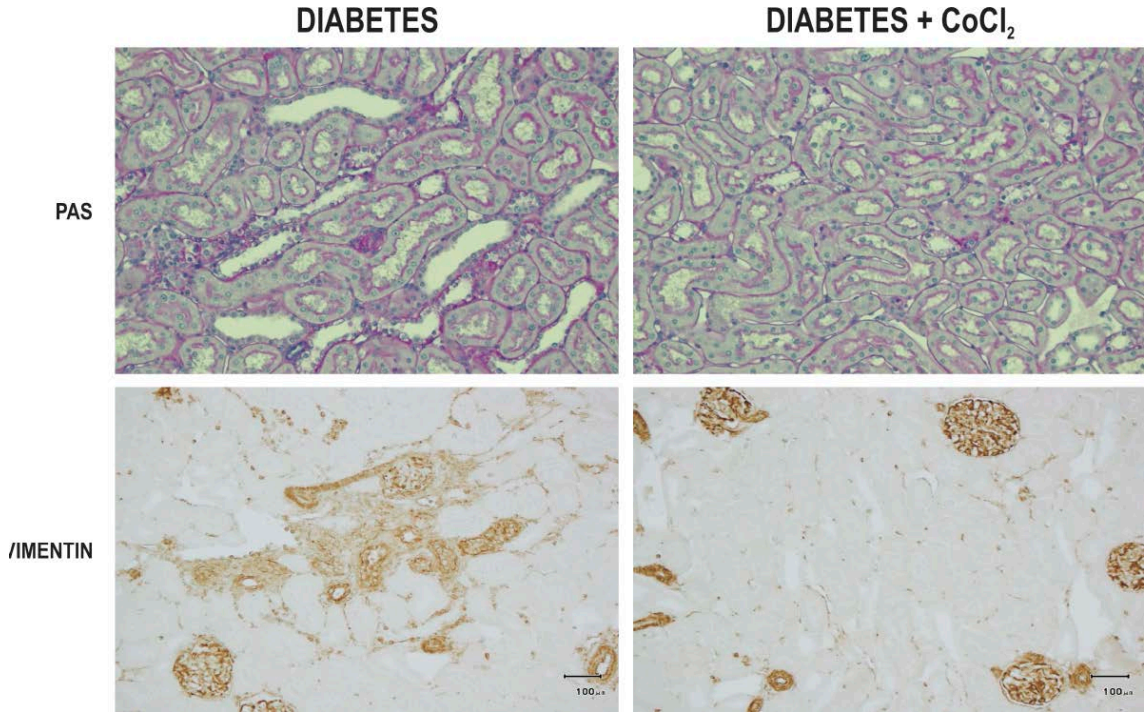


Figure 1. Activation of the hypoxic gene response by chronic $CoCl_2$ treatment prevented the diabetes-inudced kidney hypoxia and the clinical signs of diabetic nephrpathy, such as tubulointerstitial fibrosis, proteinuria and glomerular hyperfiltration.

We have also approached the problem with kidney hypoxia from another angle by chronically treating diabetic animals with $CoCl_2$, which activates the hypoxic gene response (HIF). The results demonstrate that HIF activation prevents the diabetes-induced kidney hypoxia and tubulointerstitial damage (Fig. 1 and 3).

Metabolic and functional alterations occurring in kidneys from diabetic animals (rats and genetically modified mice) are studied using in vivo techniques and molecular biology. Mitochondrial function and internal defence mechanisms are studied in diabetic animals and kidney tissue from diabetic patients. Renal blood flow and oxygen metabolism are studied using Magnetic Resonance Imaging (MRI) in animals as well as in diabetic patients.

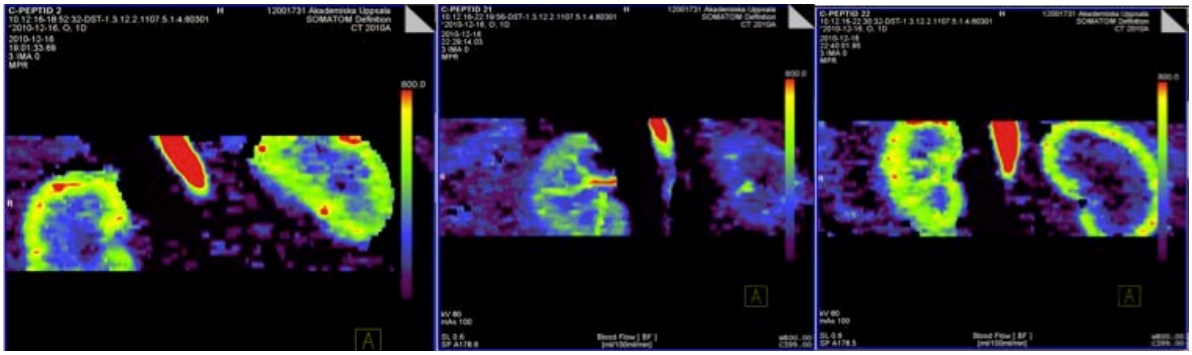


Figure 2. Thirty minuites of warm ischemia to the left kidney (right kidney on the images) did not alter kidney function or the intrarenal blood flow (images abow measured by computed tomography) in control rats four weeks after the ischemic insult. However, the same ischemic insult caused markedly reduced kidney function (glomerular filtration rate about 10% of normal), atrophy and and hypoperfusion of the left kidney. Diabetic rats administered a bolus dose of C-peptide before the ischemic insults were protected against the increased susceptibility to the ischemia-reperfusion injury.

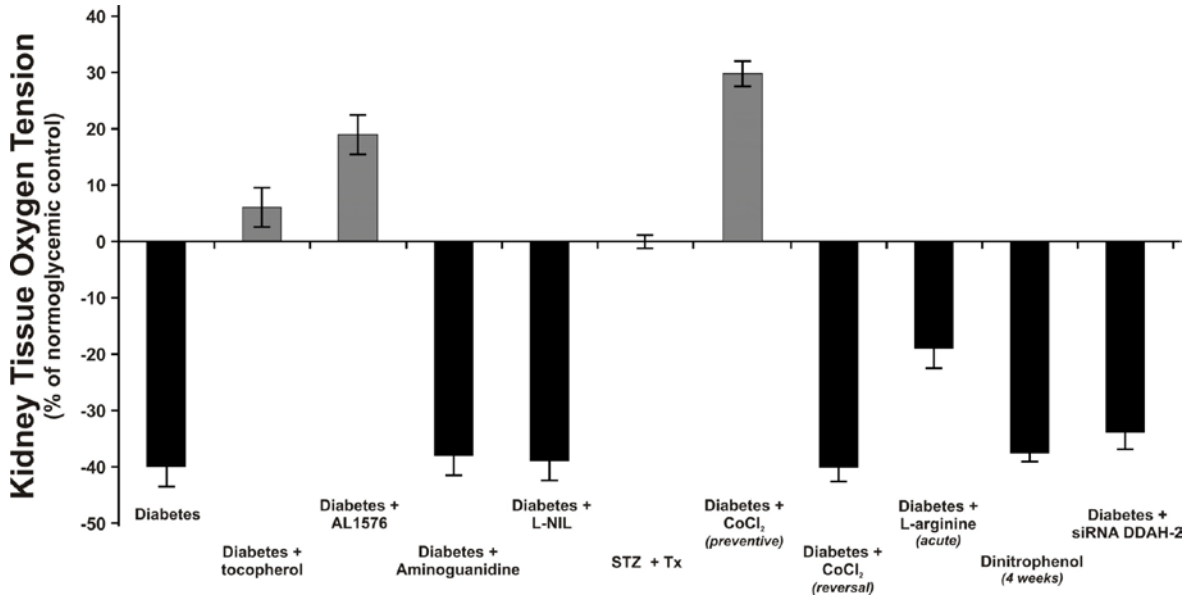


Figure 3. Summary of all investigated mechanisms affecting intrarenal oxygenation in diabetes. Tocopherol (vitamin E) is an antioxidant, AL1576 inhibits aldose reductase and presents activation of the polyol pathway, aminoguanidine inhibits AGE formation, L-NIL inhibits iNOS, STZ + Tx denotes animals administered streptozotocin (to induce diabetes) and 24h thereafter received enough islets of Langerhan's to reverse the hyperglycemia, $CoCl_2$ activates HIF and prevents kidney hypoxia if starting treatment early (preventive) but fails to reverse already established nephropathy (reversal), acute L-arginine administration induces NO release and partly restores kidney oxygenation, chronic dinitrophenol administration results in excessive oxygen utilization causing kidney hypoxia and clinical signs of nephropathy, siRNA directed against DDAH-2 reduced DDAH-2 protein expression by more than 60% but failed to normalize kidney oxygenation.

