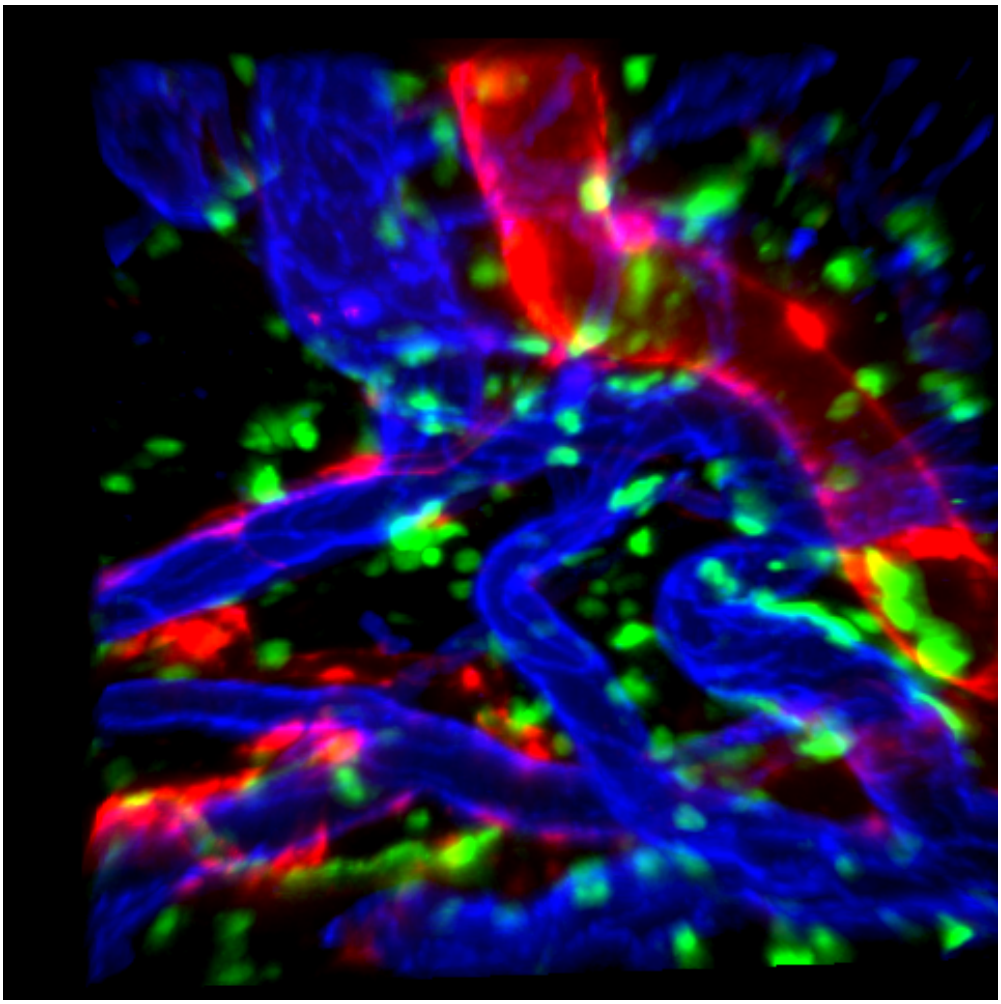
UPPSALA UNIVERSITET

Department of Medical Cell Biology

ANNUAL REPORT

2015



Fastställd av Institutionsstyrelsen 2016

Department of Medical Cell Biology

ANNUAL REPORT

2015

Introduction

First, I would like to thank professor Ulf Eriksson and senior lecturer Parri Wentzel, who both retired in 2015, for the many years of great work at the department. The contributions of Ulf and Parri in both research and teaching have been highly esteemed by colleagues at the department and by many students. One permanent position as lecturer was appointed at MCB during 2015 to Martin Blixt, who is very much welcomed.

During the year of 2015 research groups at the department of MCB have obtained several new prestigious research grants. For example, investigators, such as Olof Idevall, Gunilla Westermark and Ingela Parmryd, were awarded new grants from the Swedish Research Council. New grants were also obtained from Diabetes Wellness (Mia Phillipson, Anders Tengholm), Cancerfonden (Michael Welsh), Vinnova (Evelina Vågesjö and Mia Phillipson), Hjärt-Lungfonden (Michael Hultström) and the Novo-Nordisk Foundation (Gunilla Westermark, Olof Idevall). A commissioned research grant from Astra Zeneca has been given to Peter Bergsten.

In addition, Kailash Singh received the Young Investigators Award from the Scandinavian Society for the Study of Diabetes for best Scandinavian publication in diabetes during the year. Professor Per-Ola Carlsson received the senior research award, Knud Lundbeck Award (to a researcher who has substantially contributed to the progress of diabetes research, mainly through work conducted within the Nordic area) from the same society. Nikhil Gandasi received the European Foundation for the Study of Diabetes (EFSD) / Lilly Research Fellowship and a SSMF 2 year research fellowship. Michael Hultström received an early Career Researcher Prize, at the Neural, Hormonal and Renal Interactions in Blood Pressure Control meeting in Mussoorie, India, December 2015. Marie E. Oskarsson's PhD theses ″Islet amyloid polypeptide (IAPP) in Type 2 diabetes and Alzheimer disease" has been recognized by the Swedish Society of Diabetology (SFD) as the best preclinical dissertation in 2015.

It is also noteworthy that the MCB investigators have eminently communicated their research achievements to the general public via the news media (“tredje uppdraget”). For example, Per-Ola Carlsson has been featured in TV, radio and newspapers for new treatments in type 1 diabetes, and held several public lectures on diabetes and research for the lay community. Sebastian Barg was interviewed for an article by Mike May in Bioopticsworld in 2015. Misty Marshall became a panel member at Norwescon Science Fiction conference in Seattle. Evelina Vågesjö was elected “SKAPA framtidens innovatör i Uppland”, and the winner of UIC Business Lab contest in fictional innovations. During 2015 Dr. Michael Hultström lectured about heart failure for the Swedish Pensioner’s Society (SPF) on three occations arranged by the Swedish Heart-Lung Foundation with between 40 and 100 attendees per lecture. Michael Hultström has been repeatedly invited to #NephJC, a Twitter-based journal club in nephrology to discuss hypertension and acute kidney injury, the associated account @NephJC has over 3000 followers of mostly nephrologists and some kidney interested doctors of other specialities. Finally, Mia Phillipson participated in SVT’s Nobel studio as a commentator of the Nobel price in medicine. Mia Phillipson and Evelina Vågesjö wrote also the article: “Ny teknologi för accelererad läkning av sår” in SårMagasinet #3, 2015.

During 2015 our teachers Per Holmfeldt and Faranak Azerbayjani have worked very hard, and successfully, to renew and develop our courses on the Programmes for Nursing and Pharmacy, respectively. Through their efforts the students now graduate from the courses at higher numbers and with more knowledge. The medical anatomy course KARL, which is headed by Mats Hjortberg, was the winner of the annual KURT contest, which means that it reached the

highest approval by the medical students. We are also happy to congratulate Monica Sandberg, who was awarded a Pedagogical Rose by the first year medical students on 2 mars 2015.

After some economical difficulties during the past 1-2 years, the economy of the department has now stabilized. This, in combination with an upcoming retirement late 2016, allows some expansion and the department initiated late 2015 recruitments of a new senior lecturer and an associate senior lecturer (BULE) position.

To further improve the quality of research and teaching of the department three new groups have been formed; one group that deals with research, one group that deals with the training of graduate students, and one group that deals the teaching of under-graduate students. These groups meet regularly and have during 2015 composed lists of goals and strategies for the department, which delineate what improvements the department strives for and how to reach these goals. The goals and strategies are now posted on the MCB web page.

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 Camilla Sävmarker, 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 mediterranean “julgransplundrings party”.

Nils Welsh, Department Head Uppsala 2016-05-12

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
Carl Johan Drott, PhD student representative, deputy
Fredrik Lyngfalk, student representative
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Camilla Sävmarker, personell administrator, adjunct

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Technical staff
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Annika Jägare
My Quach
Lisbeth Sagulin
Monica Sandberg
Jan Saras

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 in recent years 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.

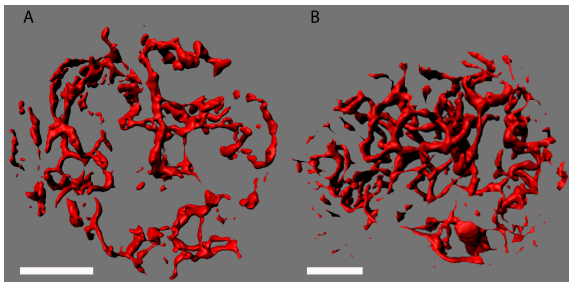


Fig 1. Two-photon confocal images of vascularity in pancreatic islets with low (A) or high (B) blood perfusion (blood perfusion identified by microsphere measurements).

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).

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. Clinical studies are performed to prevent development of type 1 diabetes in patients, e.g. by autologous mesenchymal stem cell transplantation, and to develop means for beta-cell imaging by positron emission tomography. We also conduct studies to improve the results of clinical islet transplantation, e.g. by encapsulation in order to avoid immune suppression of the patients.

. In one of these studies, 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.

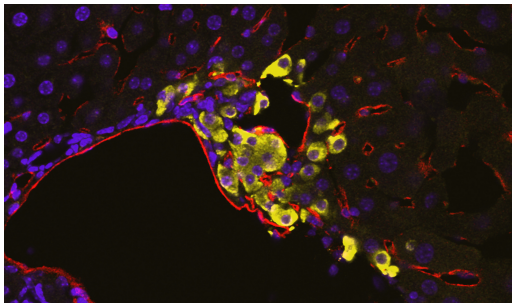
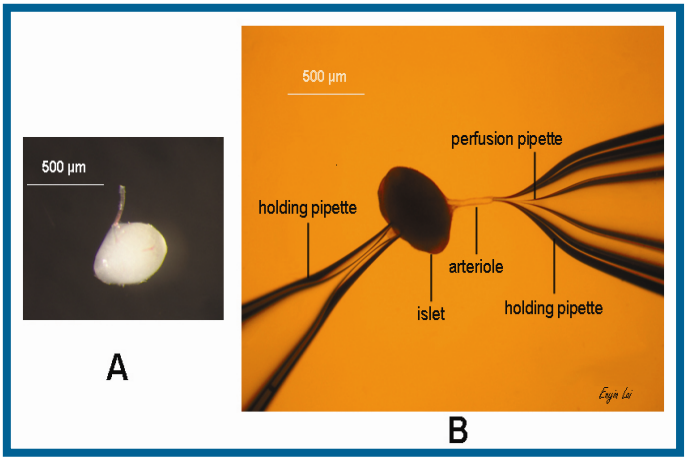


Fig 2. Micrograph showing vascularization of intraportally transplanted islet with disrupted integrity in the wall of a portal vein tributary. Yellow depicts insulin; red CD31 staining for blood vessels and blue DAPI.

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, associate professor

Monica Sandberg, post-doc

Sara Bohman, post-doc

José Caballero, MD, post-doc

Xiang Gao, post-doc

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Zhanchun Li, laboratory engineer

My Quach, laboratory engineer

Lisbeth Sagulin, laboratory engineer

Petra Franzen, laboratory engineer

Birgitta Bodin, laboratory technician

Eva Törnelius, laboratory technician

Violeta Armijo Del Valle, research nurse

Rebecca Hilmius, research nurse

Karin Kjellström, research nurse

Publications 2013-

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Agencies that support the work

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The European Foundation for the Study of Diabetes

The Swedish Research Council

The Swedish Diabetes Association

The Torsten Söderberg Foundation

The Diabetes Wellness Foundation

AFA

The Swedish Juvenile Diabetes Fund

The Novo Nordisk Foundation

The Knut and Alice Wallenberg 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 Forsknin

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The Family Ernfors Foundation

Goljes Memorial Fund

Islet function in childhood obesity and type 2 diabetes mellitus

Peter Bergsten

Background

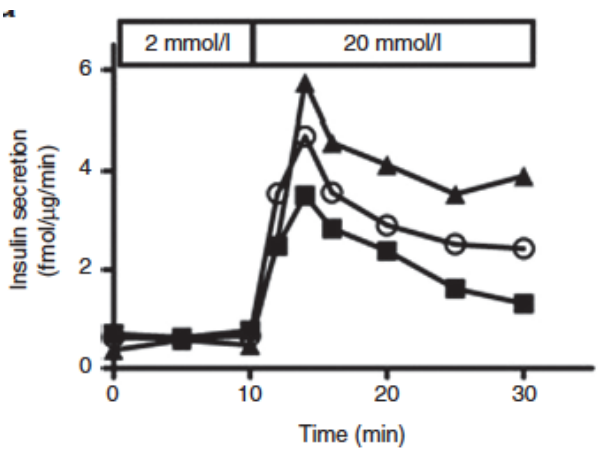
The prevalence of persons with metabolic disease including type 2 diabetes mellitus (T2DM) is expected to rise from 3% in 2000 to almost 5% in 2030. Since obesity is strongly linked with T2DM, the increasing prevalence of over-weight and obesity especially among children, reaching 20% in Sweden, is of particular concern. The rise in obesity has a multi-factorial background, where both genetic and environmental factors contribute. Our research focuses on the role and function of the islet of Langerhans in the early stages of obesity and obesity-related complication including T2DM.

