

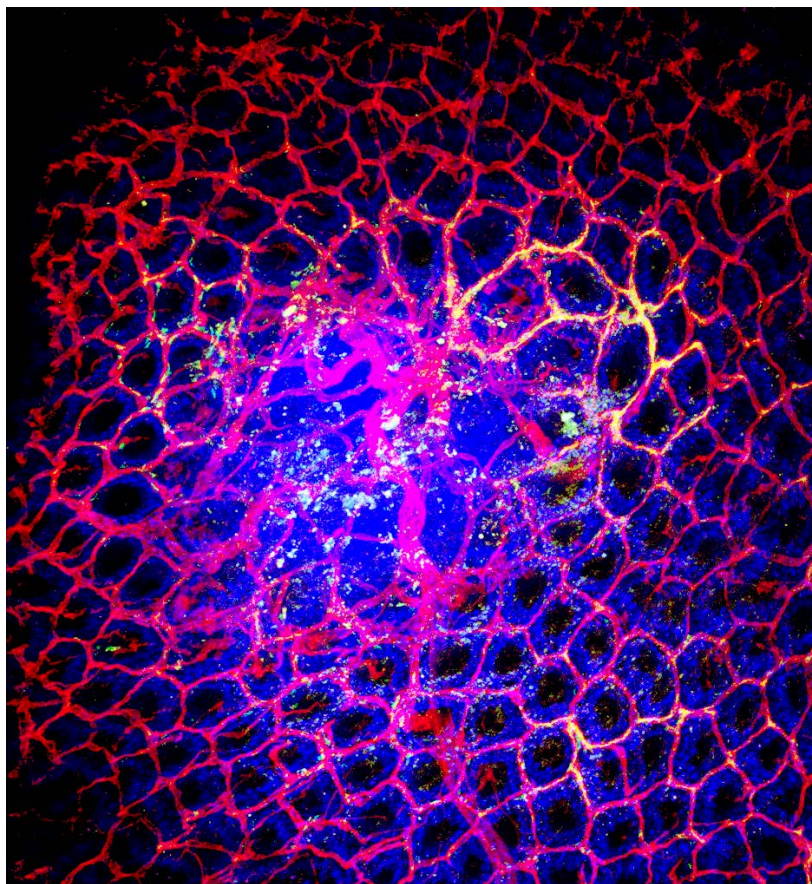


UPPSALA
UNIVERSITET

Department of Medical Cell Biology

ANNUAL REPORT

2013



Fastställd av Institutionsstyrelsen 2014-04-25

Department of Medical Cell Biology

ANNUAL REPORT

2013

Introduction

The past year 2013 was somewhat mixed with regard to the economic situation for MCB. The total financial turnover increased 16% from 78 to 92 million SEK. The government subsidies for basic education increased 10% due to increased teaching volume, whereas the subsidies for research and research education decreased 13%. MCB was less successful in the performance-based distribution of research subsidies, which was attributable to reduced number of examinations, a reduction of MCBs relative contribution to the total number of scientific publications and low use of grants from external sources. These research subsidies are based on MCBs performance during the preceding 4 years and therefore change with some delay. However, during 2013 the use of external grants increased as much as 56% or almost 13 million SEK, which should improve MCBs share of future government subsidies. This increase is due to use of Peter Bergsten's European Union grant and Mia Phillipson's Söderberg grant but also to finishing 6 Research Council (VR) grants. These 6 grants were scheduled for renewal 2014 but only one was funded and no other new VR grant was approved. Our department was not unique in losing VR grants. Many prominent scientists throughout the country lost their grants due to changed and heavily criticised VR policy. Major factors are increased average VR grants, increased grant period for up to 5 years, poorly controlled redistribution of money to young scientists and special VR-professorships with very large grants etc. Also 2014 is expected to be a meagre VR-year but the situation may improve somewhat in 2015. Unfortunately, the application pressure is also expected to increase making VR support a lottery. Current VR policy is consequently a source of considerable concern for MCB and science in general. I hope that the heavy criticism will have effects and will change things to the better. VR itself points out that its relative importance has decreased in relation to other sources of external grants. This is apparent also at MCB, whose major new support comes from non-VR organizations. Therefore I urge all scientists consider alternative grant sources like EU, ERC, Wallenberg, Söderberg and others in addition to bombarding VR with applications

There was also good news. Mia Phillipson's 5-year Wallenberg grant starts running in 2014, and at the end of 2013 Michael Hultström was awarded Swedish Society for Medical Research's major grant. Among the younger collaborators Gustaf Christoffersson was awarded both the Rolf Luft scholarship and an Anniversary scholarship from the Swedish Diabetes Fund. Although the economy differs between research groups it is generally good and, as evident below, permits recruitment of new staff.

After a promotion reform in the late 1990ies many senior lecturers became promoted to professors and after that very few old type professorships (previously denoted chairs) have been advertised for general application. At MCB it was almost 20 years since chairs were last announced but in 2013 we advertised two professorships in Secretion Research and Physiology. Anders Tengholm was appointed to the position in Secretion Research from March 2014 and Mia Phillipson and Fredrik Palm are the most highly ranked applicants for the Physiology position.

Three senior lectureships were also established at MCB in 2013. Mia Phillipson was awarded one in Physiology motivated by her highly ranked research and remarkable success in obtaining major research grants. Mats Hjortberg won the competition for a senior lectureship in Anatomy and Ingela Parmryd and Johan Kreuger are the top ranked applicants for a senior lectureship in Medical Cell Biology. Two Lectureships in Medical Cell Biology were assigned to Farnak Azarbayjani and Per Holmfeldt. The Disciplinary Domain of Medicine and Pharmacy announced six Assistant Professorships for open competition, which attracted

138 applicants. Olof Idevall-Hagren, who returned to MCB in 2013 after a two-year postdoctoral period at Yale University, was one of the lucky six, who will now be employed for 4 years. Despite the many new positions I can foresee that additional recruitments are required in the next few years due to retirements. During 2013 there was only one retirement our Laboratory Engineer Heléne Dansk, who has continued to work part-time. In 2014 Håkan Borg retires in April and I in June. Nils Welsh will succeed me as Chairman and, as mentioned above, Anders Tengholm as Professor of Secretion Research.