These non-invasive techniques were used in a recent study, in which we studied the effect of ischemia-reperfusion injury in diabetic kidneys (Fig. 2). It is well-known that diabetic kidneys are increasingly susceptible to an ischemic insult, but we were able to show that administration of a bolus dose of C-peptide had pronounced renoprotective effects in diabetes. Interstingly, we have previously shown that C-peptide reduces oxygen utilization in the diabetic kidney and this might therefore explain the renoprotective effects against the ischemic insult in these kidneys. This fidning might have important clinical implications since C-peptide is an endogenous substance, which therefore only needs relatively minor administrative work before moving into clinical practice.

By combining basic renal and diabetic research, we believe we can contribute to increase the understanding of the mechanisms involved in diabetic nephropathy, which will facilitate development of novel therapies. Additionally, metabolic alterations always precede histological changes, which potentially can be used as a clinical diagnostic tool when identifying patients at increased risk to develop diabetic nephropathy. This would hopefully enable early treatment modalities before the seemingly irreversible histological changes occur with manifest nephropathy.

Our results so far suggest:

A) Diabetic rats display kidney hypoxia, which is linked to excessive oxygen utilization.
B) Mitochondrial uncoupling results in excessive oxygen utilization and development of nephropathy.
C) C-peptide protects the diabetic kidney against ischemic insults, which may in part be

explained by the oxygen utilization-lowering effects of C-peptide in diabetes.
D) By using non-invasive imaging techniques, we may be able to transfer our knowledge from our experimental settings into clinical use.
E) Intrarenal hypoxia per se causes kidney disease.

Agencies that support the work

Swedish Research Council

Swedish Diabetes Association

Family Ernfors Foundation

Magnus Bergwall Foundation

Åke Wiberg Foundation

ERC Marie Curie IRSES

Uremic Toxins (core director: Lina Nordquist)

In uremic patients, losses of kidney function are accompanied by deteriorating organ function attributable to the accumulation of uremic retention solutes. Compounds that exert an adverse biologic impact are called uremic toxins

Indoxyl sulfate is a representative uremic toxin made by the liver from indole produced by gut bacteria from tryptophan. In addition to causing uremic symptoms, indoxyl sulphate per se accelerates the progression of renal failure. Our recent study for the first time demonstrated that indoxyl sulfate increases oxygen consumption and aggravates local hypoxia in renal tubular cells via enhancement of oxidative stress (Fig. 4). Uremic states per se may accelerate progression of renal dysfunction via aggravation of chronic hypoxia as a final common pathway to end stage renal disease.

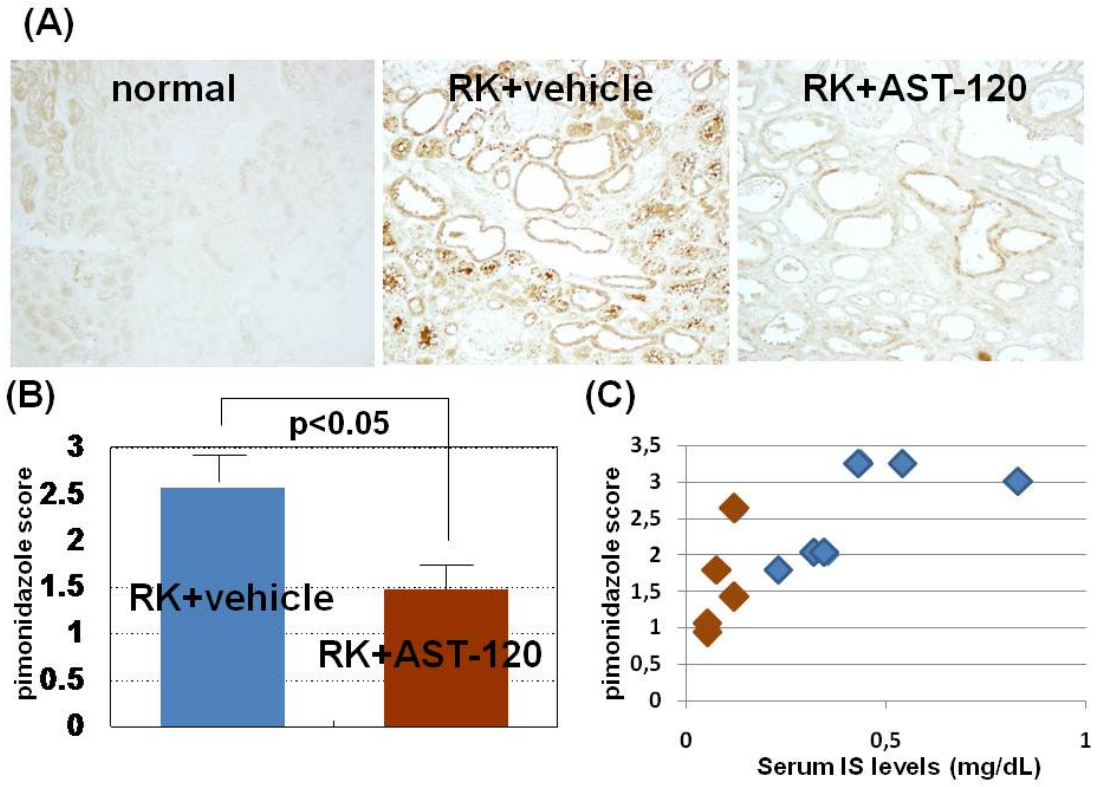


Figure 4. Improvement of oxygenation of the remnant kidney by reduction of uremic toxins. (A) Immunohistochemical staining of pimonidazole accumulation in the cortex showed improvement of oxygenation of the remnant kidney in animals treated with the oral absorbent AST-120 that reduces plasma levels of indoxyl sulfate. No pimonidazole accumulation was observed in cortical tubules of normal animals. (B) Semi-quantitative analysis of pimonidazole accumulation confirmed better oxygenation of the remnant kidney in rats treated with AST-120. (C) Pimonidazole accumulation, an indicator of hypoxia, showed a good correlation with serum IS levels in RK rats.

Agencies that support the work

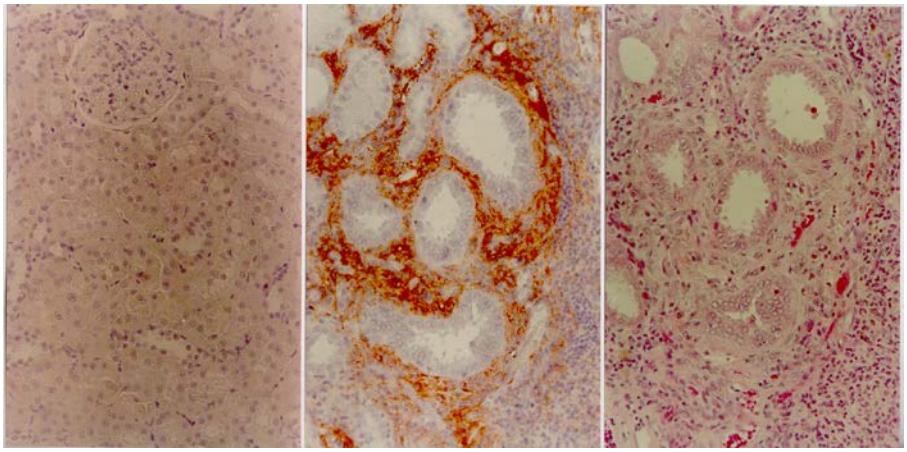
Swedish Society for Medical Researsh

Lars Hierta Foundation

Intrarenal Hyaluronan in the Regulation of Fluid Balance. Pathophysiological Relevance to Renal Damage during Diabetes and Ischemia-Reperfusion. (core director Peter Hansell)

The kidney is a main determinant of fluid/electrolyte balance and of mean arterial blood pressure. Hypertension is often caused by a renal inability to regulate fluid balance. The present research focuses on a matrix component (hyaluronan, HA) with extreme water attracting properties in the regulation of fluid balance. The proinflammatory property of HA is also evaluated in pathophysiological models. In contrast to the renal cortex which is almost void of HA, the interstitium of the renal medulla contains high amounts of HA during normal physiological conditions which changes depending on the body hydration status and, more severely, during pathological conditions.

We have found that HA has an important dynamic role in normal renal water-handling (hydration/dehydration) and that the intrarenal distribution of HA is severely altered during diabetes and after ischemia-reperfusion injury which correlates to renal dysfunction and inflammation. We have also demonstrated that the normal intrarenal distribution of HA is severely altered if angiotensin II tonus is diminished neonatally (during nephrogenesis) in the rat which correlates to renal dysfunction and inflammation. We aim to: a) determine the physiological relevance of the glycosaminoglycan hyaluronan (HA) in the regulation of renal fluid/electrolyte balance; b) determine the pathophysiological relevance of HA in the renal dysfunction during diabetes (diabetic nephropathy) and after ischemia-reperfusion injury; c) determine if hyaluronidase-treatment and siRNA improves renal function during diabetic nephropathy and following renal ischemia-reperfusion; d) elucidate the time frame and mechanisms in the development of the intrarenal heterogenous distribution of HA which occur neonatally in the rat and its angiotensin II dependency.