Aim

The overall aim is to find therapeutic approaches to halt the rise in childhood obesity and related metabolic disease including T2DM. This will be attempted by applying a translational approach, where obese and lean patients are examined and characterized and underlying mechanisms investigated in islet cellular systems.

Beta-cell function in juvenile type 2 diabetes and obesity (Beta-JUDO)

The FP7 project "Beta-cell function in JUvenile type 2 diabetes Diabetes and Obesity (Beta-JUDO)" started 2012 and will end 2016 and is coordinated from MCB. In the project the role of the beta-cell in development of obesity is addressed. Beta-JUDO encompasses both *in vitro* work, where isolated human islets and beta-cell lines are used, and *in vivo* work, where obese and lean children are examined.



Elevated palmitate concentrations

When isolated islets are exposed to prolonged elevated palmitate levels, as observed in obese subjects and T2DM, insulin secretion is impaired (Fig 1). However, this impaired insulin sceretion is preceded by islet insulin hypersecretion (Fig 1; Kristinsson et al 2013). Thus, it appears that before palmitate-induced impairment of insulin secretion and loss of beta-cell mass occur, enhanced insulin secretion is 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

In young obese and lean children belonging to the "Uppsala Longitudinal Study of Childhood Obesity" (ULSCO) (Forslund et al 2014), we have 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, which together with similar patient cohorts in Salzburg, Leipzig and Cambridge form the Beta-JUDO childhood obesity cohort. Circulating palmitate concentrations were determined in the lean and obese subjects (Ubhayasekera et al, 2013). 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.

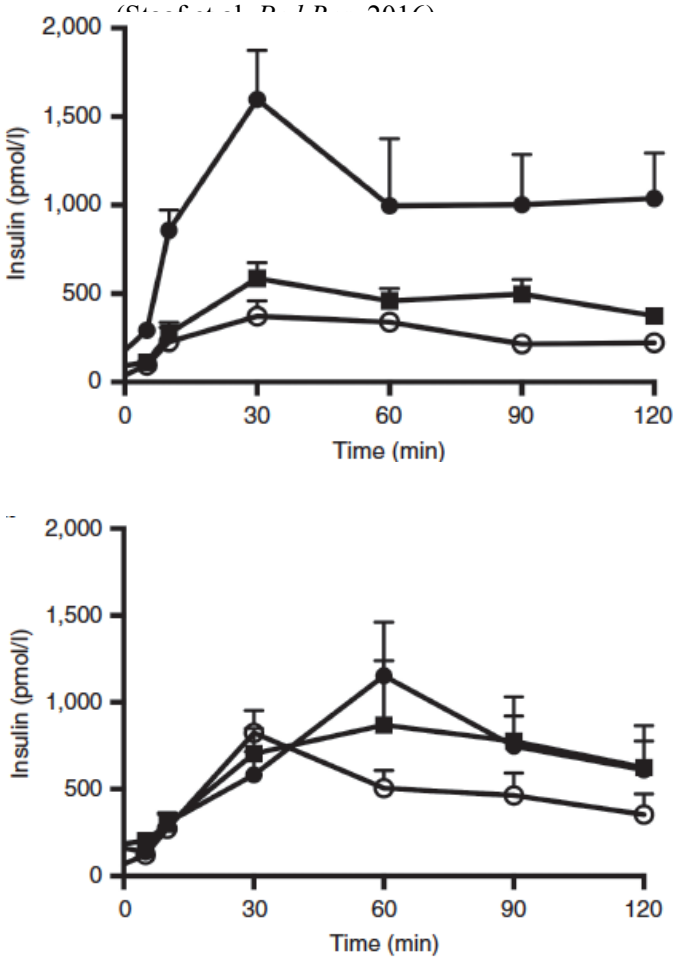


Figure 2: Oral glucose tolerance test in obese pre-pubertal (top panel) and pubertal (bottom panel) children with high palmitate (closed circles), low palmitate (closed squares) and lean controls (open circles) (Staaf et al, *Ped Res*, 2016).

Attenuation of insulin hypersecretion

In isolated islets approaches to attenuate beta-cell hypersecretion are 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 are expected to give information on pharmacological treatment alternatives for the obese children. In this way antagonist of the G-protein coupled receptor FFAR1, which has palmitate as ligand, was investigated for its effects on insulin hyeprsecretion (Kristinsson et al, *Endocrinology*, 2013; Kristinsson et al, *BBA*, 2015).

Glucagon/Insulin processing

Accentuated glucagon secretion, was also observed in obese children with high circulating palmitate concentrations (Manell et al, JCEM, 2016). We have investigated how elevated levels of glucagon correlate with glucose tolerance in children with obesity. Also, in isolated human islets mechanisms for glucagon hypersecretion are investigated.

Sphingolipids

When palmitate concentrations are elevated the formation of the sphingolipid ceramide is increased. Since this sphingolipid has been implicated in apoptosis we have investigated how sphingolipid metabolism is affected in obesity. This was done by measuring multiple shingolipid species by GC-MS both in beta-cell exposed to elevated palmitate concentrations (Manukyan et al, 2014) and in the circulation of obese and lean children.

Significance

The results of the project are expected to identify novel principles of normalizing hypersecreting beta-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-cell function and apoptosis in juvenile obesity appears to be limited.

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Swedish Diabetes Association

Regional Research Council

Gillberg's Foundation

Family Ernfors' Foundation

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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).

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.

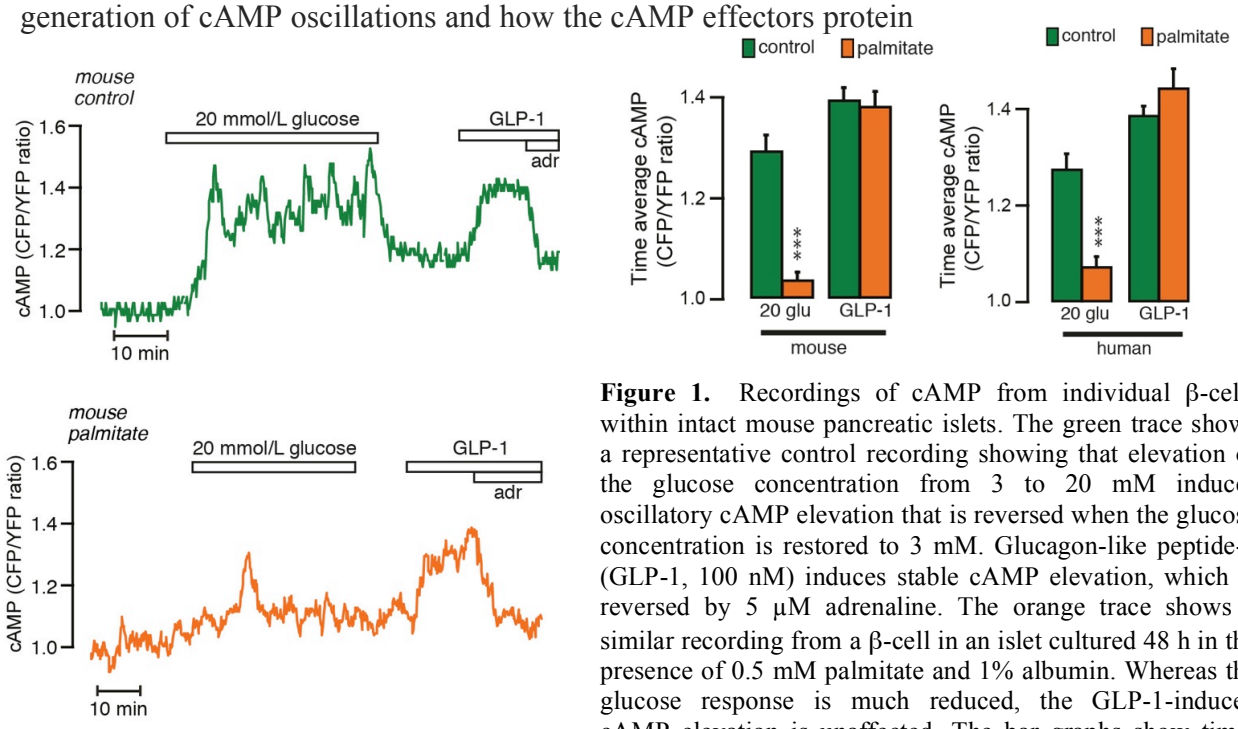

Figure 1. Recordings of cAMP from individual β-cells within intact mouse pancreatic islets. The green trace shows a representative control recording showing that elevation of the glucose concentration from 3 to 20 mM induces oscillatory cAMP elevation that is reversed when the glucose concentration is 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.

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.

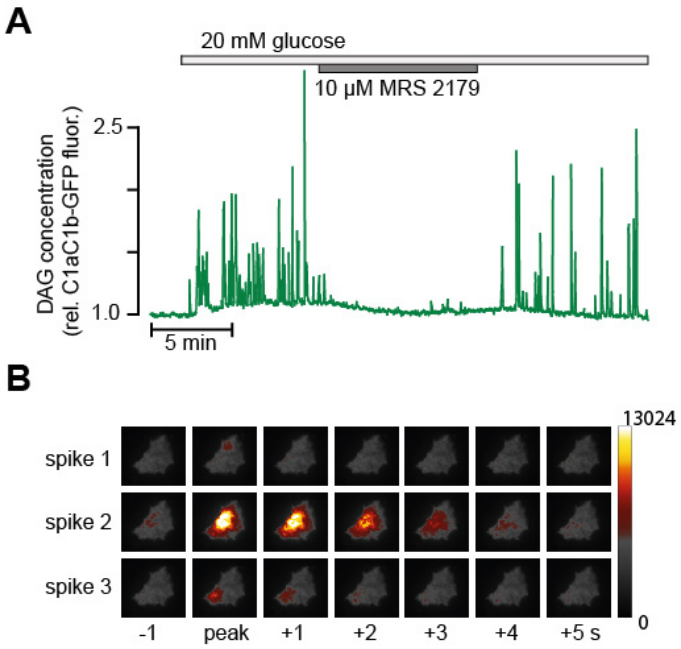

Figure 2. (A) Glucose stimulation of a mouse β-cell triggers pronounced DAG spiking in the plasma membrane that is monitored with a fluorescent DAG reporter. The response is reversibly inhibited when the autocrine action of ATP is blocked with the purinergic receptor antagonist MRS2179.

(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.

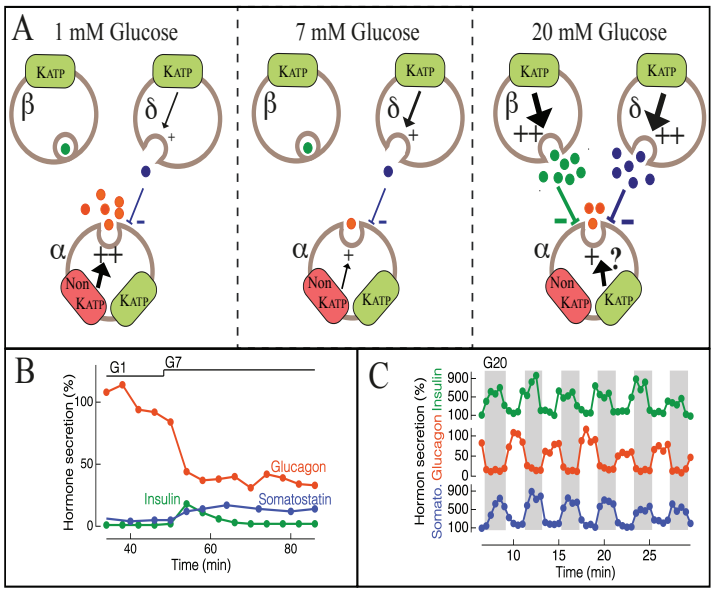

Figure 3. Model for glucose regulation of glucagon release. (A) In the 1-7 mM range (G1, G7), glucose controls glucagon release via an intrinsic non-K_{ATP} channel-dependent mechanism in α-cells and paracrine release of somatostatin from δ-cells has only a tonic inhibitory effect. (B) The graph showing 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.