The decrease of MCB's share of government subsidies for research and research education depends to a considerable extent on a dramatic reduction of the PhD students reaching a minimum of 16 at the end of 2010. However new PhD students have been recruited and the number during 2013 was 34, four of whom were new recruitments (Jing Cen, Hanna Liljebäck, Qian Yu and Ye Wang). Also the number of examinations has increased from 0 dissertations and 5 licentiate theses in 2011 to 6 dissertations and 2 licentiate theses in 2013. Since the government subsidies are based on 4-year periods, there is good hope that MCB will increase its share of government support in coming years. The number of post-doctoral fellows active within MCB has increased and was as high as 20 during 2013.

MCB is well represented in important University boards. Stellan Sandler is Dean of the Medical Faculty and my Deputy Chairman Peter Hansell is a member of the board of the Disciplinary Domain of Medicine and Pharmacy. My Vice-Chairwoman Gunilla Westermark is also Deputy Director of the Biomedical Centre. Indeed, MCBs general influence has never previously been as high. I would like to thank all collaborators for contributing to a good MCB climate and working for success in science as well as in teaching. From an administrative perspective I would particularly like to mention the deputy chairman Peter Hansell, who is also assistant chairman dealing with basic teaching, and Gunilla Westermark, who is assistant chairwoman with responsibility for PhD studies and work environment. Our Dean Stellan Sandler is important in keeping us informed and he facilitates the communication between the Department and the Faculty/Disciplinary Domain. I am fortunate to have such wise constellation of persons around to discuss all difficult matter. Then of course little would happen without an engaged administrative staff and I am most grateful for the dedicated work of Shumin Pan, Camilla Sävmarker, Lina Thorvaldson, Björn Åkerblom, Erik Sandin, Oleg Dyachok and Göran Ståhl. Finally I would like to congratulate all those mentioned above who got new positions and grants, welcome new collaborators and finish by wishing MCB and my successor as chairman Nils Welsh all the best for the future.

Uppsala 2014-04-25

Erik Gylfe

Chairman

List of Contents

Introduction	2
List of Contents	4
Organization	5
Scientific Reports	7
Islet vascular physiology and cell therapy	7
Islet function in childhood obesity and type 2 diabetes mellitus	12
Physiology of pancreatic islet hormone secretion	17
Mechanisms of regulated exocytosis	22
The functional organisation of the plasma membrane	26
Importance of Shb-dependent signaling for glucose homeostasis, angiogenesis, hematopoiesis and reproduction	28
Complications in pregnancy	31
Pathogenesis of type 1 Diabetes Mellitus	33
Role of tyrosine kinases in β -cell apoptosis and diabetes	38
Intrarenal Hyaluronan in the Regulation of Fluid Balance. Pathophysiological Relevance to Renal Damage during Diabetes and Ischemia-Reperfusion.	41
Renal Physiology	44
Gastro-intestinal protection mechanisms studied in vivo	46
Leukocyte recruitment during inflammation and angiogenesis	49
Diabetic Nephropathy and Uremic Toxins	52
Studies of the pathophysiological mechanisms behind protein aggregation and formation of organ and cell toxic amyloid	59
Dissertations 2013	64
Licentiate theses 2013	64
Economy	65
Undergraduate Teaching	66
Graduate Teaching	67
MD/PhD programme	67
Centres and Facilities	67
BMC Electron Microscopy Unit	67
Advanced light microscopic imaging facilities	68
Other equipment	69
Prizes and awards 2013	70
E-mail address list	70

Organization

Chairman

Erik Gylfe

Deputy chairman

Peter Hansell

Vice chairmen

Peter Hansell (Director of undergraduate studies)

Gunilla Westermark (Director of graduate studies)

Department board

(At the end of 2013)

Peter Hansell, teacher representative

Mia Phillipson, teacher representative

Stellan Sandler, teacher representative

Anders Tengholm, teacher representative

Per-Ola Carlsson, teacher representative, deputy

Lena Holm, teacher representative, deputy

Leif Jansson, teacher representative, deputy

Gunilla Westermark, teacher representative, deputy

Nils Welsh, teacher representative, adjunct (chairman starting 2014-06-01)

Lisbeth Sagulin, representative for technical/administrative personnel

Björn Åkerblom, representative for technical/administrative personnel, deputy

Daniel Espes, PhD student representative

Ebrahim Anvari, PhD student representative deputy

Linn Ingvall, student representative

Shumin Pan, economy administrator, adjunct

Camilla Sävmarker, personell administrator, adjunct

Professors emeriti

Ove Nilsson

Bo Hellman

Erik Persson

Örjan Källskog

Jan Westman

Mats Wolgast

Arne Andersson

Administration

Shumin Pan
Erik Sandin
Göran Ståhl
Camilla Sävmarker
Lina Thorvaldson
Björn Åkerblom

Computers/IT

Oleg Dyachok
Peter Öhrt (BMC computer department)

Technical staff

Parvin Ahooghalandari
Helené Dansk
Angelica Fasching
Antoine Giraud
Annika Jägare
Marianne Ljungkvist
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 also to 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. 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.

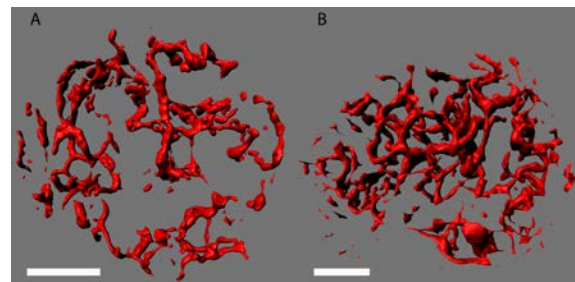


Fig 1. Two-photon confocal images of vascularity in pancreatic islets with low (A) or high (B) blood perfusion (blood perfusion identified by microsphere measurements).