Both in vivo and in vitro experiments are performed. Diabetes, ischemia, hydration, dehydration, hormones, pharmacological and biomolecular intervention activate/deactivate the systems. Human renal tissue from resections is also analysed. Rats and genetically modified mice are used during in vivo conditions whereafter the renal tissue is analysed using molecular biology to follow HA (amount, size), HA synthases, hyaluronidases and CD44 expression. Renomedullary interstitial cells in culture are used in parallel to follow similar parameters during interventions. In cooperation with the section of diagnostic radiology (assoc prof Per Liss) the mechanisms underlying diabetic nephropathy is to be validated and the increased sensitivity of the diabetic kidney to radiological contrast agents is elucidated. Cardiovascular disease is a dominant cause for invalidity and mortality. The results of the present projects may give rise to basic understanding of, and new treatment modalities in, fluid balance disorders and cardiovascular diseases.

Members of the group

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Peter Hansell, Ph.D., core director

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Carla Carvalho, Ph.D-student

Oskar Nensén, MD/Ph.D-student

Per Eckerbom, Ph.D-student

Publications 2014-

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Dissertations

Stephanie Franzén "The role of hypoxia for the development of diabetic nephropathy: Temporal relationship and involvement of endothelin receptor signaling" PhD May 2016.

Circulatory function in acute and chronic kidney injury

Michael Hultström

Cardiovascular disease is the most common cause of death. The strongest treatable risk factor for cardiovascular disease is hypertension. In patients with hypertension end-organ damage in the form of kidney failure, heart failure and vascular dysfunction is the cause of disease progression and predicts mortality driving events. Our research group focuses on interactions between the cardiovascular system and kidney function in the development of acute and chronic kidney injury using systems physiology and genome centric methods.

Kidney in hypertension

The kidneys are important in hypertension both as central regulators of blood pressure, and as one of the most important target organs for hypertensive kidney damage. We study the role of the kidney in the development of hypertension and progression of hypertensive renal damage using physiological methods, genomics and bioinformatics.

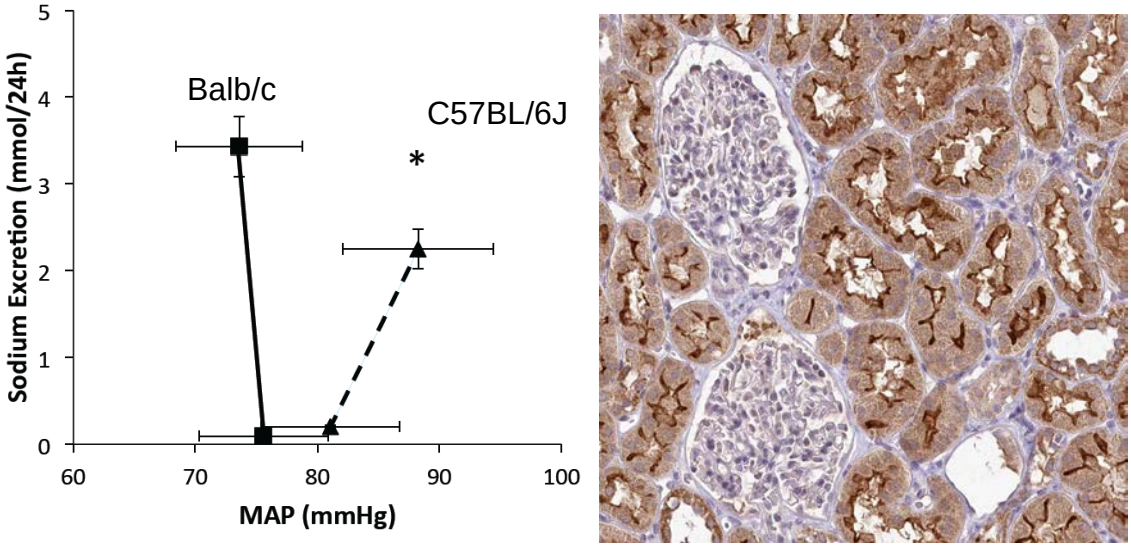

Figure 1: Left: Renal function curves showing how C57BL/6J blood pressure increases when the animals are challenged with a high salt diet, while Balb/c can excrete the salt load without increasing blood pressure. Right: ABCC2 expression in the kidney from http://proteinatlas.org.

We recently found that the salt sensitive hypertension that develops (Fig 1) in C57BL/6J is associated with a mutation in ABCC2, which is also implicated in human hypertension through genome wide association studies (GWAS). Although ABCC2 is mostly known as a bile acid transporter, it is actually expressed in renal proximal tubuli, and we are working on validating its effect on blood pressure. This would be a new causal gene for human hypertension if we can validate its functional significance, like we were previously involved in for uromodulin. Beyond translating single genes we have worked with using high-level bioinformatics to

translate whole groups or networks of genes. Recently, we developed a genetic network from the in-common genes in three different rat models with hypertensive kidney damage that translates surprisingly well to gene expressions in human kidney damage (Fig 2).

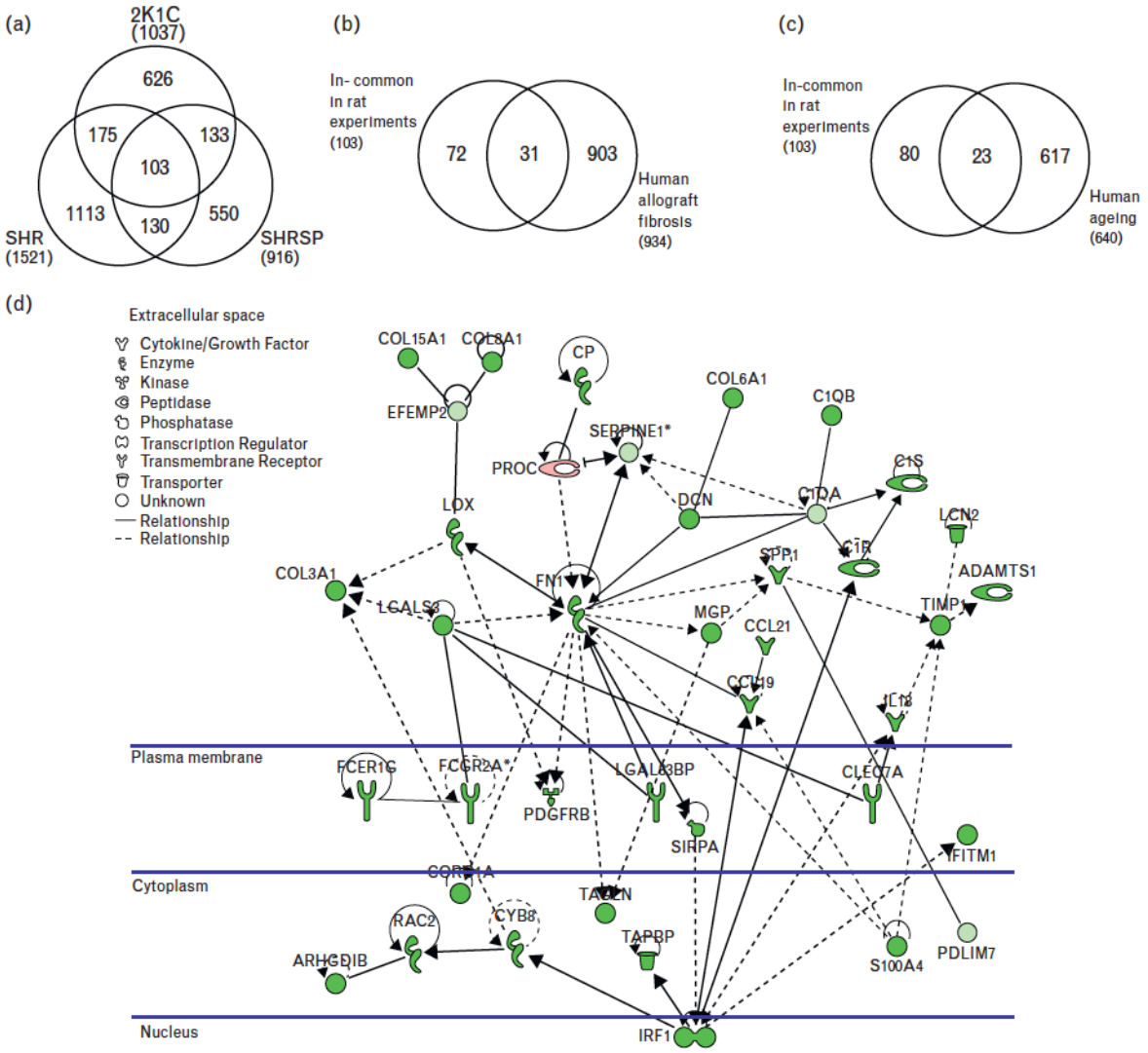

Figure 2: a) Gene expression changes in three hypertensive rat models with kidney damage showing 103 in-common genes. b) 31 in-common genes with human allograft nephropathy, and c) 23 with gene expression changes in elderly humans with kidney fibrosis. d) Genetic network of 44 highly connected genes centereda round the fibronectin/complement system that also translate to human kidney damage.