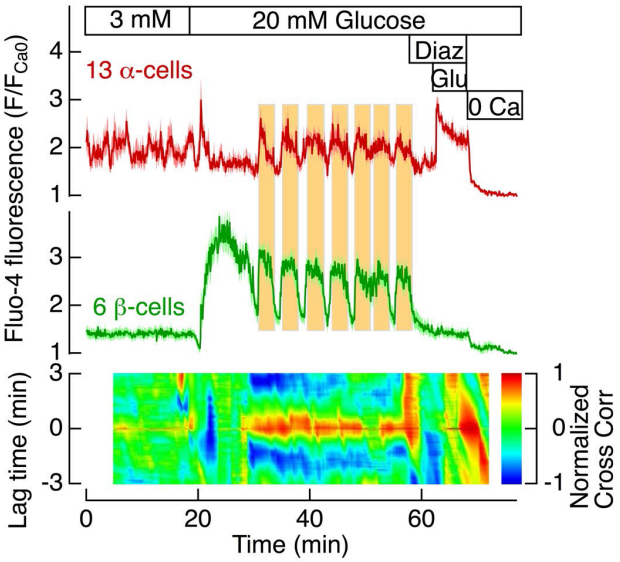

Figure 4. The graphs show average cytoplasmic Ca^{2+} concentrations (red for α- and green from β-cells ± s.e.m. (pink for α- and light green for β-cells) for all 13 α-cells and 6 β-cells in a single mouse islet loaded with the fluorescent Ca^{2+} indicator fluo-4 and with red fluorescent protein expression 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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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.

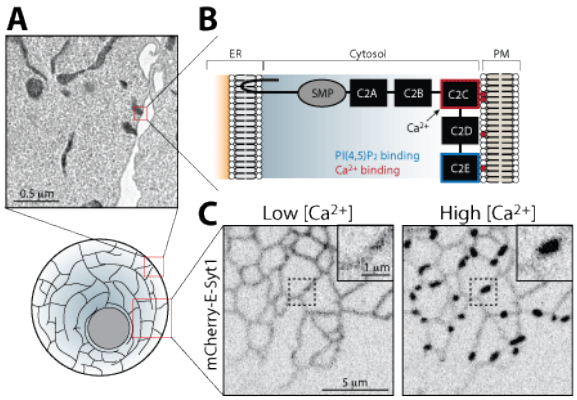

***Figure 1**: **A**. Electron micrograph of a fibroblast where the ER is stained black. Notice how parts of the ER are in close proximity of the cell periphery (red box). **B**. Schematic illustration depicting Extended-Synaptotagmin-1 (E-Syt1), an ER-anchored protein that also binds the plasma membrane 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.*

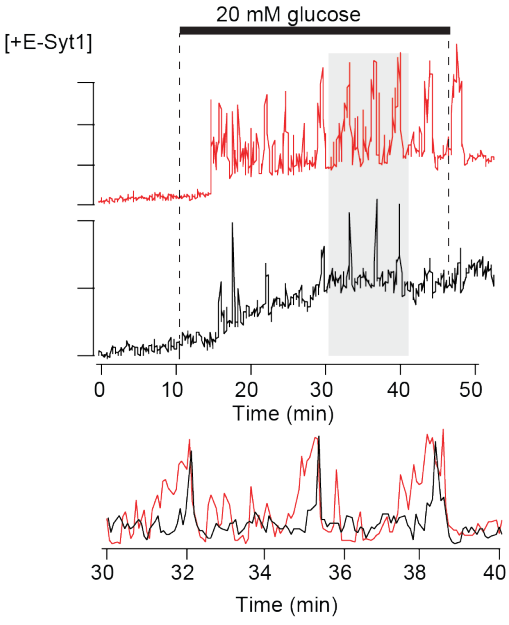

***Figure 2**: Figure shows glucose insduced oscillations of the cytosolic Ca^{2+} concentration (red) and the corresponding recruitment of the ER-localized E-Syts (back) to the plasma membrane. The gray area is shown below on an expanded time axis. Notice the close association between changes in Ca^{2+}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

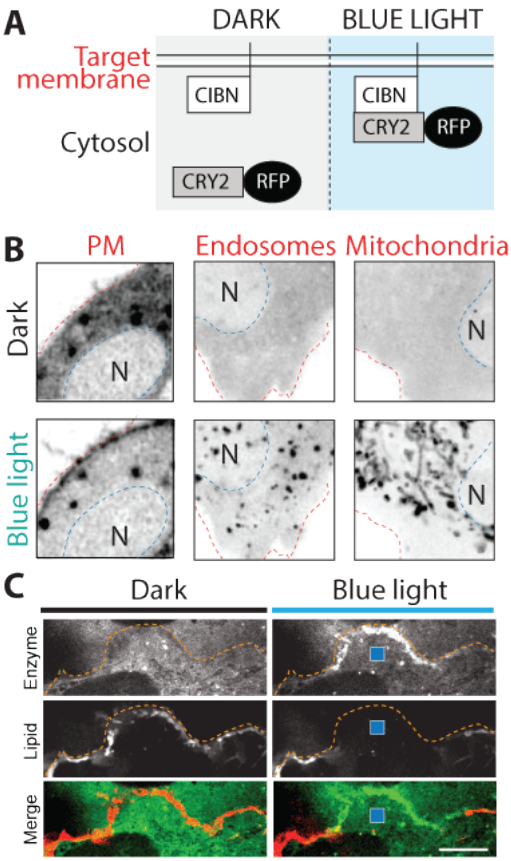

Figure 2: A,B. *Drawing showing the principle of light-induced hetero-dimerization. One part of the optogenetic module (CIBN) can be anchored to any cellular membrane (target membrane) whereas the other part (CRY2) can be fused to a protein of interest (here a Red Fluorescent Protein). Blue-light illumination 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).*

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Agencies that support the work

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STINT

Magnus Bergwalls 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

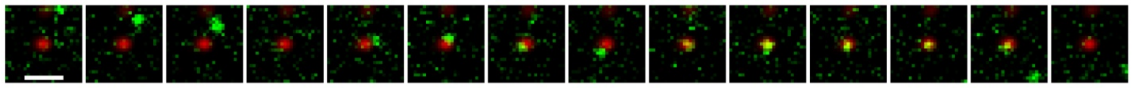
Every ß-cell contains thousands of secretory granules that store insulin. When blood glucose is elevated, these granules undergo regulated exocytosis and release the hormone into the blood stream. Before this can happen, granules have to reach the plasma membrane, where they "dock" and then assemble 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?

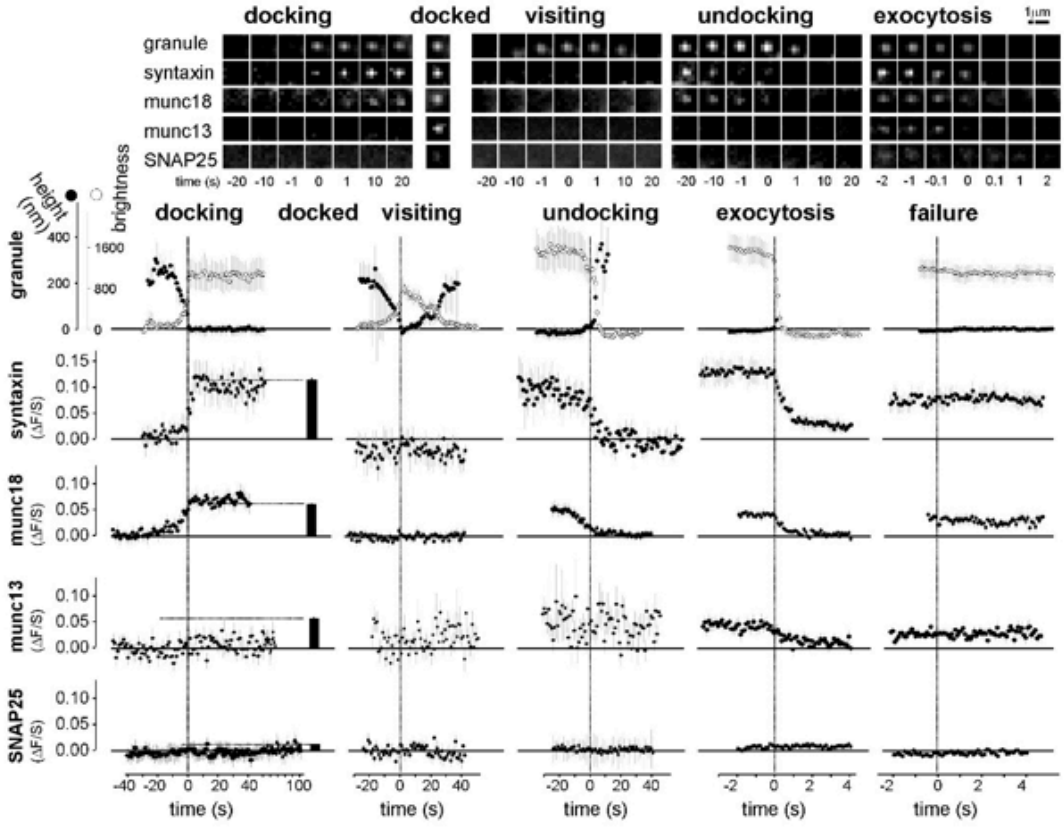
The three SNARE proteins syntaxin, SNAP25 and synaptobrevin are central to membrane fusion during exocytosis. Since two of these, the t-SNAREs syntaxin and SNAP-25 inhabit the plasma membrane, one expects them to collect at the exocytic site before a vesicle or granule can fuse there. Indeed, we found 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. However, individual molecules of both proteins diffuse rapidly in the plasma membrane and are only occasionally captured beneath a granule, for a short time (<1s). Thus, the protein composition of individual granule-associated nanodomains is remarkably dynamic and correlates with the granules' ability to exocytose. This organization is established during or just after granule docking, which suggests that granules approaching the plasma membrane might induce the formation of their own docking site. Dynamic association of exocytosis proteins with individual granules occurs on a timescale consistent with rapid cellular signaling, and may be important for the short-term regulation of insulin secretion (Barg et al PNAS 2010; Knowles et al PNAS 2010). We have until now quantified to over 20 other exocytosis proteins (syntaxin, SNAP25, munc18, munc13, rab3 etc) , and established the time course of their recruitment to the the insulin

granule release site. 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. We could also show that the proteins SNAP25 and munc13 are recruited to the docking site with a delay of at least a minute, consistent a role in granule priming rather than docking. We conclude that secretory vesicles dock by inducing syntaxin1/munc18 clustering in the target membrane, and find no evidence for preformed docking receptors.

Our work is unique in that we have correlated release probability or individual docking/undocking events with local protein recruitment. We provide the first quantitative timecourse for the assembly of the release site, which puts constraints on molecular models of docking and exocytosis. For example, we show that Ca^{2+}-channels, SNAP25 and munc13 are not detectable at the release site for at least 30 s after docking, implying that these proteins are recruited as part of secretory vesicle priming, rather than docking. This is not due to limits in sensitivity, as our method can detect specific association of on average less than one labeled molecule with the docking site To our knowledge, this is also the first time that the formation of protein-containing membrane rafts has been directly visualized and quantified in living cells.



A single molecule of Syx-EGFP (green) binds to a secretory granule (NPY-cherry, red). Scalebar 1µm; 20ms per frame.