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. Experimental studies are conducted to elucidate the importance of islet endothelial cells and neural cells for beta-cell regeneration and function. Other studies investigate the adaptation of pancreatic islets to the implantation organ, i.e. the so called engraftment process, following transplantation, and develop strategies 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

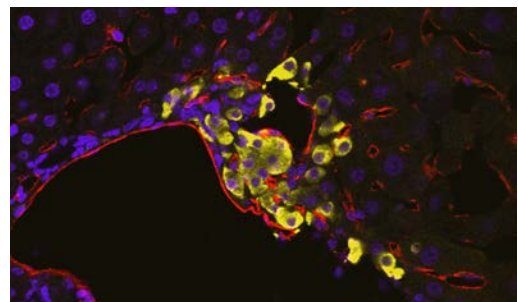


Fig 2. Micrograph showing vascularization of intraportally transplanted islet with disrupted integrity in the wall of a portal vein tributary. Yellow depicts insulin; red CD31 staining for blood vessels and blue DAPI.

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 we are also involved in studies to improve the results of clinical islet transplantation.

Pancreatic islet blood flow and endocrine function (Leif Jansson)

Disturbances in carbohydrate and lipid metabolism during impaired glucose tolerance and type 2 diabetes are associated with an endothelial dysfunction favouring vascular disease. The role of the regulation of the blood circulation for the normal function of the islets of Langerhans, especially under pathological conditions, is still incompletely understood.

We have previously demonstrated aberrations in islet blood perfusion during impaired glucose tolerance or type 2 diabetes. These blood flow changes may affect islet function by impairing endothelial function. Furthermore, most of the treatment regimes for type 2 diabetes decrease the increased islet blood flow suggesting a role for the blood perfusion in the pathogenesis of the disease.

By a combination of studies *in vivo*, on isolated single islets with attached arterioles and *in vivo* studies we intend to study disturbances in blood flow regulation of islet and white adipose tissue and how to amend these. A careful analysis of the factors responsible for the regulation of islet and adipose tissue blood perfusion in type 2 diabetes will provide knowledge on the role of these factors in the pathogenesis of islet functional deterioration, and hopefully open up new possibilities for treatment of this serious and disabling disease.

Members of the group

Per-Ola Carlsson, MD, professor

Leif Jansson, MD, professor

Arne Andersson, MD, professor em.

Joey Lau, post-doc

Monica Sandberg, post-doc

Sara Bohman, post-doc

Guangxiang Zang, post-doc

Ulrika Pettersson, Post-doc

Svitlana Vasylovska, post-doc

Carl Johan Drott, MD, PhD student

Daniel Espes, MD, PhD student

Liza Grapensparr, PhD student

Sara Ullsten, PhD student

Xiang Gao, PhD student

Ulrika Pettersson, PhD student

Hanna Liljebäck, MD/PhD student

Astrid Nordin, laboratory engineer

Ing-Britt Hallgren, laboratory engineer
Zhanchun Li, laboratory engineer
My Quach, laboratory engineer
Lisbeth Sagulin, laboratory engineer
Birgitta Bodin, laboratory technician
Eva Törnelius, laboratory technician
Violeta Armijo Del Valle, research nurs

Publications 2011-

1. Källskog Ö. and Jansson L.: Pancreatic islet grafts under the renal capsule autoregulate their blood flow in concert with the implantation organ. *Journal of Surgical Research* 171:865–870, 2011.
2. Sandberg M., Carlsson F., Nilsson B., Korsgren O., Carlsson P-O. and Jansson L.: Syngeneic islet transplantation into the submandibular gland of mice. *Transplantation*, 91:e17-19, 2011.
3. Purins K., Sedigh A., Molnar C., Jansson L., Korsgren O., Lorant T., Tufveson G., Wennberg L., Wiklund L., Lewén A. and Enblad P.: Standardized experimental brain death model for studies of intracranial dynamics, organ preservation and organ transplantation in the pig. *Critical Care Medicine* 39:512-517, 2011.
4. Jansson L., Carlsson, P.-O., Bodin B. and Källskog Ö.: Splanchnic flow distribution during infusion of UW-solution in anesthetized rats. *Langenbeck's Archives for Surgery* 396:677–683. 2011.
5. Westermark G, Andersson A, Westermark P. Islet amyloid polypeptide, islet amyloid and diabetes mellitus. *Physiol. Rev* 91:795-826, 2011
6. Lau J, Zang G and Carlsson P-O. Pancreatic islet transplantation to the liver: how may vascularization problems be resolved? *Diabetes Management* 1:219-227, 2011
7. Pettersson US, Henriksnäs J and Carlsson P-O. Endothelin-1 markedly decreases the blood perfusion of transplanted pancreatic islets in rats. *Transpl Proc* 43:1815-1820, 2011
8. Svensson J, Lau J, Sandberg M and Carlsson P-O. High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle. *Cell Transplant* 20:783-788, 2011
9. Christoffersson G, Carlsson P-O, and Phillipson M. Intramuscular islet transplantation promotes restored islet vascularity. *Islets* 3:69-71, 2011
10. Grapensparr L, Olerud J, Vasylovska S and Carlsson P-O. The therapeutic role of endothelial progenitor cells in type 1 diabetes mellitus. *Regen Med* 5:599-605, 2011
11. Carlsson P-O. Vilande Langerhanska öar-en funktionell reserve I bukspottkörteln. *BestPractice diabetes* 1:7-9, 2011
12. Espes D, Eriksson O, Lau J and Carlsson P-O. Striated muscle as implantation site for transplanted pancreatic islets. *J Transpl* 2011:352043, 2011
13. Olerud J, Johansson M, Christoffersson G, Lawler J, Welsh N and Carlsson P-O. Thrombospondin-1: An islet endothelial cell signal of importance for beta-cell function. *Diabetes* 60:1946-1954, 2011
14. Olsson R and Carlsson P-O. A low oxygenated subpopulation of pancreatic islets constitutes a functional reserve of endocrine cells. *Diabetes* 60:2068-2075, 2011
15. Olsson R, Olerud J, Pettersson U and Carlsson P-O. Increased numbers of low oxygenated pancreatic islets after intraportal transplantation. *Diabetes* 60:2350-2353, 2011