Acute kidney injury

A sudden worsening of kidney function is a common and dangerous complication for example in trauma, sepsis and surgery. In the setting of chronic kidney disease an acute kidney injury (AKI) is often the precipitating event to end stage renal disease (ESRD), which is when the patient requires transplantation or dialysis to survive. We study the mechanisms behind acute cellular injury in the kidney and loss of renal function in several models focused on the role of hormonal regulation of oxygen delivery and utilisation as well as inflammatory processes and gene regulation (Fig 3).

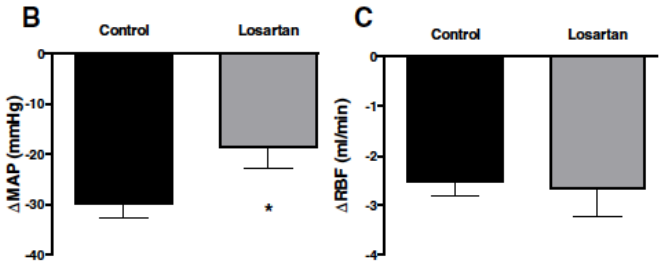

Figure 3: Treatment with the anti hypertensive drug Losartan decreases blood pressure as expected, but it does not aggrevate renal perfusion beyond than of haemorrhage in itself since it relaxes the afferent arteriole and reduces renal vascular resistance.

A major riskfactor for acute kidney injury is surgery. Up to 50% of patients fulfill the criteria for AKI after major gastrointestinal or cardiac surgery. We have addressed this question using renal gene expression in rats, either after a quick sacrifice and tissue collection, or after 3-4 hours of surgery and measurement of renal autoregulation with open abdomen. This insult in itself if enough to cause massive changes in gene expression in the kidney, and is associated with infiltration of inflammatory cells (Fig 4). This is important because it would normally be thought of as a control situation to be compared with a disease model such as haemorrhage. Perhaps the prolonged surgery actually predisposes the kidney to AKI more than many disease models in themselves.

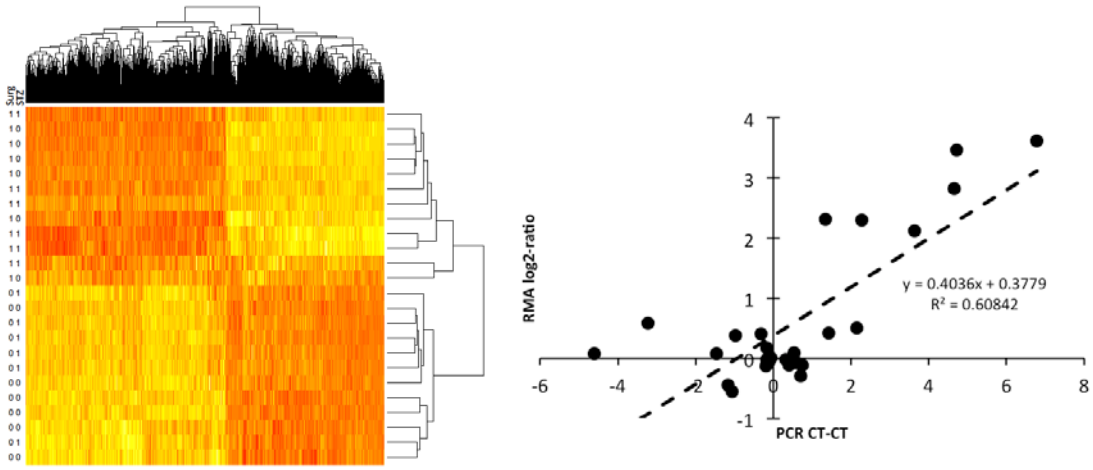

Figure 4: Left: Heat map of over 7000 differentially expressed kidney genes after prolonged surgery. Right: PCR validation of 22 selected genes with a wide expression difference show good correlation with microarray data.

AKI can be caused by a large number of diseases, we have recently identified a network of genes that are activated in AKI in six experimental models with different underlying diseases. This network is regulated through the transcription factor MYC and may be a common molecular pathway by which AKI develops into progressive chronic kidney disease. The future perspectives for our study of AKI will be to further study the effects of hormonal intervention in experimental models, but importantly to translate the findings into humans in an intervention study.

Cardiorenal syndrome

Kidney function is directly dependent on arterial pressure, and heart function on the venous filling pressure. This makes patients with combined kidney and heart failure, so called cardio-renal syndrome, particularly fragile and hard to treat. We have developed a new experimental model with a genetic difference in susceptibility to ADHF in two mouse strains (Fig 5).

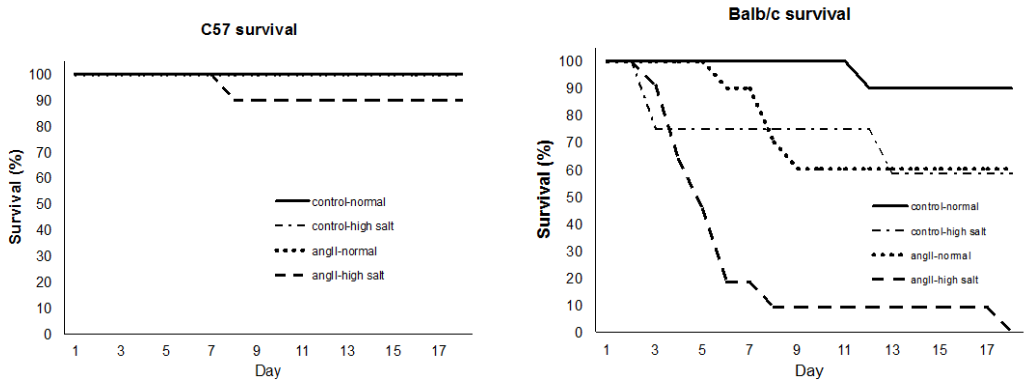

Figure 5: Survival curves for the two strains. Left: C57BL/6J that show minimal morbidity and mortality, and Right: BALB/c that are more sensitive and several animals have to be sacrificed early already with only AngII treatment or high-salt diet. With the combination treatment 80% develop symptoms of heart failure within the first six days.

BALB/c mice were found to be very sensitive and most had to be sacrificed within the first six days of treatment. In contrast, C57BL/6J mice were protected. In order to better understand the genetic difference between the mouse strains we performed a genome wide mRNA expression analysis of both heart and kidney (Fig 6).

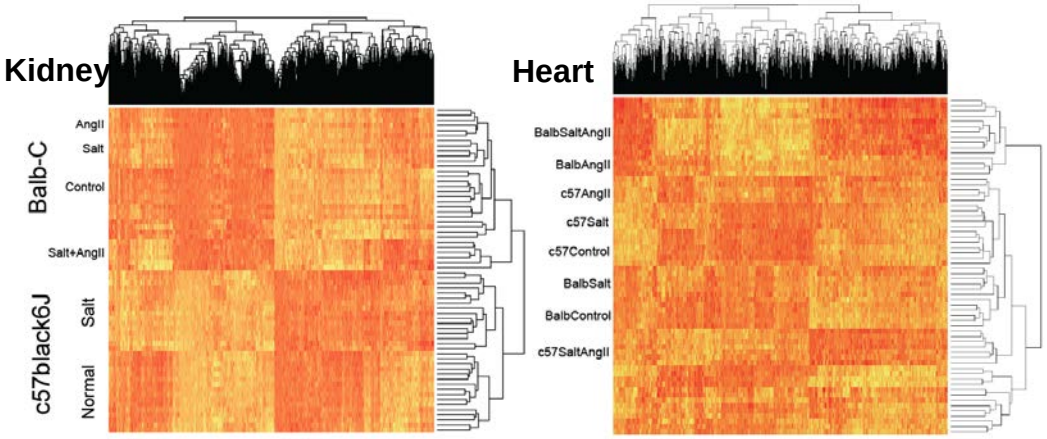

Figure 6: Differentially expressed genes between the strains during combined AngII and salt treatment in the kidney (left) and heart (right).

By comparing the the two mouse strains in the Mouse Genome Database to identify 6253 mutations, Single Nucleotide Polymorphisms (SNP), that differ between the mouse strains out of 63439 that the initial mouse diversity study focused on. This strategy identified the antioxidant system glutathion S-transferase as potentially important, which we were able to verify by investigating heart and kidney function under N-acetylcystein. Surprisingly, the higher oxidative stress in C57BL/6J seems to be a protective mechanism in the setting of fluid overload.

This project is interesting as a novel model for ADHF, and in particular since it appears to show a beneficial, or at least previously underappreciated physiological effect of oxidative stress in acute volume regulation.