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Agencies that support the work

Diabetes Research Wellness Foundation

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Novo Nordisk Foundation

European Foundation for the Study of Diabetes/MSD

European Foundation for the Study of Diabetes/BI

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The Göran Gustafsson Foundation

Family Ernfors Foundation

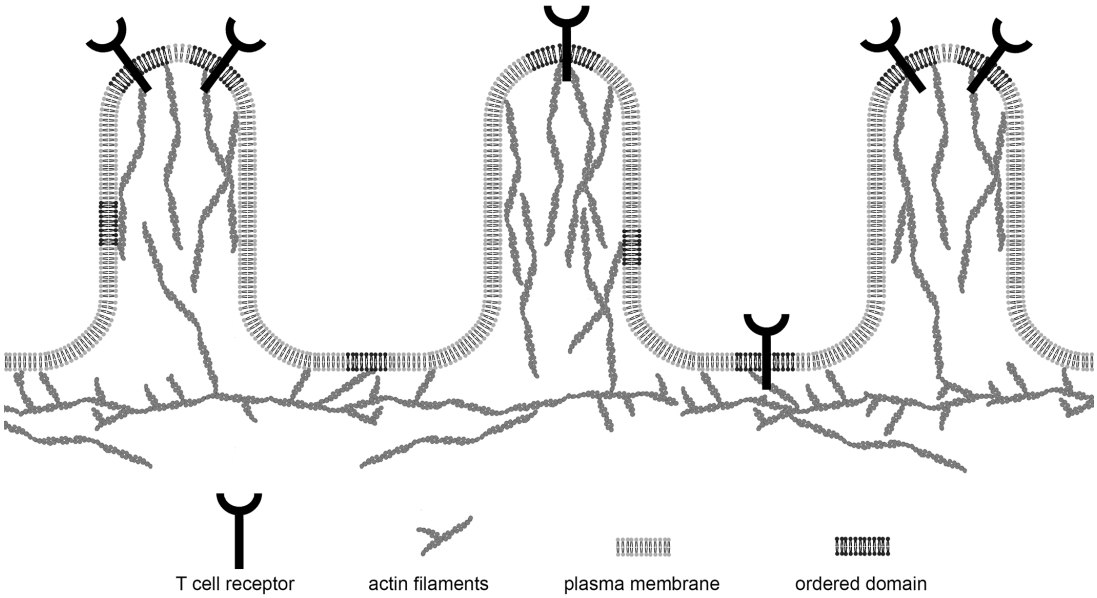
OE och Edla Johanssons stiftelse

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, which are more ordered than the rest of the plasma membrane. The high order is generally considered homologous to the tight packing of cholesterol and sphingolipids observed in model membranes. 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 now together with collaborators at CBA at UU developing a method to analyse diffusion coefficients on non-flat surfaces.

γ9δ2 is a T cell subset that is activated by phosphoantigens, small organic compounds with phosphate groups. Together with collaborators we have found that media from erythrocytes infected with *P. falciparum* can stimulate γ9δ2 T cell prolifieration (Lindberg et al., 2013) suggesting that phosphoantigens both are produced in and secreted from these cells. We now address at which parasite stage this production occurs and what metabolic pathway is responsible for the production and how phosphoantigens affect the plasma membrane organisation of signalling molecules.

We also 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 different intracellular distributions of

molecules are represented by our division of colocalisation analysis into co-occurrence and correlation.

Members of the group

Ingela Parmryd, associate professor

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Parham Ashrafzadeh, graduate student

Chenxiao Liu, graduate student

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Chenxiao Liu: Vγ9Vδ2 T cell activation by phosphoantigens released by *Plasmodium falciparum* infected erythrocytes. December 2015.

Agencies that support the work

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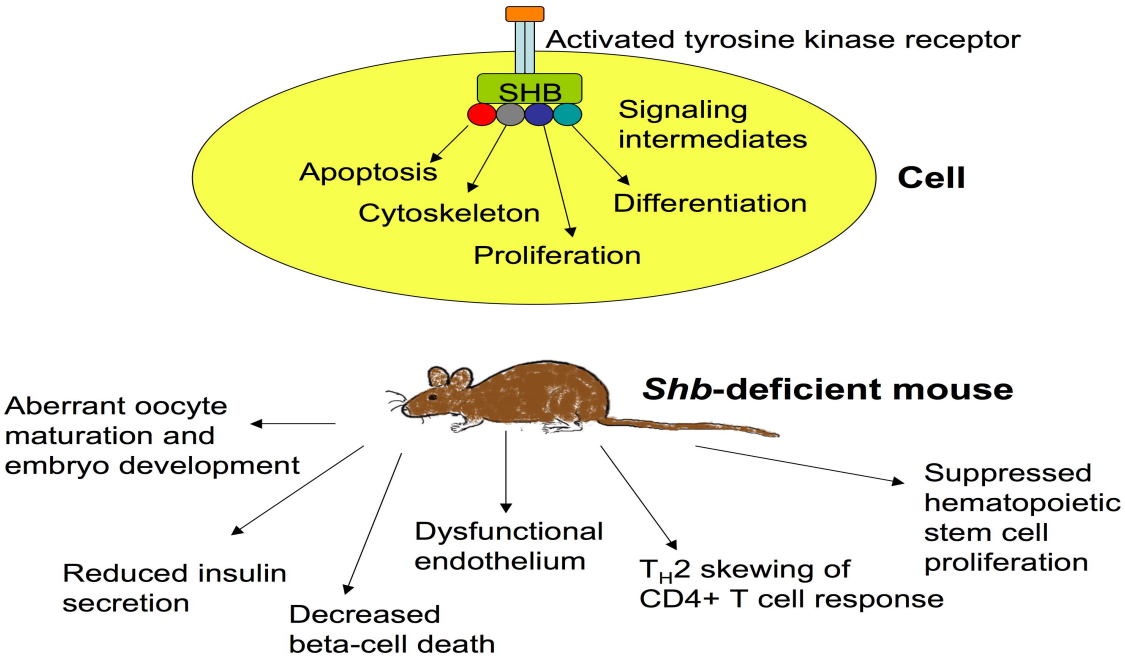
Importance of Shb-dependent signaling for angiogenesis and hematopoiesis

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.

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 for tumor metastasis

B) The development of leukemia in relation to Shb deficiency

Members of the group

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Maria Jamalpour – PhD-student

Xiujuan Li- Post-doc

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Agencies that support the work

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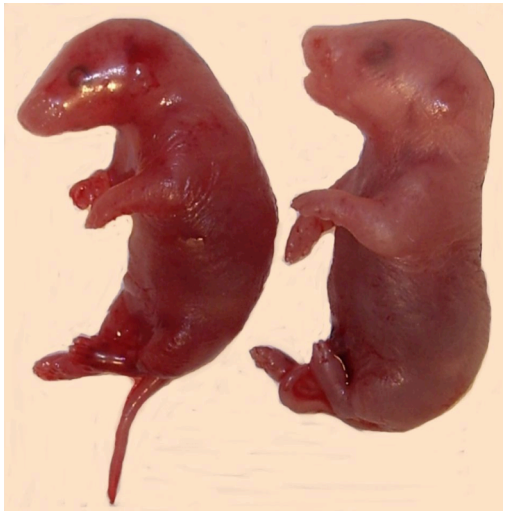
Stiftelsen Familjen Ernfors fond

EXODIAB

Complications in pregnancy

Ulf Eriksson, Parri Wentzel

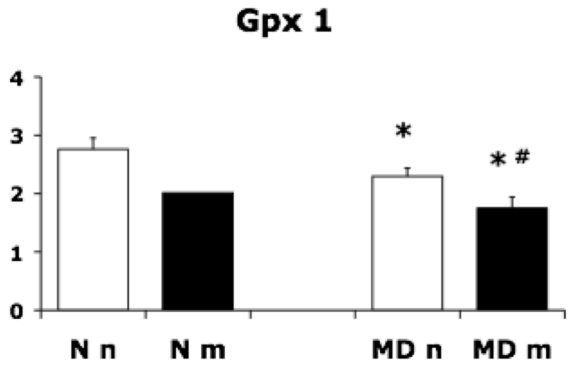
We are studying different types of pregnancy complications, resulting in disturbed embryo-fetal development as a consequence of altered maternal metabolism (caused by diabetes, obesity, or ethanol intake). Our short-term aims are to clarify and understand the mechanisms and patterns of dysmorphogenesis; 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.



Fetuses with facial malformation (left) and normal morphology (right), from diabetic rats.

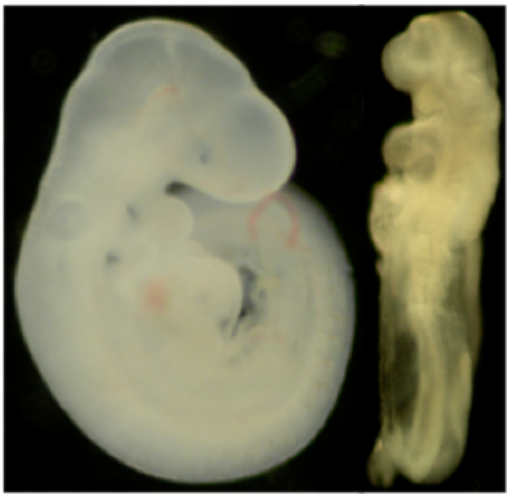
Diabetes in the pregnant women is associated with an increased risk for malformations in the offspring and preeclampsia in the mother. We have studied the mechanisms behind the disturbed development of the offspring in animal models, embryo culture, as well as by *in vitro* culture of embryonic tissues 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.

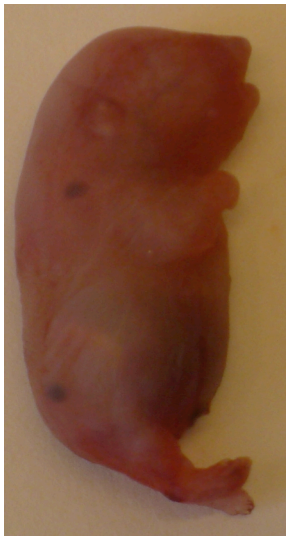


Decreased expression of Gpx-1 in malformed compared to non-malformed offspring of diabetic rats.

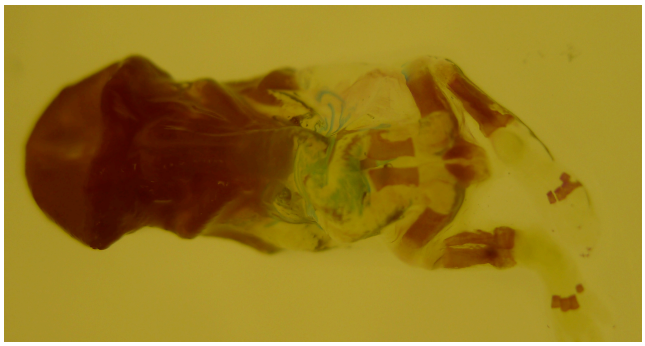
Recently we have found evidence for a new teratological pathway in diabetic pregnancy, activation of the receptor for advanced glycation end products (RAGE). We will persue this line of research by identifying the ligand(s) causing the RAGE activation, and by investigating the possible therapeutical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

Members of the group

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Andreas Ejdesjö, postdoc

Collaborators

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2. Post-implantation Diabetic Embyopathy. Eriksson UJ & Wentzel P, Chapter 41. Textbook of Diabetes and Pregnancy, Informa Healthcare, 2015

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.