16. Wu L., Ölverling A, Huang Z., Jansson L., Chao H, Gao X and Sjöholm Å.: GLP-1, exendin-4 and C-peptide regulate pancreatic islet microcirculation, insulin secretion and glucose tolerance in rats. *Clinical Science* 122:375-384, 2012.
17. Westermark GT, Davalli AM, Secchi A, Folli F, Kin T, Toso C, Shapiro AM, Korsgren O, Tufveson G, Andersson A, Westermark P. Further evidence for amyloid deposition in clinical pancreatic islet grafts. *Transplantation* 93:219-223, 2012
18. Henriksnäs J, Lau J, Zang G, Berggren P-O, Köhler M and Carlsson P-O. Markedly decreased blood perfusion of pancreatic islets transplanted intraportally into the liver: disruption of islet integrity necessary for islet revascularization. *Diabetes* 61:665-673, 2012
19. Barbu A, Johansson Å, Bodin B, Källskog Ö, Carlsson P-O, Sandberg M, Lau J and Jansson L. Blood perfusion of endogenous and transplanted pancreatic islets in anesthetized rats after administration of lactate and pyruvate. *Pancreas* 41:1263-1271, 2012
20. Pettersson U., Christoffersson C., Massena S., Jansson L., Henriksnäs J. and Phillipson M.: Increased recruitment but impaired function of leukocytes during inflammation in mouse models of type 1 and type 2 diabetes. *PLoS One* 6:e22480, 2012
21. Barbu A.; Johansson Å., Bodin B., Källskog Ö., Carlsson P-O., Sandberg M., Lau J. and Jansson L.: Blood perfusion of endogenous and transplanted pancreatic islets in anaesthetized rats after administration of lactate and pyruvate. *Pancreas* 41:1263-1271, 2012
22. Grouwels G., Vasylovska S., Olerud J., Leuckx G., Ngamjarriyawat A., Jansson L., Van De Castele M., Kozlova E.N. and Heimberg H.: Differentiating neural crest stem cells induce proliferation of cultured rodent islet beta cells. *Diabetologia* 55:2016-2025, 2012
23. Lau J, Svensson J, Grapensparr L, Johansson Å and Carlsson P-O. Superior beta-cell proliferation, function and gene expression in a subpopulation of islets identified by high blood perfusion. *Diabetologia* 55:1390-99, 2012
24. Pettersson US, Waldén TB, Carlsson PO, Jansson L, Phillipson M. Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. *PLoS One* 7:e46057, 2012
25. Drott CJ, Olerud J, Emanuelsson H, Christoffersson G, Carlsson PO. Sustained beta-cell dysfunction but normalized islet mass in aged thrombospondin-1 deficient mice. 7:e47451, 2012
26. Pettersson U.S., Sandberg M. and Jansson L.: Two-week treatment with the β_3 -adrenoceptor antagonist SR59230A normalizes the increased pancreatic islet blood flow in type 2 diabetic GK rat. *Diabetes, Obesity and Metabolism* 14:960-962, 2012.
27. Sun Z, Li X., Massena S., Kutschera S., Padhan N., Gualandi L., Sundvold-Gjerstad V., Gustafsson K., Choy W.W., Zang G., Quach M., Jansson L., Phillipson M., Abid Md.R., Spurkland A., Xiujuan X., and Claesson-Welsh L.: VEGFR2 induces c-Src signaling and vascular permeability *in vivo* via the adaptor protein TSA. *Journal of Experimental Medicine* 209:1363-1377, 2012
28. Sandberg M., Pettersson U., Henriksnäs J. and L. Jansson L.: The α_2 -adrenoceptor antagonist yohimbine normalizes the increased islet blood flow in GK rats, a model of type 2 diabetes. *Hormone and Metabolic Research* 45:252-254, 2013
29. Högberg N, Carlsson P, Hillered L, Meurling S, Stenbäck A. Intestinal ischemia measured by intraluminal microdialysis. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2012;72(1):59-66.

30. Högberg N, Carlsson P, Hillered L, Stenbäck A, Engstrand Lilja H. Intraluminal intestinal microdialysis detects markers of hypoxia and cell damage in experimental necrotizing enterocolitis. *Journal of Pediatric Surgery*. 2012;47(9):1646-1651.
31. Espes D, Engström J, Reinius H, Carlsson P. Severe diabetic ketoacidosis in combination with starvation and anorexia nervosa at onset of type 1 diabetes : A case report. *Upsala Journal of Medical Sciences*. 2013;118(2):130-133.
32. Högberg N, Stenbäck A, Carlsson P, Wanders A, Engstrand Lilja H. Genes regulating tight junctions and cell adhesion are altered in early experimental necrotizing enterocolitis. *Journal of Pediatric Surgery*. 2013;48(11):2308-2312.
33. Vågesjö E, Christoffersson G, Waldén TB, Carlsson PO, Essand M, Korsgren O, Phillipson M. Immunological shielding by induced recruitment of regulatory T lymphocytes delays rejection of islets transplanted to muscle, *Cell Transplant* 2013, in press
34. Espes D, Lau J, Carlsson PO. Increased circulating levels of betatrophin in individuals with long-standing type 1 diabetes. *Diabetologia* 2013, in press
35. Chu X., Gao X., Jansson L., Skogseid B. And Barbu A.: Multiple microvascular alterations in pancreatic islets and neuroendocrine tumors of a Men 1 mouse modelneuroendocrine tumors of the Men1 mouse model. *American Journal of Pathology* 182:2355-2367, 2013.
36. Jansson L., Kampf C. and Källskog Ö.: Reinnervation of pancreatic islet grafts in rats: functional stimulation of nerves have minor effects on transplant endocrine function. *Upsala Journal of medical Sciences* 118:209-216, 2013.
37. Sandberg M., Quach M., Bodin B., Johansson L. and Jansson L.: Effects of Mn-DPDP and manganese chloride on hemodynamics and glucose tolerance in anesthetized rats. *Acta Radiologica*, in press.
38. Gao X., Jansson L., Persson A.E.G. and Sandberg M.: Short-term glucosamine infusion increases islet blood flow in anesthetized rats. *Islets* 5:1-6, 2013.