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- Daniela Patinha, University of Bristol, England.
- Enyin Lai, Zhejiang University, Hangzhou, China.
- Jyrki Tenhunen, Anaesthesia and Intensive Care, Uppsala university.
- Kristofer Rubin, IMBIM, Uppsala university.
- Martin McBride, University of Glasgow, Scotland.
- Michael Fähling, Alex Paliege, Andreas Patzak, Charité, Berlin, Germany.
- Miklós Lipcsey, Anaesthesia and Intensive Care, Uppsala university.
- Per Liss, Radiology, Uppsala university.
- Renjun Wang, Jilin Normal University, China.
- Robert Frithiof, Anaesthesia and Intensive Care, Uppsala universitet.
- Rolf Reed, Department of Biomedicine, University of Bergen, Norway.
- Olav Tenstad, Department of Biomedicine, University of Bergen, Norway.
- Hans-Peter Marti, Department of Clinical Medicine, University of Bergen, Norway.
- Stefan Nilsson, Department of Bedical Biosciences, Umeå universitet.
- Will Cupples, Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Vancouver, Canada.
- Wojciech Weigl, Anaesthesia and Intensive Care, Uppsala university

Publications 2014-

1. Mortality rate is higher in Polish intensive care units compared to other European countries. Wojciech Weigl, Jan Adamski, Paweł Goryński, Andrzej Kański, Michael Hultström. Intensive Care Medicine. Accepted for publication.
2. c-Jun N-terminal Kinase mediates prostaglandin-induced sympathoexcitation in rats with chronic heart failure by reducing GAD1 and GABRA1 expression.Wang R, Zhang W, Dong Z, Qi Y, Hultström M, Zhou X, Lai EY. Acta Physiol (Oxf). 2017 Feb;219(2):494-509. doi: 10.1111/apha.12758. Epub 2016 Aug 12.
3. Increased hydrogen peroxide impairs angiotensin II contractions of afferent arterioles in mice after renal ischaemia-reperfusion injury.Huang Q, Wang Q, Zhang S, Jiang S, Zhao L,

Yu L, Hultström M, Patzak A, Li L, Wilcox CS, Lai EY. Acta Physiol (Oxf). 2016 Oct;218(2):136-45. doi: 10.1111/apha.12745. Epub 2016 Jul 15.

4. Renal oxygenation during haemorrhage is not aggarvated by Angiotensin II AT1-receptor blockade. Jacqueline M. Melville, Fredrik Palm, Michael Hultström. Acta Physiol (Oxf). 2016 Feb;216(2):153-5.
5. Sympathoexcitation in Rats with Chronic Heart Failure Depends on Homeobox D10 and MicroRNA-7b Inhibiting GABBR1 Translation in Paraventricular Nucleus. Renjun Wang, Qian Huang, Zengxiang Dong, Yunfeng Qi, Hua Li, Xiaowei Wei, Hui Wu, Huiping Wang, Christopher S. Wilcox, Michael Hultström, Xiaofu Zhou, En Yin Lai. Circulation Heart Failure. 2016 Jan;9(1):e002261.
6. Unilateral renal ischemia in rats induces a rapid secretion of inflammatory markers to renal lymph and increased peritubular capillary permeability. Liliana Monica Bivol, Bjarne M Iversen, Michael Hultström, Paal William Wallace, Rolf K Reed, Helge Wiig, and Olav Tenstad. J Physiol. 2015 Nov 20. [Epub ahead of print].
7. Matrix metalloproteinase-2 knockout and heterozygote mice are protected from hydronephrosis and kidney fibrosis after unilateral ureteral obstruction. Maria K. Tveitarås, Trude Skogstrand, Sabine Leh, Frank Helle, Bjarne M. Iversen, Christos Chatziantoniou, Rolf K. Reed, Michael Hultström. PLoS One. 2015 Dec 16;10(12):e0143390.
8. Lack of effect of dietary nitrate in elite athletes may be due to dilation of non-prioritized vascular beds in Commentaries on Viewpoint: Can elite athletes benefit from dietary nitrate supplementation? Michael Hultström. J Appl Physiol (1985). 2015 Sep 15;119(6):762-9.
9. Caloric restriction reduces age-related but not all-cause mortality. Michael Hultström. Acta Physiol (Oxf). 2015 May;214(1):3-5.
10. Norepinephrine enhances angiotensin II responses via p38 MAPK activation after hypoxia/re-oxygenation in renal interlobar arteries. Jana Kaufmann, Peter Martinka, Olivia Moede, Mauricio Sendeski, Andreas Steege, Michael Fähling, Michael Hultström, Matthias Gaestel, Ivana de Moraes da Silva, Olga Zavaritskaya, Andreas Patzak. Acta Physiol (Oxf). Acta Physiol (Oxf). 2015 Apr;213(4):920-32.
11. Identification of a common molecular pathway in hypertensive renal damage: comparison of rat and human gene expression profiles. Trude Skogstrand, Sabine Leh, John McClure, Mohammed Dashti, Bjarne M. Iversen (deceased), Delyth Graham, Martin W. McBride, Michael Hultström. J Hypertens. 2015 Mar;33(3):584-96.
12. Distinct Protein Signature of Hypertension-induced Damage in the Renal Proteome of the Two-Kidney, One-Clip (2K1C) Rat Model. Heidrun Vethe, Kenneth Finne, Trude Skogstrand, Marc Vaudel, Bjørn E. Vikse, Michael Hultström, Sandrine Placier, Andreas Scherer, Olav Tenstad, Hans-Peter Marti. J Hypertens. 2015 Jan;33(1):126-35.
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14. Renal neurohormonal regulation in heart failure decompensation. Sofia Jönsson, Mediha Becirovic Agic, Fredrik Narfström, Jacqueline M. Melville, Michael Hultström. Am J Physiol Regul Integr Comp Physiol. 2014 Sep;307(5):R493-R497.
15. Validation of uromodulin as a candidate gene for human essential hypertension. Lesley A Graham, Sandosh Padmanabhanm Niall J Fraser, Satish Kumar, James M Bates, Hajamohideen S Raffi, Paul Welsh, Wendy Beattie, Nicholas R Ferreri, Shoujin Hao, Sabine Leh, Michael Hultström, Anna F. Dominiczak, Delyth Graham, Martin W McBride. Hypertension. 2014 Mar;63(3):551-8.
16. Intradermal Insulin Delivery: A Promising Future for Diabetes Management. Hultström M, Roxhed N, Nordquist L. J Diabetes Sci Technol. 2014 Apr 10;8(3):453-457.

Agencies that support the work

Swedish Society for Medical Research (SSMF)

Swedish Heart-Lung Foundation

Åke Wiberg Foundation

Magnuns Bergvall Foundation

Swedish Society of Medicine

Lars Hierta Foundation

Marcus Borgström Foundation

Prizes and awards 2016

Michael Hultström: Young Investigator Travel Award at the Federation of American Societies of Experimental Biology (FASEB) science research conference on "Renal Hemodynamics and Cardiovascular Function in Health & Disease".

Studies of the pathophysiological mechanisms behind protein aggregation and formation of organ and cell toxic amyloid

Gunilla T Westermark

With our research we aim to pinpoint mechanisms that precede the formation of beta-cell toxic islet amyloid, and also characterize the endogenous mechanism involved in resolution of amyloid. Amyloid defines a fibrilar aggregate where beta strands of protein monomers are assembled perpendicularly to the fibrilar axis. Initiation of amyloid fibrils involves the formation of smaller intermediates, so called protofibrils that has been ascribed the cell toxic activity. Today, 30 different amyloid forming proteins have been isolated from amyloid deposits in man.

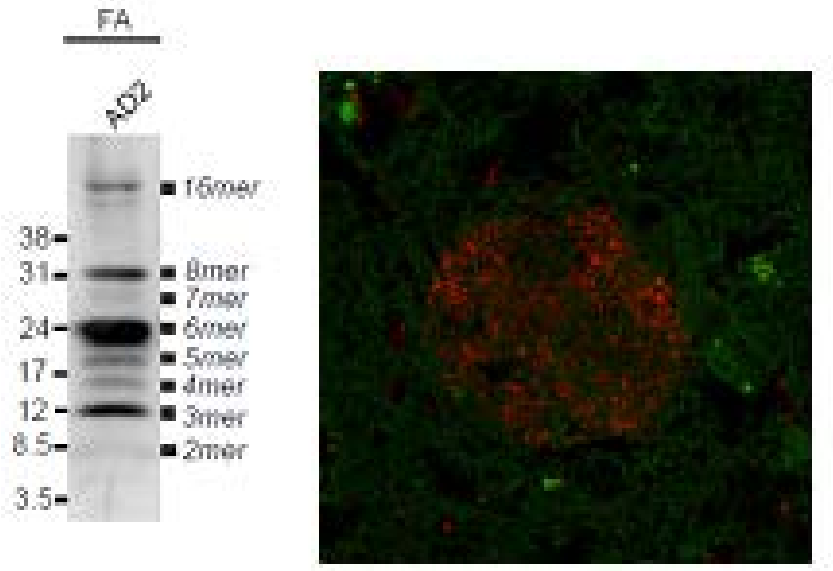
Islet amyloid and beta-cell death

The beta-cell hormone Islet Amyloid Polypeptide (IAPP) is the major amyloid component present in the islets of Langerhans in almost all individuals with type 2 diabetes. IAPP is synthesised as a larger proIAPP and arises after posttranslational processing that comprises the removal of an N-terminal and a C-terminal flanking peptide, formation of a disulfide bond and N-terminal amidation. Processing is performed by the prohormone convertases PC2 and PC1/3 and takes place in the secretory granules. Proinsulin is processed to insulin by the same convertases at the same location. ProIAPP and incomplete processed proIAPP can be detected in amyloid deposits formed in vivo, and at present we investigate how expression of PC2 and PC1/3 is affected by conditions that trigger islet amyloid formation. Mouse and rat do not develop IAPP-amyloid due to sequence variations in the IAPP molecule. Therefore, this work is performed on our human IAPP transgenic mouse strain where islet amyloid develops in male mice fed a diet high in fat for 12 months.