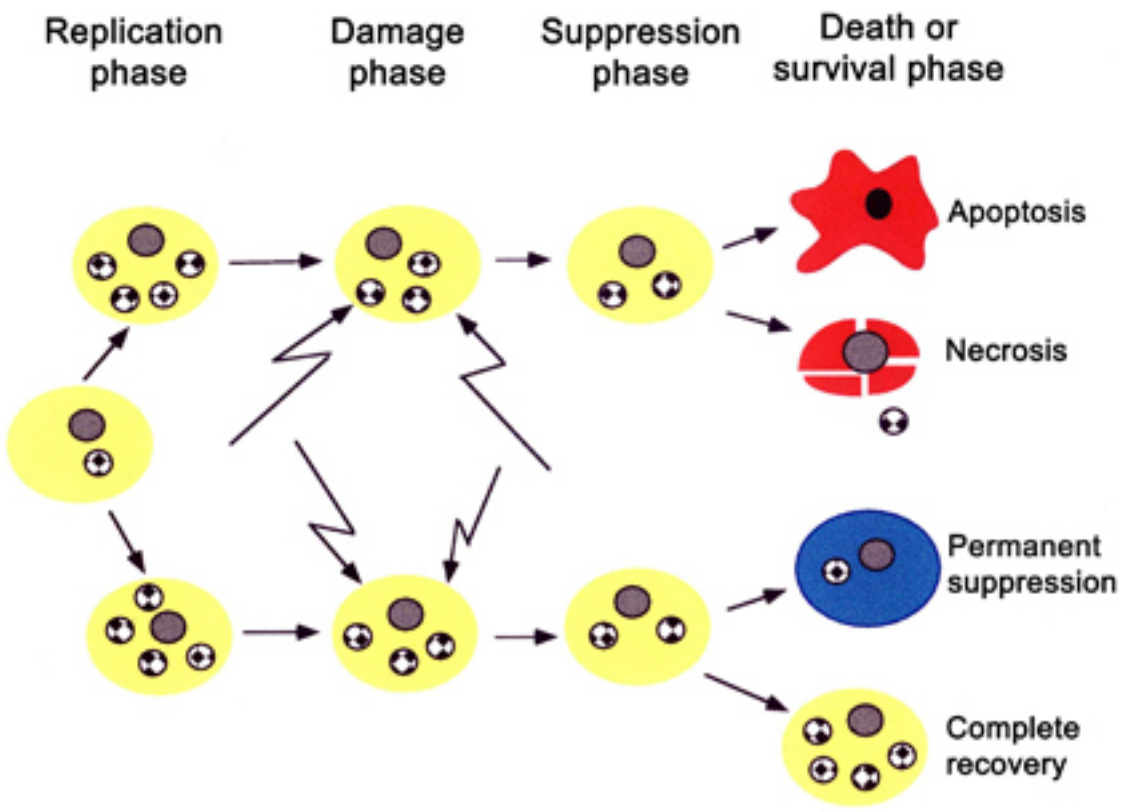

Fig. 1. Schematic view of the β-cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β-cell replication is increased, but later it becomes restricted. After birth β-cells acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate 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)

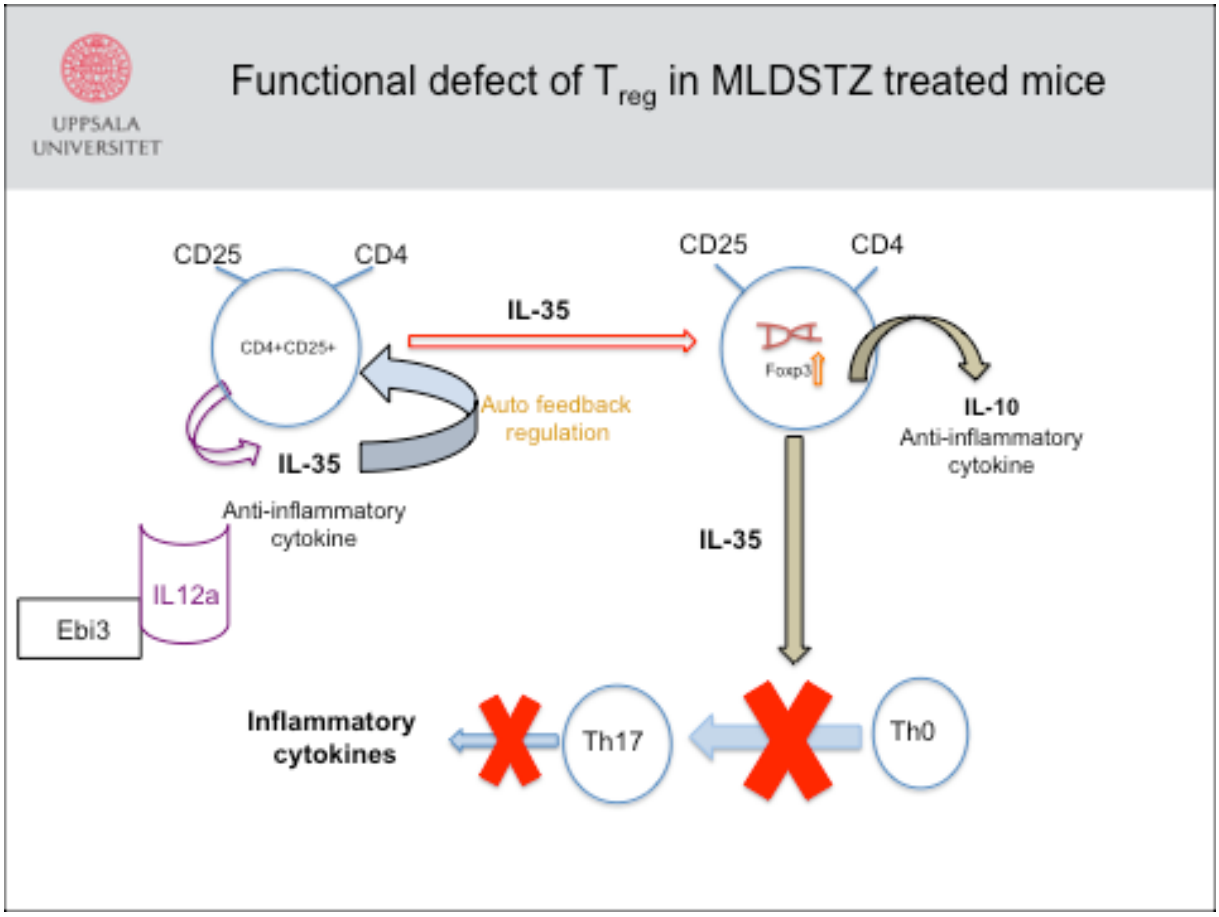

Fig. 2. Based of a number of different experiments we propse that Tregs in the multiple low dose streptozotocin (MLDSTZ) model of T1DM are functionally impaired, since a key cytokine (IL-35) is not being up-regulated in response to the proinflammatory environment induced by MLDSTZ.

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

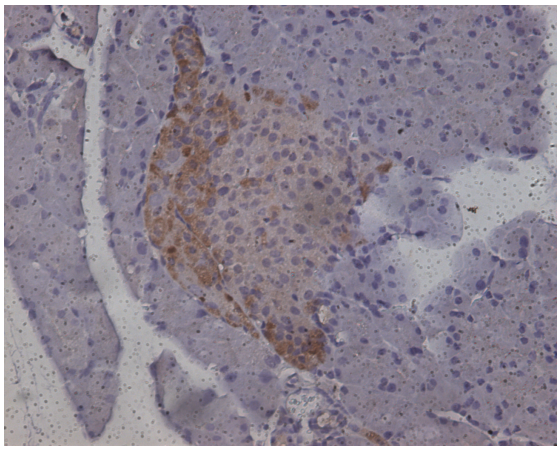

*Fig. 3. Pancreatic islet of a female colonized bank vole 18 weeks of age. The bank vole was hyperglycemic (17.4 mM) 120 min after the IPGTT and serum insulin was elevated (2.34 mg/ml). The section was IHC stained with an Ljungan virus-specific antibody (brown colour) showing strong staining in some 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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Interleukin-35 adminstration counteracts established murine type 1 diabetes – possible involvement of regulatory T-cell.

Sci. Rep. 5:12633, DOI: 10.1038/srep12633 pp. 1-19, 2015

16. Blixt M, Niklasson B and Sandler S.

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:

PhD Martin Blixt (adjunt, part-time research)

PhD (Lina Thorvalson (part time post-doc)

Laoratory technician IngBritt Hallgen (part-time)

PhD Student Kailash Singh

PhD student Gustaf Arbrant

Professor Stellan Sandler

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

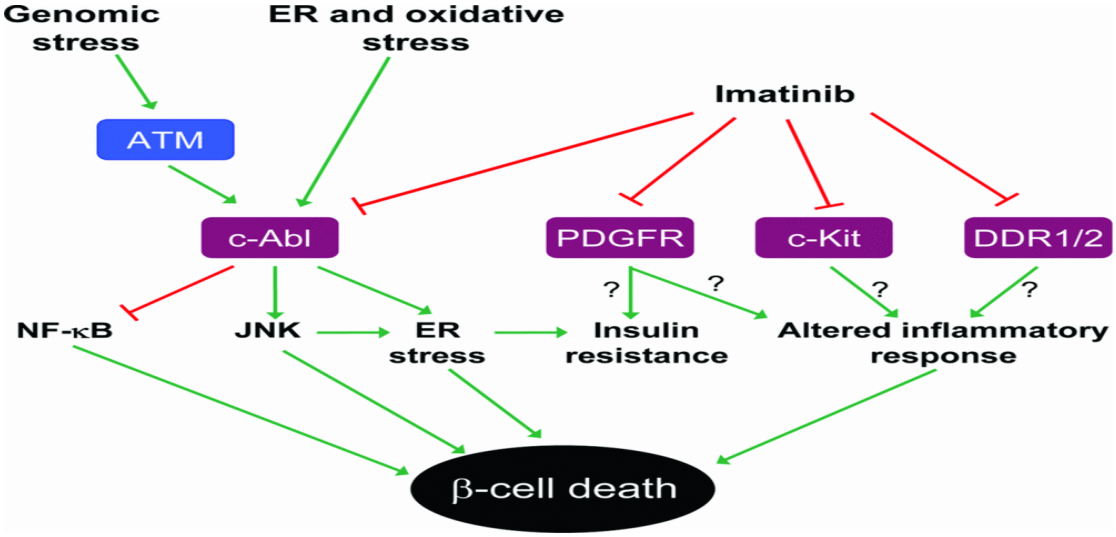
Role of tyrosine kinases in β-cell apoptosis and diabetes

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

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.

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 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 (part-time) – Post-doc

Andris Elksnis – Project student

Zhao, Lijun (part-time) - technician

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Agencies that support the work

The Swedish Diabetes Association

Stiftelsen Familjen Ernfors Fond

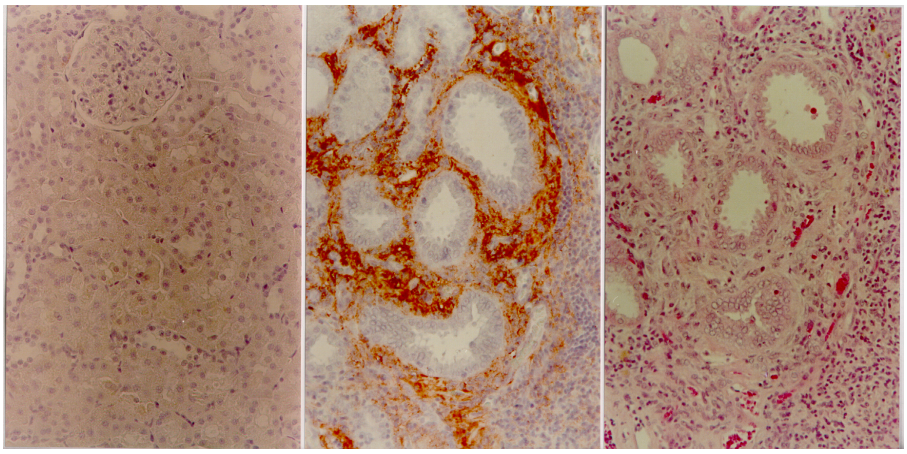
Diabetes Wellness

EXODIAB

Intrarenal Hyaluronan in the Regulation of Fluid Balance. Pathophysiological Relevance to Renal Damage during Diabetes and Ischemia-Reperfusion.

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



Histochemical staining for HA demonstrating the absence of HA in the normal renal cortex of rats (left). Patchy accumulation of interstitial HA in the ischemia-reperfusion damaged renal cortex (middle). Accumulated HA is found mainly in the same areas as infltrating immune competent cells, as seen by parallel staining with haematoxylin-eosin (right).

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

Peter Hansell – Professor

Angelica Fasching - Laboratory Engineer

Fredrik Palm – Professor

Per Liss – Assoc Professor

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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.

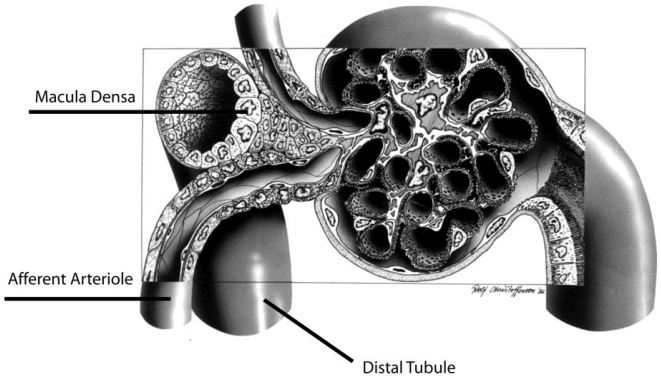

Figure 1. Schematic illustration of the juxtaglomerular apparatus (JGA) with the macula densa cells in wall of the distal tubule and the glomerular arterioles.