Agencies that support the work

Juvenile Diabetes Research Foundation
 European Foundation for the Study of Diabetes
 The Swedish Research Council
 The Swedish Diabetes Association
 The Diabetes Wellness Foundation
 AFA
 The Swedish Juvenile Diabetes Fund
 Novo Nordisk Foundation
 The Knut and Alice Wallenberg Foundation
 Olle Engkvist Byggmästare Foundation
 The Gunvor & Josef Ane's Foundation
 The Thuring Foundation

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 secretion 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

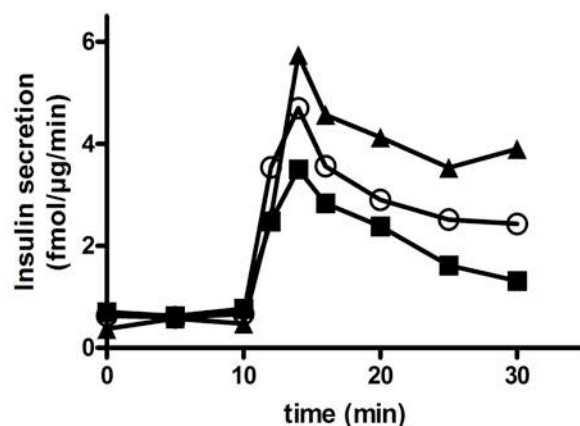


Figure 1: Glucose-stimulated insulin secretion from isolated human islets exposed to 0.5 mM palmitate for 0 (open circles), 2 (closed triangles), or 7 (closed squares) days.

of beta-cell mass occur, enhanced insulin secretion is observed.

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 elevated 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.

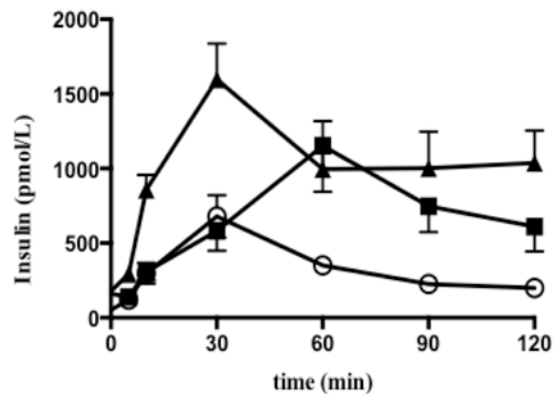


Figure 2: Oral glucose tolerance test in obese pre-pubertal (closed triangles), pubertal (closed squares) children with high palmitate and lean controls (open circles).

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 a collaborative, 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.

Insulin processing

Accentuated insulin secretion, as observed in isolated islets after 2 days exposure to elevated palmitate concentrations and in obese children with high circulating palmitate concentrations, puts high demands on the insulin biosynthetic machinery. We have investigated how the amount of fully processed insulin and non-processed proinsulin is affected in obesity. Measurements of insulin and proinsulin were conducted both in isolated islets exposed to palmitate and in obese and lean children. In islets expression of enzymes responsible for cleavage of proinsulin to insulin were also measured.

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 sphingolipid 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.

Islet architecture

The islet of Langerhans is a complex organ containing different cell types, The interaction between these cell types is essential for proper function. We have investigated the role of coupling between beta-cells for glucose-stimulated insulin secretion (Chowdhury et al 2013a) and also how signalling is altered if such coupling is disrupted (Chowdhury et al 2013b).

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.

Members of the group

Peter Bergsten, professor

Anders Forslund, MD, PhD

Ernest Sargsyan, researcher

Levon Manukyan, postdoctoral person

Anders Alderborn, PhD

Azazul Chowdhury, graduate student

Johan Staaf, graduate student (MD/PhD-programme)

Hjalti Kristinsson, graduate student

Hannes Ohlsson, graduate student (MD/PhD-programme)

Jing Cen, graduate student

Henrik Ström, undergraduate student

Iris Ciba, MD

Marie Dahlbom, research nurse

Malte Lidström, research nurse

Helena Vilén, research dietician

Malin Meirik, research psychologist

Emmelie Brandt, research physiotherapist

Grants

European Commission, FP7, Beta-JUDO

Swedish Medical Research Council

Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning

Swedish Governmental Agency for Innovation Systems

Swedish Diabetes Association

Regional Research Council

Gillberg's Foundation

Family Ernfors' Foundation

Selander's Foundation

Collaborations

Uppsala University:

- Fredrik Ahlsson (Womens's and Children's Health)
- Håkan Ahlström (Radiology)
- Jonas Bergquist (Analytical Chemistry)
- Barbro Diderholm (Womens's and Children's Health)
- Jan Gustafsson (Womens's and Children's Health)
- Mats Gustafsson (Medical Sciences)

Other universities:

- Ali Moazzami (Swedish University of Agricultural Sciences)
- Antje Körner (University of Leipzig, Germany)
- Reinhard Schneider (EMBL, Germany)
- Daniel Weghuber (Paracelsus Medical University, Salzburg, Austria)
- Kurt Widhalm, (University of Vienna, Austria)
- Jean-Charles Sanchez (University of Geneva, Switzerland)
- Sadaf Farooqi, (University of Cambridge, Great Britain)
- Dave Smith (AstraZeneca, Great Britain)
- Ulrika Hammarström (Scandnavian CRO, Uppsala)