The transgenic hIAPP mouse model is used for studies including prevention or blocking of amyloid propagation. At present we analyse the inhibitory effect that heparin related molecules exert on amyloid formation. Also, we have established a new mouse strain that over-express heparanase and show that this reduce formation of IAPP amyloid. This work is done in collaboration with Jin-ping Li, IMBIM, UU.

Islet amyloid is also a frequent finding in transplanted islet, and we use isolated islets from the hIAPP transgenic strain and human islets from the *Nordic Network* for clinical islet transplantation to investigate if IAPP amyloidogeneity is influenced by the transplantation local. We have shown that amyloid develops to the same degree in grafts implanted under the kidney or spleen capsule or to the liver. Other locations are under investigation.

Fibrils formed from different amyloid precursor proteins appear to be morphological inseparable. Therefore, it is possible that fibrils formed by one protein can seed amyloid made up by a second amyloid protein. We have seeded islet amyloid in human IAPP transgenic mice through administration of preformed fibrils made up by Aβ protein. Aβ and IAPP exhibits 50% sequence identity and using a high sensitive detection method, proximity ligation assay (PLA) we have identified IAPP in the brain of patients with Alzheimer's disease. The finding is interesting because type 2 diabetes increases the risk of developing Alzheimer's disease. At present, in collaboration with dr Martin Ingelsson, Uppsala and Bradley Hyman, and collegues at Alzheimer Disease Research Center at Harvard Medical School, Massachusetts we compare amyloid plaque composition in AD pateints with and without type 2 diabetes.



Western blot analysis of brain extract from an AD patient with IAPP antiserum shows a ladder like pattern. PLA performed with a combination of IAPP and Aβ antibodies identifies IAPP reactivity throughout the Aβ-paque.

We have established a new model in *Drosophila melanogaster* for studies of proIAPP/IAPP amyloid formation. In transgenic flies expression of human proIAPP or IAPP amyloid is detected already in 20 days old flies. As expected, amyloid does not develop in control flies expressing non-amyloid-forming mouse IAPP.

The *Drosophila melanogaster* system is used for pinpointing the intracellular events that result in amyloid-linked cell death. We analyse important pathways such as ER-stress, ERAD and autophagy.

Insulin is in vitro a potent inhibitor of IAPP-aggregation and the two peptides co-localize in the secretory granules where they undergo enzymatic processing. A disturbance in cleavage and/or folding in any of the precursors might initiate amyloid aggregation. We use the Drosophila model to investigate if induction of human proinsulin or any of its processing metabolites in flies expressing the amyloidogenic proIAPP or IAPP will prevent amyloid formation.

The *Drosophila melanogaster* will also be used as a tool for analysis of amyloid inhibitors.

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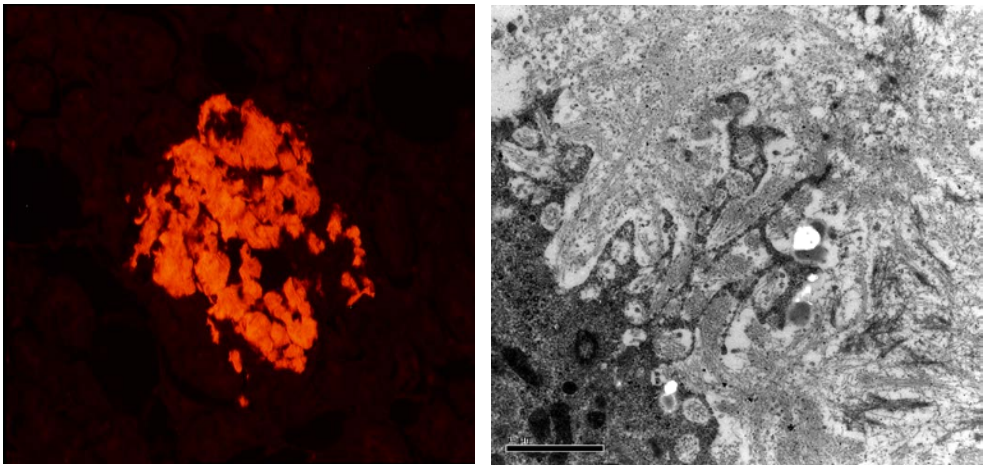


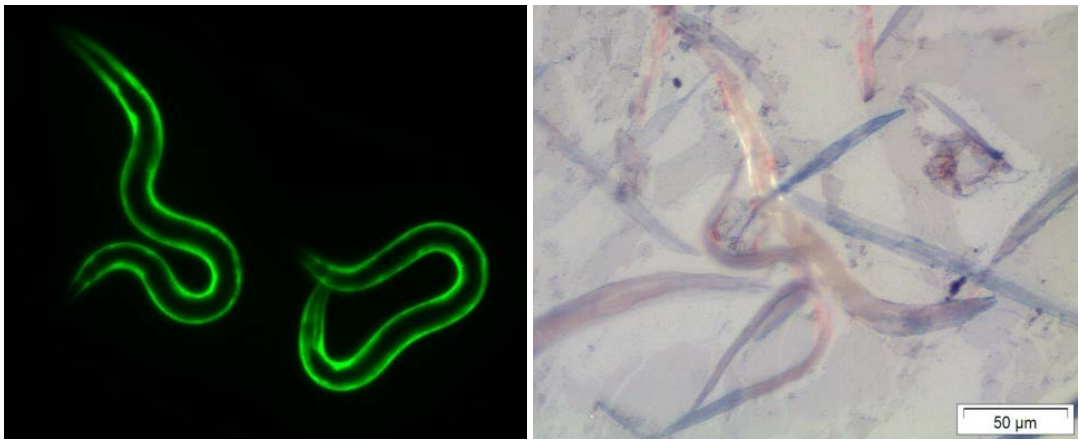
A human islet stained for amyloid by Congo red. The amyloid deposits replace most of the beta-cells. The section is viewed at 546 nm. The electron micrograph shows the border between a beta-cell and extracellular amyloid. Note the close association between the amyloid bundles and the cell membrane.

We have identified autophagy as an important mechanism that link amyloid and cell death. In collaboration with Annica Rönnbeck, KI is autophagy's role in neuronal cell death explored. This work is performed using Aβ-transgenic mice, human brain tissue and Aβ transgenic flies

There is a well-established mouse model for reactive amyloidosis (AA-amyloidosis) where N-terminal fragments (protein AA) of serum amyloid A (SAA) deposit as amyloid. We have used this model and studied resolution of amyloid. This process depends on formation of AA reactive antibodies and activation of macrophages.

We have also used this model to study transmission of amyloid and have recently shown that monocytes from a diseased mouse can prime for the disease in a recipient animal. This result points to a prion-like mechanism for spreading of amyloid. With the model, we have also shown that non-amyloid fibrilar structures can prime for AA-amyloidosis. This finding is interesting and points to a possible environmental component in the pathogenesis of the disease. To reduce the numbers of mice used for our transmission studies we explore the

possibility to establish a model for AA amyloidosis in C elegans. This work is ongoing and we have now transgenic worms that express human protein AA, and in these develops amyloid. Feeding worms on OP50 bacteria mixed with amyloid fibrils leads to disturbance in mobility and is indicative for transmission.



The transgenic C. elegans express GFP and an amyloid protein (e.g. AA 45). The expression is driven to the body wall muscle. The presence of the green GFP allows us to monitor the movements of the worm. Aged worms, for amyloid with Congo red exert green birefringence when viewed in polarised light.