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

Publications 2013-

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Dissertation examination

Xiang Gao: ”Local purinergic control of arteriolar reactivity in pancreatic islets and renal glomeruli” October 2014

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. 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

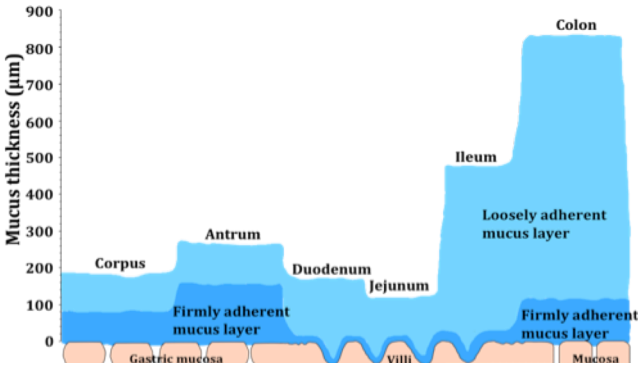
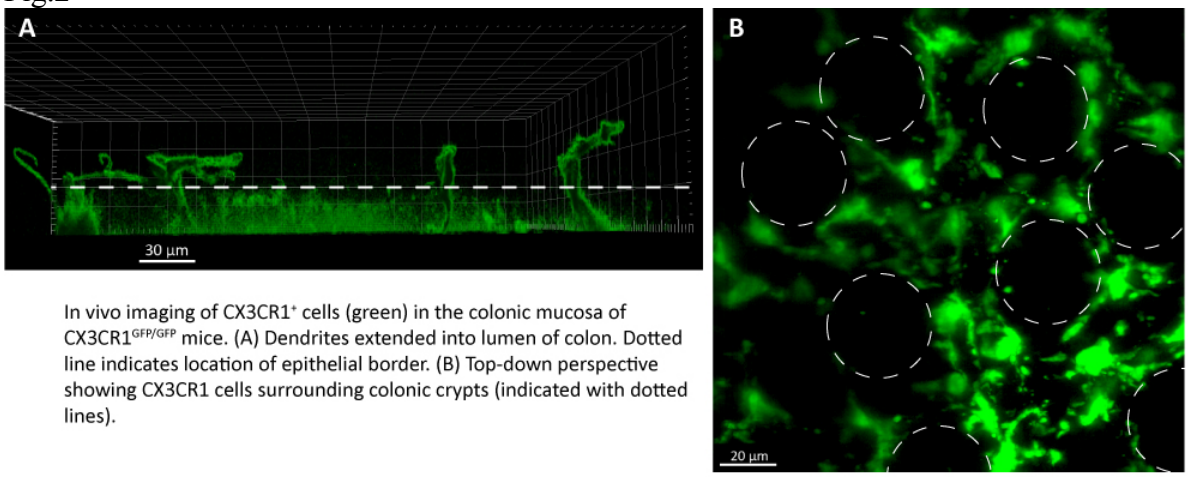


Fig 1. The mucus layers in different parts of the GI tract

confocal microscopy available in our lab (Fig.2). Furthermore, with a newly 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

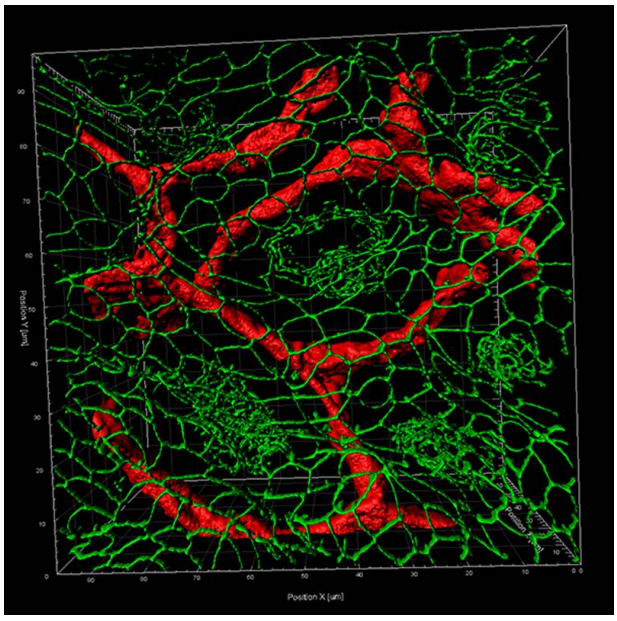


Fig 3. 3D-visualization of capillaries (stained with CD31: red) surrounding colonic crypts just below the luminal epithelial layer (tight junctions stained with ZO-1: green).

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* 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.

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Agencies that support the work

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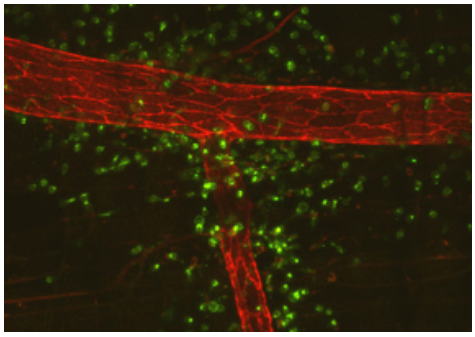
BioGaia AB

Targeting leukocytes in health and disease

Mia Phillipson

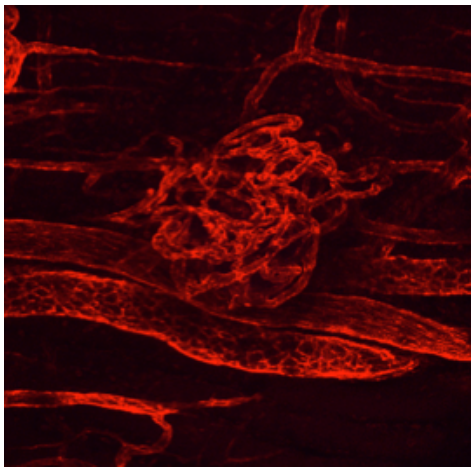
The capacity of circulating leukocytes to arrest on the surface of inflamed endothelium, transmigrate, and penetrate into the underlying tissue are key steps in response to infections as well as other inflammatory processes. The importance of recruited as well as tissue resident leukocytes also during 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.

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Dissertations

Sara Massena "A close-up on neutrophils: Visualizing the mechanisms of their in vivo recruitment and function" PhD December 2015

Gustaf Christoffersson ”Leukocytes in Angiogenesis: Learning from Transplanted Pancreatic Islets” PhD April 2013

Agencies that support the work

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Diabetic Nephropathy and Uremic Toxins

Fredrik Palm

Diabetic Nephropathy (core director: Fredrik Palm)

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.

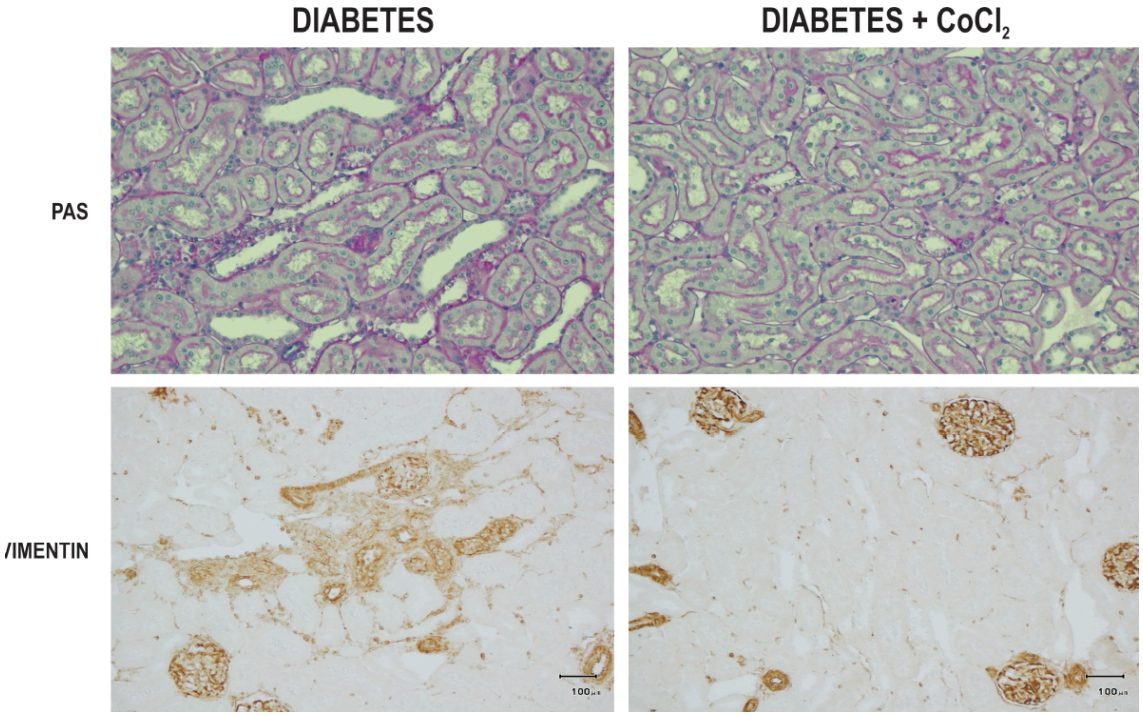

Figure 1. Activation of the hypoxic gene response by chronic $CoCl_2$ treatment prevented the diabetes-inudced kidney hypoxia and the clinical signs of diabetic nephrpathy, such as tubulointerstitial fibrosis, proteinuria and glomerular hyperfiltration.

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.

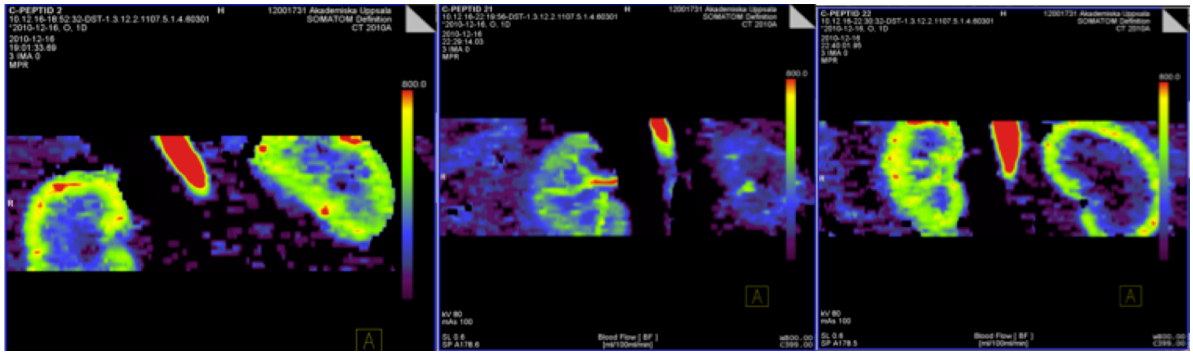

Figure 2. Thirty minuites of warm ischemia to the left kidney (right kidney on the images) did not alter kidney function or the intrarenal blood flow (images abow measured by computed tomography) in control rats four weeks after the ischemic insult. However, the same ischemic insult caused markedly reduced 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.

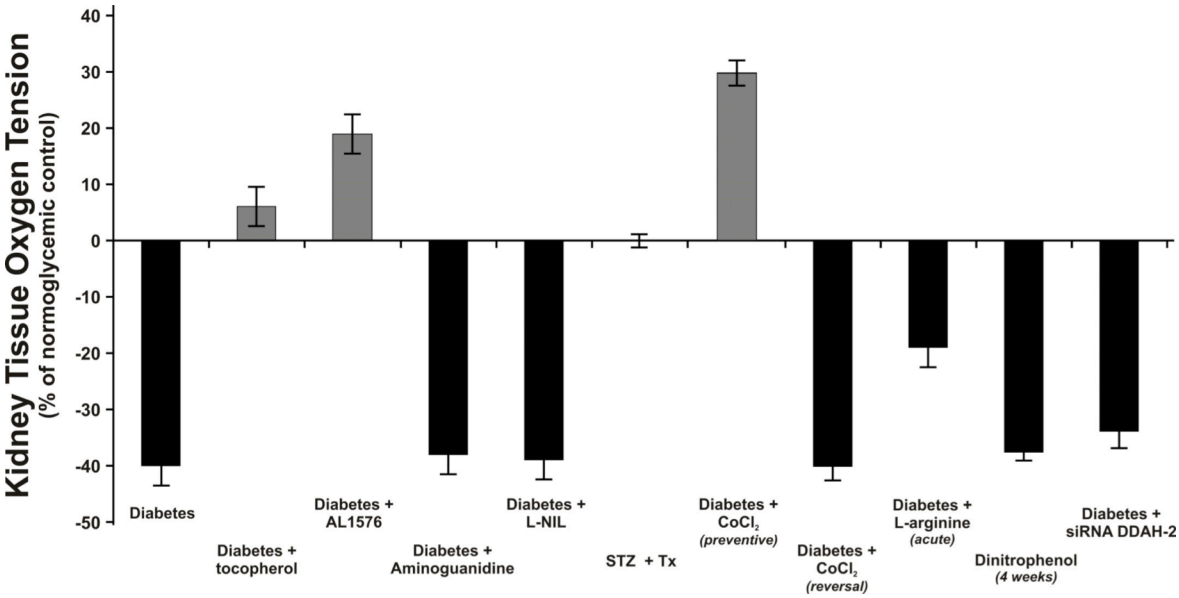

Figure 3. Summary of all investigated mechanisms affecting intrarenal oxygenation in diabetes. Tocopherol (vitamin E) is an antioxidant, AL1576 inhibits aldose reductase and presents activation of the polyol pathway, aminoguanidine inhibits AGE formation, L-NIL inhibits iNOS, STZ + Tx denotes animals 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.