Publications 2011-

1. Manukyan L, Ubhayasekera SJ, Thörn K, Bergquist J and Bergsten P. Palmitate increases levels of ceramide in MIN6 cells by shifts in multiple ceramide turnover pathways. *Lipids* (in revision)
2. Forslund A, Staaf J, Kullberg J, Ciba I, Dahlbom M and Bergsten P. Uppsala Longitudinal Study of Childhood Obesity – a pediatric cohort addressing childhood obesity. *Pediatrics*, Jan 13, 2014.
3. Kristinsson H, Smith DM, Bergsten P and Sargsyan E. FFAR1 is involved in both the acute and chronic effects of palmitate on insulin secretion. *Endocrinology*, 154:4078-4088, 2013
4. Chowdhury A, Satagopam VP, Manukyan L, Artemenko KA, Fung YM, Schneider R, Bergquist J and Bergsten P. Signaling in insulin-secreting MIN6 pseudoislets and monolayer cells. *J Proteome Res*, 12:5954-62, 2013
5. Chowdhury AI, Dyachok O, Tengholm A, Sandler S and Bergsten P. Functional differences between aggregated and dispersed insulin-producing cells. *Diabetologia* 56:1557-1568, 2013
6. Ubhayasekera SJ, Staaf J, Forslund A, Bergsten P, Bergquist J. Free fatty acid determination in plasma by GC-MS after conversion to Weinreb amides. *Anal Bioanal Chem* 405: 1929-35, 2013.
7. Topf F, Schwartz D, Gaudet P, Priego-Capote F, Zufferey A, Turck N, Binz PA, Fontana P, Wiederkehr A, Finamore F, Xenarios I, Goodlett D, Kussmann M, Bergsten P and Sanchez JC. The Human Diabetes Proteome Project (HDPP): From network biology to targets for therapies and prevention. *Translational Proteomics* 1:3-11, 2013
8. Ahlsson F, Diderholm B, Ewald U, Jonsson B, Forslund A, Stridsberg M, Gustafsson J: Adipokines and their relation to maternal energy substrate production, insulin resistance and fetal size. *Eur J Obstet Gynecol Reprod Biol* 168:26-9, 2013
9. Staaf J, Åkerström T, Ljungström V, Larsson S, Karlsson T, Skogseid B and Bergsten P. Early bridge between preclinical and clinical research. *Lakartidningen* 109: 898, 2012.
10. Sargsyan E, Sol EM and Bergsten P. UPR in palmitate-treated pancreatic beta-cells is not affected by altering oxidation of the fatty acid. *Nutr Metab*, 8:70, 2011.
11. Sargsyan E and Bergsten P. Lipotoxicity is glucose-dependent in INS-1E cells but not in human islets and MIN6 cells. *Lipids Health Dis*, 10:115, 2011.

Physiology of pancreatic islet hormone secretion

Erik Gylfe, Anders Tengholm

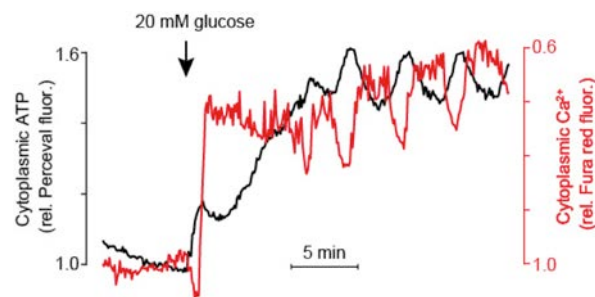
The research in our group aims at clarifying the mechanisms regulating the release of insulin, glucagon and other hormones from the islets of Langerhans. Insufficient secretion of blood-glucose-lowering insulin and dysregulated secretion of blood-glucose-elevating glucagon secretion are hallmarks of 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 signaling biosensors and live cell imaging methods, we study the spatio-temporal dynamics of signaling processes regulating secretion in single cells and intact mouse and human pancreatic islets. At present we are focusing specifically on the following issues:

Intracellular dynamics of ATP, Ca²⁺ and cAMP and the generation of pulsatile insulin secretion from pancreatic β -cells

Insulin is released from β -cells in response to glucose, other nutrients, hormones and neural factors. The hormone is normally released in pulses with the kinetics determined by a complex interplay between second messengers and signaling proteins beneath the β -cell plasma membrane. Glucose is the main stimulator of insulin secretion. Uptake and metabolism of the sugar in β -cells result in elevation of the ATP/ADP ratio, closure of ATP-sensitive K⁺ (K_{ATP}) channels in the plasma membrane, depolarization and voltage-dependent Ca²⁺ influx, which triggers exocytosis of insulin secretory granules. The exocytosis response is amplified by the messenger cAMP, which is generated in β -cells after activation of glucagon and incretin hormone receptors as well as after glucose stimulation.

Our lab has discovered that glucose triggers coordinated oscillations of Ca²⁺ and cAMP in β -cells, and that this response is important for pulsatile insulin secretion. However, the mechanisms underlying the generation of these oscillations are not clear. ATP plays a central role, linking metabolism to electrical activity by blocking the K_{ATP} channels, and variations in metabolism may underlie the Ca²⁺ oscillations in glucose-stimulated cells. There are also feedback effects of Ca²⁺ on cell metabolism and we are currently employing various imaging tools to investigate the relationship between ATP and Ca²⁺ in β -cells.

We use various cell signaling biosensors to clarify the mechanisms underlying the generation of cAMP oscillations and how the cAMP targets protein kinase A and Epac are involved in the regulation of insulin secretion. For example, we have found that protein kinase A, in addition to potentiating exocytosis in response to cAMP-elevating hormones, is



Relationship between the intracellular concentrations of ATP (black trace) and Ca²⁺ (red trace) beneath the plasma membrane of a β -cell within a mouse islet. When the glucose concentration is increased from 3 to 20 mM (arrow) there is an immediate rise of ATP followed by increase of Ca²⁺ that triggers insulin secretion. After 5-10 minutes there are pronounced antiphase oscillations of ATP and Ca²⁺, which reflect interactions between the two messengers important for generating pulsatile insulin secretion.