Members of the group

Gunilla T Westermark, PI

Lakshim Kotegala, Post Doc

Camilla Krappe, Post Doc

Marie Oskarsson, PhD Student

Gu Xiaohong, PhD Student

Ye Wang, Ph.D. Student

Marianne Ljungkvist, Laboratory engineer

Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

Family Ernfors Foundation

Alzheimer fonden

Novo Nordisk

Gamla Tjänarinnor

Publications 2014-

1: Oskarsson ME, Singh K, Wang J, Vlodavsky I, Li JP, Westermark GT. Heparan Sulfate Proteoglycans Are Important for Islet Amyloid Formation and Islet Amyloid Polypeptide-induced Apoptosis. J Biol Chem. 290:15121-15132, 2015

2: Westermark GT, Oskarsson M, Andersson A, Westermark P. Eighty years of research on islet amyloidosis in Uppsala. Ups J Med Sci. 120:117-123, 2015

3: Oskarsson ME, Paulsson JF, Schultz SW, Ingelsson M, Westermark P, Westermark GT. In vivo seeding and cross-seeding of localized amyloidosis: a molecular link between type 2 diabetes and Alzheimer disease. Am J Pathol. 185:834-846, 2015

4: Westermark GT, Fändrich M, Westermark P. AA amyloidosis: pathogenesis and targeted therapy. Annu Rev Pathol. 10:321-344, 2015 Review

5. Hermansson E, Schultz SW, Crowther D, Linse S, Winblad B, Westermark GT, Johansson J, Presto J. The chaperone domain BRICHOS prevents CNS toxicity of amyloid-β peptide in Drosophila melanogaster. Dis Model Mech. 7:659-65, 2014

6: Paulsson JF, Ludvigsson J, Carlsson A, Casas R, Forsander G, Ivarsson SA, Kockum I, Lernmark Å, Marcus C, Lindblad B, Westermark GT. High plasma levels of islet amyloid polypeptide in young with new-onset of type 1 diabetes mellitus.PLoS One;9(3):e93053, 2014

7: Westermark P, Westermark GT, Suhr OB, Berg S. Transthyretin-derived amyloidosis: probably a common cause of lumbar spinal stenosis. Ups J Med Sci. 119:223-228, 2014

8: Caballero F, Siniakowicz K, Hollister-Lock J, Duran L, Katsuta H, Yamada T, Lei J, Deng S, Westermark GT, Markmann J, Bonner-Weir S, Weir GC. Birth and death of human β-cells in pancreases from cadaver donors, autopsies, surgical specimens, and islets transplanted into mice. Cell Transplant. 23:139-151, 2014

You've Got Mail: Message Delivery Within and Between Cells

Johan Kreuger

The main goal of our research program is to address the question of how messages and packages are sent to the right addresses within and between cells in our bodies. This is a fundamental question. It is very important for the around 100,000 billion cells in the human body to communicate, in order to collaborate and sustain life. Malfunctioning package delivery in this context may lead to inappropriate cell behaviour, and in the worst case to disease such as cancer. We are particularly interested in cellular packages and messages that direct the collective movements of cell clusters. Cells move together during normal development and wound healing, but also during cancer progression.

The main project family in our lab deals with the roles of the exocyst complex in the recruitment of intracellular vesicles to the plasma membrane. Proteins of the exocyst complex are attached to vesicles containing information (i.e. different molecules) that move using motorproteins along cytoskeletal highways in the cell en route to their final destinations. At the final destination (the target membrane) other exocyst components are awaiting, and binding between vesicle-bound and membrane-bound exocyst proteins leads to vesicle tethering. The tethering step is then followed by fusion of the vesicle with the plasma membrane resulting in release of vesicular cargo. Importantly, components of the exocyst undergo alternative splicing, yielding a variety of isoforms, some of which have been associated with distinct cellular behaviors such as epithelial-mesenchymal transition and migration/invasion. The different exocyst protein isoforms are probably central to how vesicles can be delivered to specific destinations and packages ultimately released in a controlled manner. We are interested in the structural and functional implications of alternative splice variants, both with respect to interactivity between exocyst components and their potential for context-specific expression. Another long-term ambition is to better understand how the molecular machineries regulating assembly and delivery of intracellular and extracellular packages originated and evolved after that life emerged on Earth some 4 billion years ago.

Most projects in the lab employ a variety of molecular biology tools (e.g. qPCR, RNAi, cloning, overexpression of tagged proteins) to study transcript splicing and to perform loss- and gain-of-function experiments, as well as microscopy techniques (including confocal and TIRF) to study exocyst and vesicle trafficking events required for proper cell behavior and cell migration. We study exocyst function in cancer cells, epithelial cells and endothelial cells. We use biochemical approaches to study protein-protein and protein-drug interactions, and microfluidic assays to study cell migration.

Several projects in the lab involve the construction of new methods for detailed studies of cell communication and collective cell migration. Previous projects in the lab have resulted in patents and industrial applications and in the formation of the spin-off company Gradientech AB.

Publications 2014-

1. Hua Huang, Elise Langenkamp, Maria Georganaki, Angelica Loskog, Peder Fredlund Fuchs, Lothar C. Dieterich, Johan Kreuger, Anna Dimberg, (2014) VEGF suppresses T-lymphocyte infiltration in the tumor microenvironment through inhibition of NF-kB-induced endothelial activation
FASEB J. 29(1):227-38.

2. Sara Massena, Gustaf Christoffersson, Evelina Vågesjö, Cédric Seignez, Karin Gustafsson, Francois Binet, Carmen Herrera Hidalgo, Antoine Giraud, Jalal Lomei, Simone Weström, Masabumi Shibuya, Lena Claesson-Welsh, Pär Gerwins, Michael Welsh, Johan Kreuger, and Mia Phillipson (2015) Identification and characterization of VEGF-A-responsive neutrophils expressing VEGFR1, CD49d and CXCR4 in mice and humans
Blood 126(17):2016-26

3. Kreuger J, Phillipson M. (2015) Targeting vascular and leukocyte communication in angiogenesis, inflammation and fibrosis.
Nature Reviews Drug Discovery 15(2):125-42.

4. Endoplasmic reticulum stress enhances fibrosis through miR-150 degradation and XBP-1 splicing (2016) Heindryckx, F, Binet, F., Ponticos, M., Robouts, K., Lau, J., Kreuger, J.* and Gerwins, P.*

EMBO Molecular Medicine 8(7):729-44.

5. Kreuger J and O'Callaghan P. Failure to genotype: A cautionary note on an elusive loxP sequence. (2016)

PLOS ONE, 11(10):e0165012

6. Hernández Vera, R., Schwan, E., Fatsis-Kavalopoulos, F., and Kreuger, J. (2016) A modular and affordable time-lapse imaging and incubation system based on 3D-printed parts, a smartphone, and off-the-shelf electronics.

PLOS ONE, 11(12):e0167583.

7. Blom, M., Reis, K., Heldin, J., Kreuger, J., and Aspenström, P. (2017) The atypical Rho GTPase RhoD is a regulator of actin cytoskeleton dynamics and directed cell migration.

Exp. Cell Research, 5;352(2):255-264

Members of the group

Johan Kreuger, senior lecturer

Paul O'Callaghan, postdoc

Rodrigo Hernández Vera, postdoc

Nikos Kavalopoulos, PhD student

Carl Andrén Lundahl, project student

Agencies that support the group

Swedish Cancer Society (JK)

The Marie Sklodowska-Curie Innovative Training Network InCeM (JK)

Alzheimerfonden (POC)

Demensförbundet (POC)

Uppsala Antibiotic Center (JK)

Dissertations

Ashrafzadeh, P.(2016).Exploring Cellular Dynamics: *From Vesicle Tethering to Cell Migration*.

Dissertations 2016

- **Alenkvist, I.(2016).Epac2 signaling at the β-cell plasma membrane.**
- **Ashrafzadeh, P.(2016).Exploring Cellular Dynamics: *From Vesicle Tethering to Cell Migration*.**
- **Espes, D.(2016).Engraftment of Pancreatic Islets in Alternative Transplantation Sites and the Feasibility of *in vivo* Monitoring of Native and Transplanted Beta-Cell Mass.**
- **Vågesjö, E.(2016).Exploring immune cell functions and ways to make use of them.**

Licentiate theses 2016

Ahl, David

Manell, Hannes

Shuai, Hongyan

Peng, Yin

Economy

(kSEK)

	2015	**2016**
Undergraduate Education appropriations	34 273	34 338
Faculty appropriations	20 921	19 442
External Grants	40 415	41 482
Contract research	180	495
Total	**95 789**	**95 923**

Undergraduate Teaching

The department participates in 7 different study programmes (utbildningsprogram): medicine, pharmacy, biomedicine (Bachelor and Master programmes), nursing, biomedical laboratory science and dieticians and dispensers. In addition, it hosts a number of single subject courses (fristående kurser). Some 1500 students per year are given education at the department. The courses given by MCB generally get very good gradings in the course evaluations by the students.

Medicine

The department contributes teaching in anatomy, cell biology and physiology with both traditional lectures and problem based learning as well as with seminars and laboratory experiments. Most of this teaching is given during terms 1-3 of the programme but extensive parts are also given in the later integrated courses. The overall objective is to provide basic knowledge of the morphology and function of the human body and to create a basis for the following clinical studies. Some 115 students are enrolled every semester.