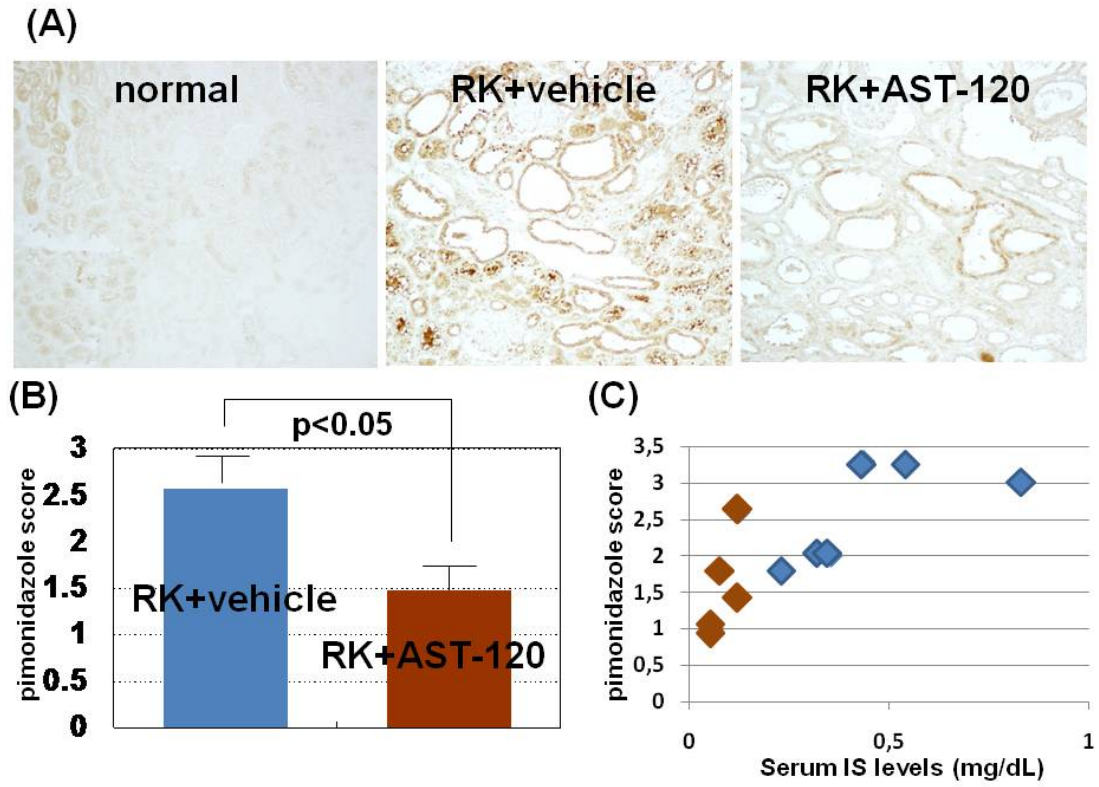

Figure 4. Improvement of oxygenation of the remnant kidney by reduction of uremic toxins. (A) Immunohistochemical staining of pimonidazole accumulation in the cortex showed improvement of oxygenation of the remnant kidney in animals treated with the oral absorbent AST-120 that reduces plasma levels of 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

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Per Eckerbom, Ph.D.-student

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Dissertations

Patrik Persson "Aspects of regulation of GFR and tubular function in the diabetic kidney - Roles of adenosine, nitric oxide and oxidative stress" PhD April 2013.

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.

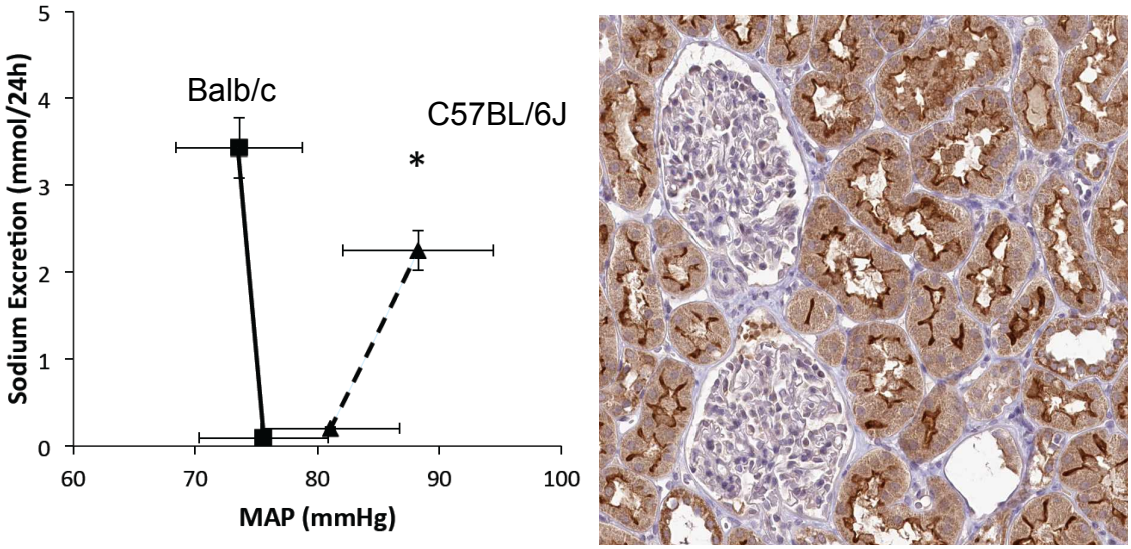

Figure 1: Left: Renal function curves showing how C57BL/6J blood pressure increases when the animals are challenged with a high salt diet, while Balb/c can excrete the salt load without increasing blood pressure. Right: ABCC2 expression in the kidney from http://proteinatlas.org.

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).

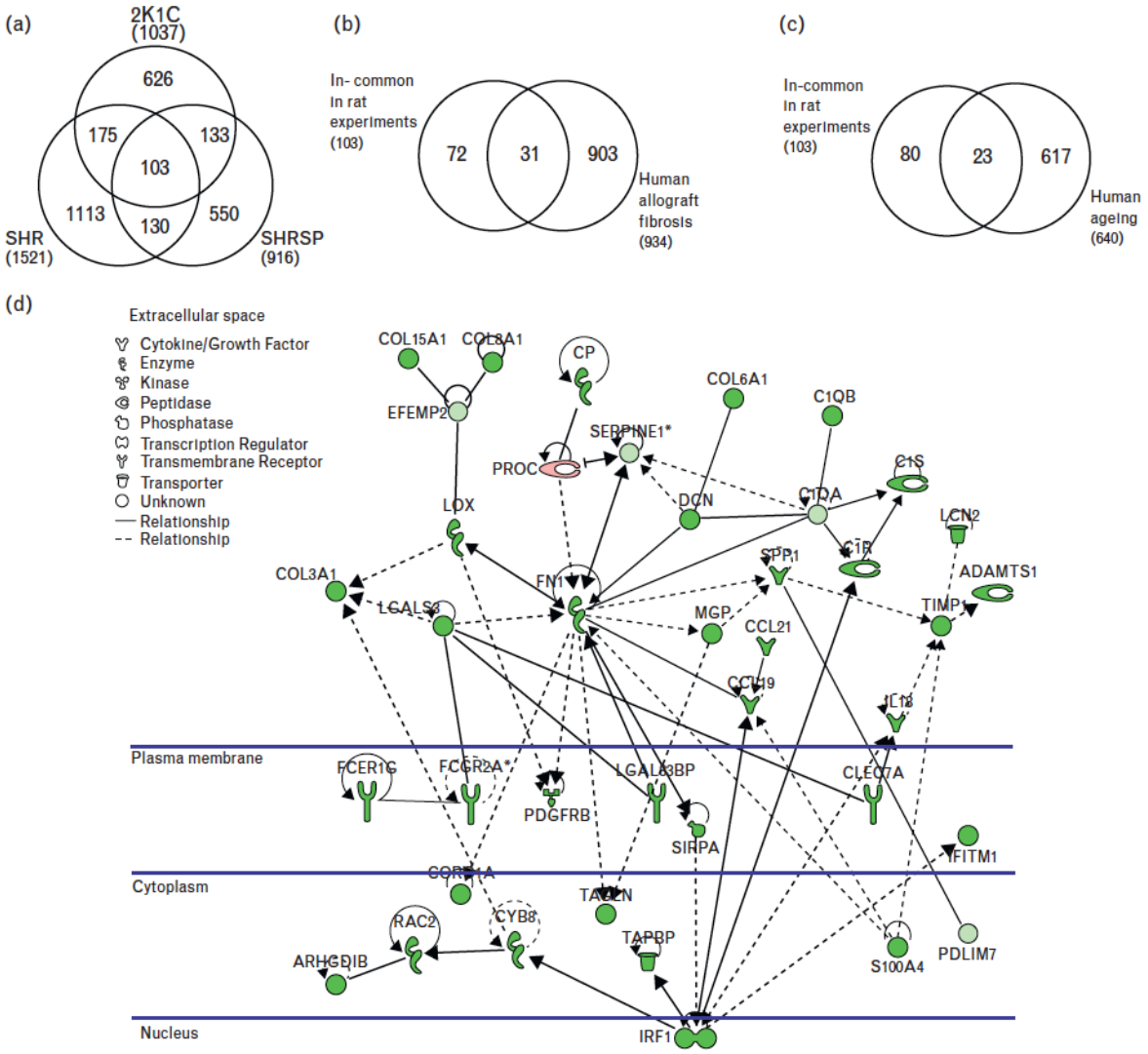

Figure 2: a) Gene expression changes in three hypertensive rat models with kidney damage showing 103 in-common genes. b) 31 in-common genes with human allograft nephropathy, and c) 23 with gene expression changes in elderly humans with kidney fibrosis. d) Genetic network of 44 highly connected genes 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).

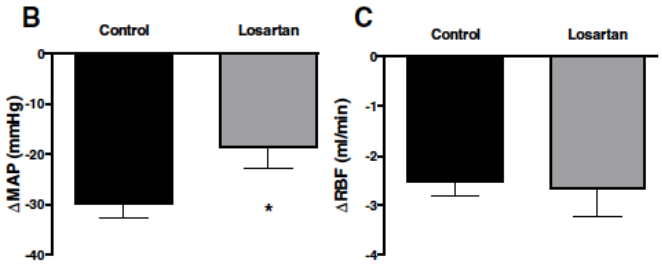

Figure 3: Treatment with the anti hypertensive drug Losartan decreases blood pressure as expected, but it does not aggrevate renal perfusion beyond than of haemorrhage in itself since it relaxes the afferent arteriole and reduces renal vascular resistance.

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.

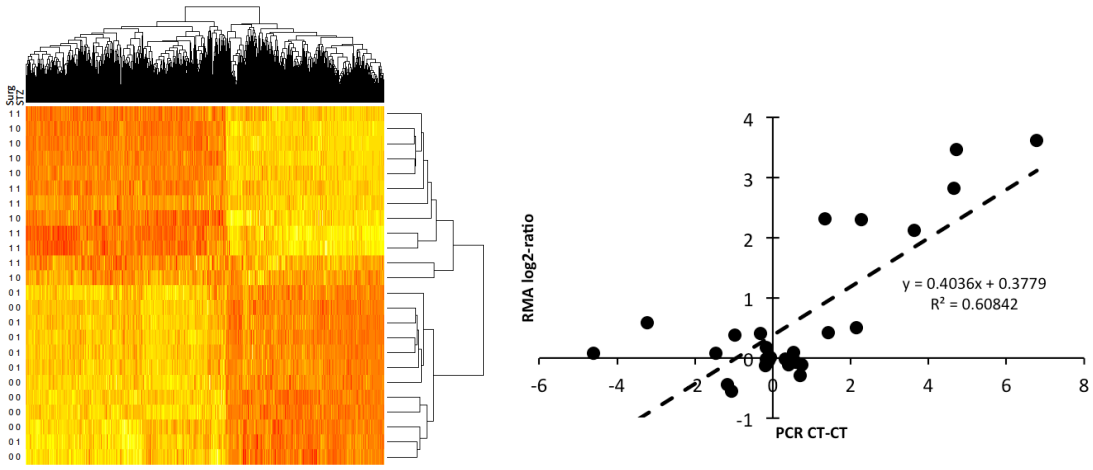

Figure 4: Left: Heat map of over 7000 differentially expressed kidney genes after prolonged surgery. Right: PCR validation of 22 selected genes with a wide expression difference show good correlation with microarray data.