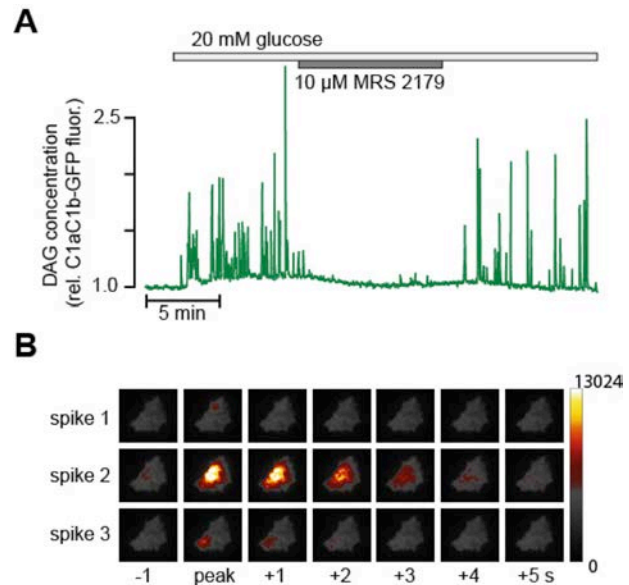
important for proper initiation of insulin secretion by glucose. Moreover, recent work from the lab has demonstrated that cAMP and Ca^{2+} signals trigger translocation of Epac, a guanine nucleotide exchange factor for Rap GTPases, to the β -cell plasma membrane. The downstream effects as well as functional importance of these signaling steps are currently under investigation.

Autocrine signaling in β -cells

Exocytosis of insulin granules not only results in the release of insulin, but also of several other granule constituents, which by autocrine actions may affect β -cell function. Activation of insulin receptors leads to PI3-kinase-mediated formation of the phospholipid $\text{PtdIns}(3,4,5)P_3$. Using fluorescent $\text{PtdIns}(3,4,5)P_3$ reporters we have demonstrated that glucose stimulation of β -cells results in pronounced oscillations of $\text{PtdIns}(3,4,5)P_3$ in the plasma membrane that reflect pulsatile insulin secretion and 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. These DAG spikes results in rapid recruitment and activation of various protein kinase C isoforms. Using various optical single-cell assays we are currently investigating how insulin, Zn^{2+} and ATP affect signaling and secretion in β -cells.

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. We were first to study Ca^{2+} signaling in all islet cell types and found that pulsatile release of the different hormones can



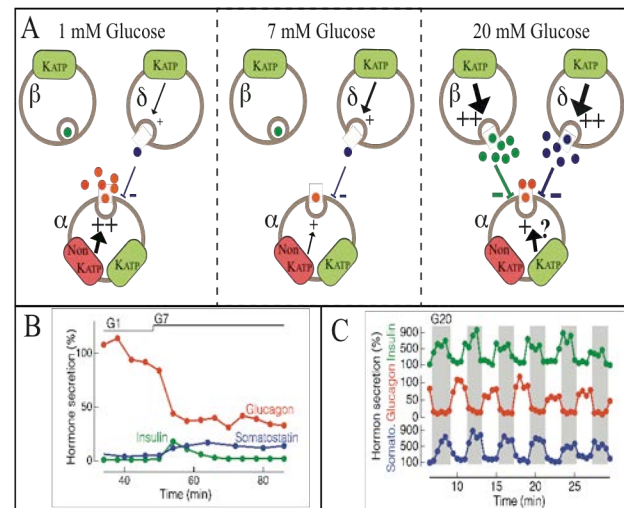
(A) Glucose stimulation of a mouse β -cell triggers pronounced DAG spiking in the plasma membrane that is monitored with a fluorescent DAG reporter. The response is reversibly inhibited when the autocrine action of ATP is blocked with the purinergic receptor antagonist MRS2179.

(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.

be explained by Ca^{2+} oscillations. More recently, we demonstrated that pulsatile release of insulin and somatostatin from mouse and human islets occur in phase, whereas pulses of glucagon occur in opposite phase. This has important implications for the understanding of the action of insulin and glucagon on glucose production in the liver. Interestingly, although glucose lowers the average levels of glucagon, the hormone release pattern is composed of alternating periods of stimulation and inhibition. At very high glucose concentrations, glucagon secretion is paradoxically stimulated. Current work is focused on understanding the mechanisms underlying the different hormone release patterns. Compared to insulin release from beta cells, little is known about the mechanisms underlying the release of the other islet hormones. We have proposed a new model for regulation of glucagon secretion. In this model a Ca^{2+} store-operated mechanisms plays a central role. The store-operated pathway contributes to alpha-cell depolarization and secretion when the Ca^{2+} stores are emptied by IP_3 -generating receptor stimuli or when there is lack of energy in the presence of low glucose concentrations. In contrast, store filling mediated by high glucose concentrations shuts off the store-operated pathway and the membrane hyperpolarizes and electrical activity and secretion ceases. We are currently investigating the molecular details of the store-operated mechanism in alpha-cells and the importance of Ca^{2+} , cAMP and ATP in the generation of pulsatile glucagon secretion.

Clinical significance

Diabetes is a widespread disease with rapidly increasing prevalence currently affecting >5 % of the world population. It is primarily due to insufficient or absent secretion of the blood glucose-lowering hormone insulin resulting in elevated blood glucose and glucose in the urine. Even if the acute symptoms of diabetes can be reversed by different therapies there are long-term complications like cardiovascular disease, stroke, kidney disease, eye complications with blindness, skin problems, nerve damage causing foot complications, gastrointestinal and sexual dysfunction.



Model for glucose regulation of glucagon release. 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. 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).