Biomedicine

This three-year Bachelor programme aims to give students a thorough understanding of normal morphology and function of the human body. The programme is given annually and provides the students training for future activity in research, information and education. The department takes part in the teaching of anatomy, embryology, cell biology and physiology. About 40 students are enrolled each year.

The two-year Master programme in Biomedicine is an international programme that aims to give a deeper knowledge in the subjects taught in the Bachelor's programme, and also offers the students an opportunity to specialize in their field of interest. The department gives the first course in the programme, Major Diseases - Homeostasis and Endocrine Disorders. The programme enrolls approximately 30 students annually.

Pharmacy

The department is responsible for the teaching in anatomy and physiology for the University Diploma of Pharmacy. The courses are in the form of lectures, seminars and laboratory experiments. Some 140 students are enrolled every semester.

Clinical dieticians

The anatomy and physiology course included in the programme for clinical dieticians is given annually by the department, and include lectures, seminars and laboratory experiments for approximately 30 students.

Nursing sciences

The department is responsible for the teaching of anatomy, cell biology and physiology in the form of lectures and seminars. Some laboratory experiments are involved as well. Some 130

students are enrolled in the spring semester and 150 students are enrolled in the autumn semester. Thus approximately 280 students are enrolled every year. A new responsibility since 2014 is the education of nurses on Gotland where MCB through data link lecture for the students in the above subjects just as for our students in Uppsala. Approximately 40 students are enrolled every year.

Biomedical laboratory sciences

The aim of this programme is to produce technicians with appropriate training for a future task in diagnostic and research laboratories. The department is responsible for the teaching in anatomy, histology, cell biology and physiology in the form of lectures, seminars and laboratory experiments. Some 35 students are enrolled each year

Medical student assistants

Three medical students are enrolled for a period of 3 year during which they teach younger medical students and participate in research.

Single subject courses (fristående kurser)

Anatomy A (evening course)

Transplantation biology (evening course)

Medical cell biology (laboratory project course)

Histology

Physiology

Basic medical physiology

Summer research school (SOFOSKO)

Major Diseases - Homeostasis and endocrine disorders

Advanced Cell Biology (lecture series with seminar)

Graduate Teaching

The department has the responsibility for two of the Mandatory Courses for Graduate Students Introduction to Scientific Research – enrolling 80 Graduate students per year and Scientific Presentation – enrolling 40-50 Graduate students per year.

MD/PhD programme

MCB is responsible for the administration and content of the MD/PhD programme. The Medical Faculty and Upsala Society of Physicians provide grants for five undergraduate medical studies per year to join the MD/PhD programme. These students pursue medical undergraduate students in parallel with a graduate research project. After finishing medical studies the MD/PhD studens have a period of full-time research leading to half-time or Licentiate exam. Students then continue with internship combined with continued graduate research project studies. After obtaining the MD a full-time research period leads to the PhD.

Centres and Facilities

Advanced light microscopic imaging facilities

Within the department there are several advanced setups for fluorescence imaging of living cells and micro-organs. These setups are based on bright field microscopy, conventional fluorescence microscopy, epifluorescence microscopy, total internal reflection fluorescence (TIRF) microscopy and confocal microscopy. We also have a laser capture microscope.

Fluorescence and intra-vital microscopy

Fluorescence imaging using epifluorescence is used for on-line monitoring of the cytoplasmic Ca2+ concentration in superfused islets of Langerhans and dispersed islet cells (Anders Tengholm, 018 471 4481). Similar studies of Ca^{2+}, nitric oxide and oxygen radicals are performed on single perfused kidney glomeruli and the juxtaglomerular apparatus (Erik Persson, 018 471 4180) and interactions between leukocytes and endothelial cells leading to leukocyte transmigration and vessel permeability are studied in the adipose tissue, gastrointestinal tract and skeletal muscle in vivo (Lena Holm, 018 4714325, Mia Phillipson, 018 471 4419). Membrane order is studied in live cells by ratiometric imaging in combination with deconvolution (Ingela Parmryd, 018 471 41 50).

TIRF microscopy

The department possesses 6 TIRF (or evanescent wave) microscopes, two of which are custom-built systems with prism-type configuration and 4 using custom-built or commercial through-the-lens illumination. The systems are differently equipped with gas and diode-pumped solid-state lasers to provide excitation at multiple lines, including 405, 442, 457, 488, 514 and 561 nm. These setups are used for on-line monitoring of cAMP, cytoplasmic Ca^{2+}, IP_3, DAG, PIP_2, PIP_3 and other signalling molecules using indicators based on different spectral variants of green fluorescent protein (Anders Tengholm, 018 471 4481) and imaging of single molecules involved in exocytosis of secretory vesicles (Sebastian Barg, 018 471 4660).

PALM and STORM superresolution microscopy

One of the multicolour TIRF microscopes is equipped for stochastic superresolution microscopy in live and fixed cells. Fluorescently labeled proteins (eg. GFP fusion proteins or antibody labelling) can be localized with a resolution of 20-50 nm. (Sebastian Barg, 018 471 4660).

Confocal microscopy

The laboratory has three inverted confocal microscopes, one fast spinning disc (Nipkow) system used for studies of living islets of Langerhans and dispersed islet cells (Anders Tengholm, 018 471 4481), one scanning confocal system mostly used for structural studies (Nils Welsh, 018 471 4212), one advanced state-of-the-art system suitable for live cell imaging (Oleg Dyachok, 018 471 4345) and an upright high speed confocal microsope for in vivo studies (Zeiss LSM5 Live, Mia Phillipson, 018 471 4419).

Laser capture microscopy

The department has a laser capture microscope (LMD6000, Leica) that can be used to isolated cells or other regions of interest from sectioned tissues for further analysis. Depending on internal use, this equipment is available for external users on a charged service basis (Per-Ola Carlsson, 018 471 4425, Sara Ullsten, 018 471 4395).

Cellular metabolism

The department has an Extracellular Flux Analyzer XF96e, which has the ability to measure oxygen consumption rates i.e estimating metabolism in cells. From the measurments estimates of ATP-coupled respiration can be derived as well as proton leak. The equipment also allows estimating glucose utilization rates.

Gel imaging

The department has a LI-COR Odyssey FC and a Kodak 4000MM gel imaging unit (Nils Welsh, 018 471 4212) and a Bio-Rad Fluor-S MultiImager system for scanning and quantification of proteins in gels and membranes (Peter Bergsten, 018 471 4923).

Digital cameras

Several of the imaging systems are equipped with ultra-sensitive state of the art cameras, some utilizing back-thinned electron multiplying charge coupled device (EMCCD) techniqe.

Other equipment

Real-time PCR (Roche Lightcycler, Nils Welsh, 018 471 4212).

Flow cytometry and cell sorting (BD FacsCalibur, Nils Welsh, 018 471 4212).

Laser Doppler blood flow measurement equipment (Lena Holm, 018 471 4325).

Patch clamp equipment for electrophysiological recordings (Sebastian Barg, 018 471 4660).

Fluoroscan supplied with detectors for luminescence and absorbanse (Gunilla Westermark, 018 471 4169).

Nanodrop for DNA/RNA and protein quantification (Gunilla Westermark, 018 471 4169).

Mesoscale multiplex immunoassays (Erik Gylfe, 018, 471 4428)

EPR (electron paramagnetic resonance) for measuring free radicals (Fredrik Palm, 018 471 4182).

EnSpire Alpha Plate Reader, Perkin Elmer (Anders Tengholm, 018 471 4481)

Small animal ultrasound (Vevo 1100) with 1000 Hz time-resolution and 30µm spatial resolution for functional cardiovascular imaging in rats and mice. (Michael Hultström 0707648454)

Cardiac output monitor for patients (LiDCO) that uses pulse-wave analysis to estimate cardiac output with a lithium-dilution calibration during surgery or critical care. (Dept. Surg. Sci. Michael Hultstöm 0707648454)

LI-COR Odyssey FC gel scanner (Nils Welsh, 018 4714212).

High-throughput imaging system, Molecular Devices ImageExpress (Sebastian Barg, 018 471 4660).

Plate reader Berthold TriStar2 with luminescence, absorbance, fluorescence, lipquid handling (Sebastian Barg, 018 471 4660).

Prizes and awards

Michael Hultström: Young Investigator Travel Award at the Federation of American Societies of Experimental Biology (FASEB) summer research conference on “Renal Hemodynamics and Cardiovascular Function in Health & Disease”.

Daniel Espes: Swedish Endocrinology Association: Best endocrinology dissertation 2016.

Omar Hmeadi: EASD Poster prize for his research entitled ”Live cell imaging of glucagon granule exocytosis in single type-2 diabetic and non-diabetic human α-cells”.

Gustaf Christoffersson: the EFSD/EASD:s Rising Star Fellowship.

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