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).

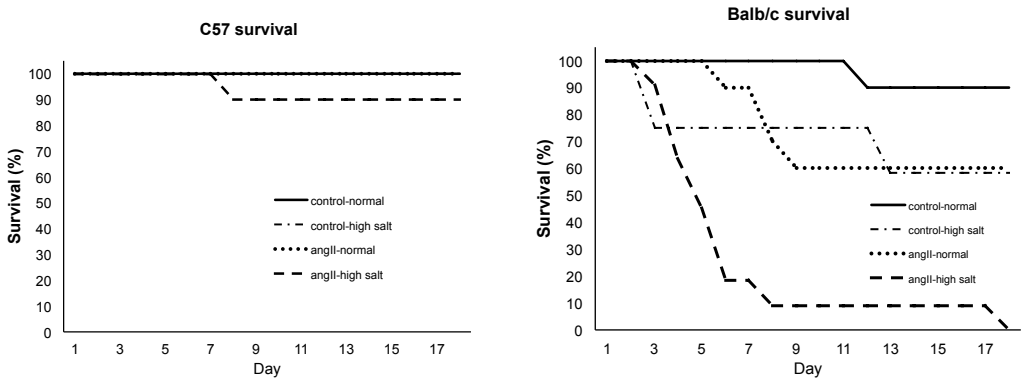

Figure 5: Survival curves for the two strains. Left: C57BL/6J that show minimal morbidity and mortality, and Right: BALB/c that are more sensitive and several animals have to be sacrificed early already with only AngII treatment or high-salt diet. With the combination treatment 80% develop symptoms of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

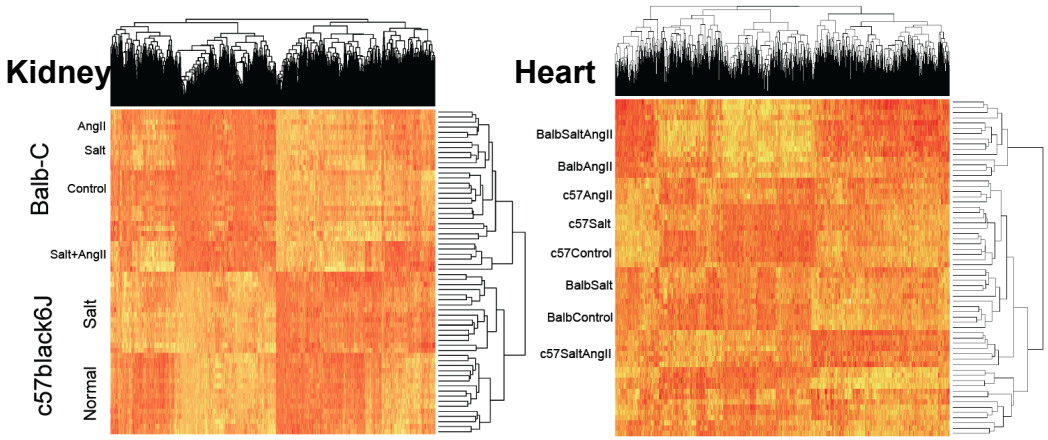

Figure 6: Differentially expressed genes between the strains during combined AngII and salt treatment in the kidney (left) and heart (right).

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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Agencies that support the work

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Åke Wiberg Foundation

Magnuns Bergvall Foundation

Swedish Society of Medicine

Lars Hierta Foundation

Marcus Borgström Foundation

Prizes and awards 2015

Michael Hultström: Early Career Researcher Prize, at the Neural, Hormonal and Renal Interactions in Blood Pressure Control meeting in Mussoorie, India, December 2015.

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.

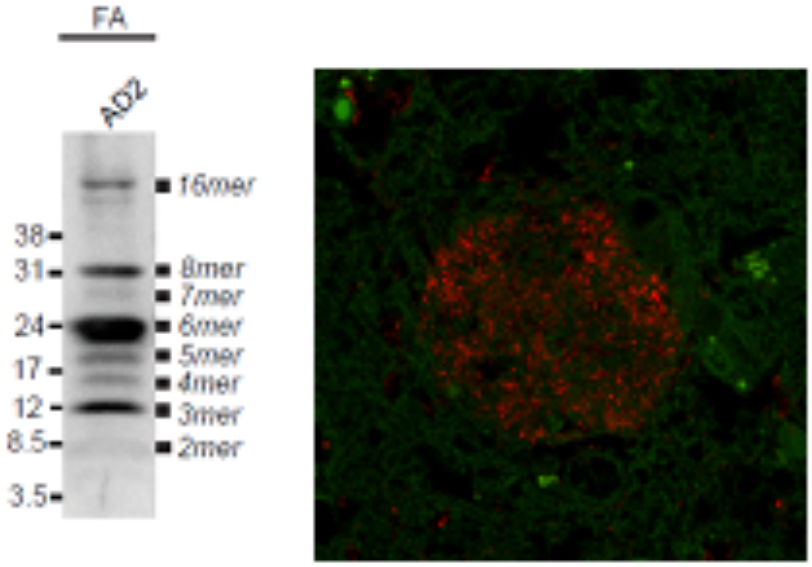
Islet amyloid and beta-cell death

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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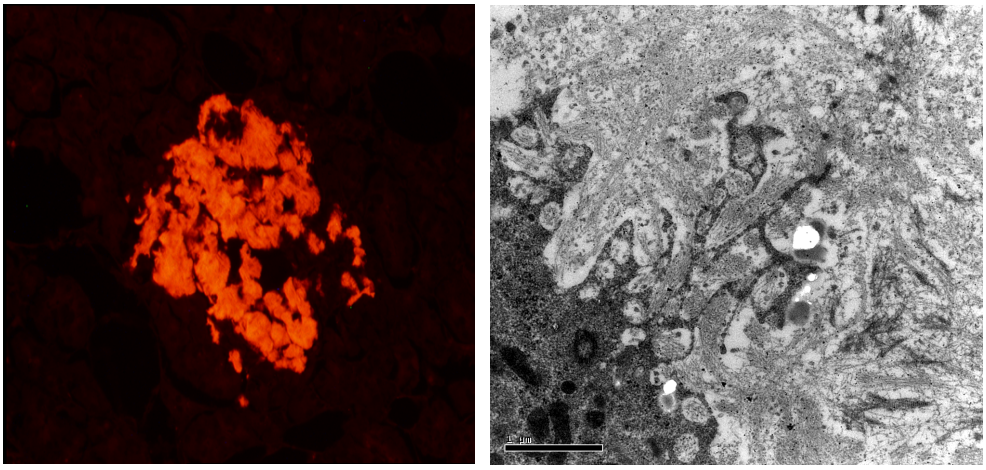
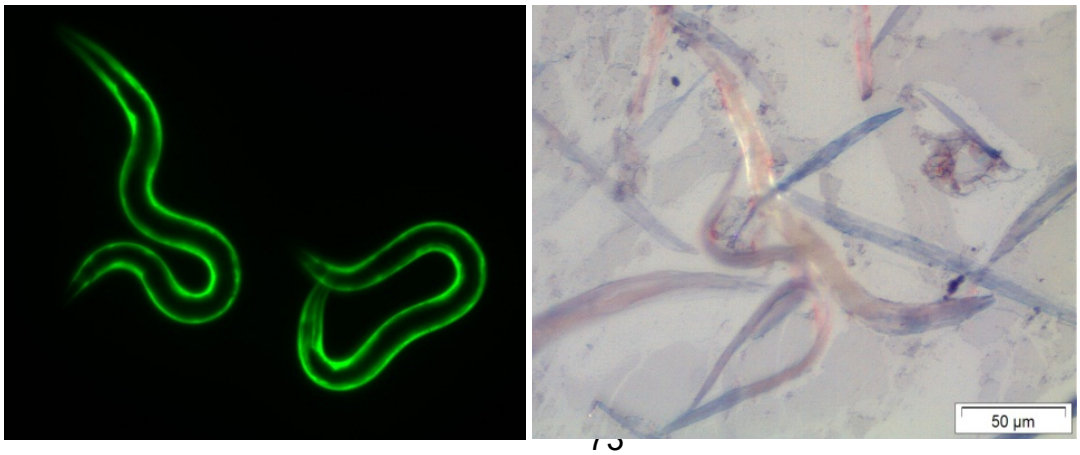


A human islet stained for amyloid by Congo red. The amyloid deposits replace most of the beta-cells. The section is viewed at 546 nm. The electron micrograph shows the border between a beta-cell and extracellular amyloid. Note the close association between the amyloid bundles and the cell membrane.

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.

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Gamla Tjänarinnor

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.

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EMBO Molecular Medicine, in press

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Astrid Karlssons Stiftelse (RHV)

Publications 2013-

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

9: Lundmark K, Vahdat Shariatpanahi A, Westermark GT. Depletion of spleen macrophages delays AA amyloid development: a study performed in the rapid mouse model of AA amyloidosis. PLoS One. 13;8:e79104. 2013

10: Westermark GT, Westermark P. Islet amyloid polypeptide and diabetes. Curr Protein Pept Sci. 14:330-337. 2013 Review.

11: Sponarova J, Nuvolone M, Whicher C, Frei N, Kana V, Schwarz P, Westermark GT, Aguzzi A. Efficient amyloid A clearance in the absence of immunoglobulins and complement factors. Am J Pathol. 182:1297-1307, 2013

Dissertations

Oskarsson, Marie. Islet amyloid polypeptide (IAPP) in Type 2 diabetes and Alzheimer disease. Diss. Uppsala Universitet.

Dissertations 2015

- **Gandasi, Nikhil R.** (2015) Molecular mechanisms of biphasic insulin secretion. Diss. Uppsala Universitet.
- **Massena, Sara.** (2015) A close-up on neutrophils: Visualizing the mechanisms of their in vivo recruitment and function. Diss. Uppala Universitet.
- **Oskarsson, Marie.** (2015) Islet amyloid polypeptide (IAPP) in Type 2 diabetes and Alzheimer disease. Diss. Uppsala Universitet.

Licentiate theses 2015

Alenkvist, I

Ashrafzadeh, P

Cen, J

Grapensparr, L

Kristinson, H

Liu, C

Singh, K

Sivertsson, E

Ullsten, S

Economy

(kSEK)

	2014	**2015**
Undergraduate Education appropriations	33 510	34 273
Faculty appropriations	20 671	20 921
External Grants	37 620	40 415
Contract research	1 082	180
Total	**92 883**	**95 789**

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)

Cell biology I and II (evening course)

Medical cell biology (laboratory project course)

Histology

Physiology

Basic medical physiology

Summer research school (SOFOSKO)

Major Diseases - Homeostasis and endocrine disorders

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 three 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).

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).

Prizes and awards 2015

Kailash Singh: the Young Investigators Award from Scandinavian Society for the Study of Diabetes

Evelina Vågesjö: SKAPA framtidens innovatör i Uppland, UIC Business Lab vinnare av fiktiva investeringar från juryn

Per-Ola Carlsson: The Knud Lundbeck Award from the Scandinavian Society for the Study of Diabetes

Nikhil Gandasi: European Foundation for the Study of Diabetes (EFSD) / Lilly Research Fellowship

Nikhil Gandasi: a two-year research fellowship från Svenska Sällskapet för Medicinsk Forskning (SSMF).

Michael Hultström: the early Career Researcher Prize, at the Neural, Hormonal and Renal Interactions in Blood Pressure Control meeting in Mussoorie, India, December 2015.

Mia Phillipson: elected member of the Swedish Young Academy (Sveriges unga akademi).

Marie E. Oskarsson's PhD theses ***"Islet amyloid polypeptide (IAPP) in Type 2 diabetes and Alzheimer disease"*** has been recognized by the Swedish Society of Diabetology (SFD) as the best preclinical dissertation in 2015.

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