Type 2 diabetes, which preferentially affects adult individuals, is the most common form and accounts for more than 90% of all diabetes. Type 2 diabetes is primarily characterized by insufficient insulin secretion from the pancreatic beta cells. Current therapy aims at maintaining or improving the secretory capacity of the beta cells and increasing the insulin sensitivity of the target organs. Improved knowledge about the mechanisms underlying insulin secretion is a prerequisite for understanding the impaired function in type 2 diabetes and for finding new strategies for restoring insulin secretion.

Type 1 diabetes mainly affects young individuals. It is a more severe disease than type 2 diabetes, since the beta cells are destroyed by an autoimmune attack. Apart from the lack of insulin, increased secretion of the blood glucose-elevating hormone glucagon contributes to rise of blood glucose in diabetes. Another dysfunction is that glucagon secretion is not appropriately stimulated when blood glucose falls to very low levels, as sometimes happens in insulin-treated diabetic patients. Clarification of the mechanisms underlying the failure of low glucose to stimulate glucagon release and the paradoxical hypersecretion of glucagon at high blood glucose may reduce acute illness and death after over-injection of insulin and help to prevent high blood glucose.

Members of the group

Parvin Ahooghalandari – Research engineer

Helene Dansk -Research engineer

Oleg Dyachok – Senior research engineer

Eva Grapengiesser - Associate professor

Erik Gylfe - Professor

Bo Hellman - Professor

Olof Idevall-Hagren - Postdoc

Ida Jakobsson – Graduate student

Lisen Kullman - Assistant professor

Jia Li – Graduate student

Hongyan Shuai – Graduate student

Anders Tengholm - Professor

Geng Tian – Graduate student

Anne Wuttke – Graduate student

Yunjian Xu - Senior research engineer

Qian Yu – Graduate student

Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

Novo Nordisk Foundation

Swedish Institute

Family Ernfors Foundation

Publications 2011-

1. Hellman B, Dansk H, Grapengiesser E. 2014. Activation of α adrenergic and muscarinic receptors modifies early glucose suppression of cytoplasmic Ca^{2+} in pancreatic β -cells. *Biochem Biophys Res Commun* 445:629-32.
2. Hellman B, Grapengiesser E. 2014. Glucose-induced inhibition of insulin secretion. *Acta Physiol* 210:479-88.
3. Gylfe E. 2013. Glucose control of glucagon secretion: there is more to it than K_{ATP} channels. *Diabetes* 62:1391-3.
4. Gylfe E. 2013. Comment on: Allister et al. UCP2 regulates the glucagon response to fasting and starvation. *Diabetes* 62:e11. Doi 10.2337/db13-0397.
5. Li J, Shuai HY, Gylfe E, Tengholm A. 2013 Oscillations of sub-membrane ATP in glucose-stimulated beta-cells depend on negative feedback from Ca^{2+} . *Diabetologia* 56:1577-86.
6. Idevall-Hagren O, Jakobsson I, Xu YJ, Tengholm A. 2013. Spatial control of Epac2 activity by cAMP and Ca^{2+} -mediated activation of Ras. *Science Signal* 6:ra29. doi: 10.1126/scisignal.2003932.
7. Høivik EA, Witsø SL, Bergheim IR, Xu YJ, Jakobsson I, Tengholm A, Døskeland SO, Bakke M. 2013. DNA methylation of alternative promoters directs tissue specific expression of Epac2 isoforms. *PLoS ONE* 8:e67925.
8. Chowdhury, Dyachok O, Tengholm A, Sandler S, Bergsten P. 2013. Functional differences between aggregated and dispersed insulin-producing cells. *Diabetologia* 56:1557-68.
9. Mokhtari D, Al-Amin A, Turpaev K, Li T, Idevall-Hagren O, Li J, Wuttke A, Fred RG, Ravassard P, Scharfmann R, Tengholm A, Welsh N. 2013 Imatinib mesilate-induced phosphatidylinositol 3-kinase signalling and improved survival in insulin-producing cells: role of Src homology 2-containing inositol 5'-phosphatase interaction with c-Abl. *Diabetologia* 56:1327-38.
10. Wuttke A, Idevall-Hagren O, Tengholm A. 2013 P2Y_1 receptor-dependent diacylglycerol signaling microdomains in β cells promote insulin secretion. *FASEB J* 27:1610-1620.
11. Zeller KS, Riaz A, Sarve H, Li J, Tengholm A, Johansson S. 2013. The role of mechanical force and ROS in integrin-dependent signals. *PLoS ONE*, 8:e64897.
12. Zang G, Christoffersson G, Tian G, Harun-Or-Rashid M, Vågesjö E, Phillipson M, Barg S, Tengholm A, Welsh M. 2013 Aberrant association between vascular endothelial growth factor receptor-2 and VE-cadherin in response to vascular endothelial growth factor-a in Shb-deficient lung endothelial cells. *Cell Signal* 25:85-92.
13. Tian G, Sågetorp J, Xu YJ, Shuai HY, Degerman E, Tengholm A. 2012. Role of phosphodiesterases in the shaping of sub-plasma-membrane cAMP oscillations and pulsatile insulin secretion. *J Cell Sci* 125:5084-95.
14. Hinke SA, Navedo MF, Ulman A, Whiting JL, Nygren PJ, Tian G, Jimenez-Caliani AJ, Langeberg LK, Cirulli V, Tengholm A, Dell'Acqua ML, Santana LF, Scott JD. 2012. Anchored phosphatases modulate glucose homeostasis. *EMBO J* 31:3991-4004.
15. Tian G, Tepikin AV, Tengholm A, Gylfe E. 2012. cAMP induces stromal interaction molecule 1 (STIM1) puncta but neither Orail protein clustering nor store-operated Ca^{2+} entry (SOCE) in islet cells. *J Biol Chem* 287:9862-9872.

16. Hellman B, Salehi A, Grapengiesser E, Gylfe E. 2012. Isolated mouse islets respond to glucose with an initial peak of glucagon release followed by pulses of insulin and somatostatin in antisynchrony with glucagon. *Biochem Biophys Res Commun* 417:1219-1223.
17. Gylfe E, Grapengiesser E, Dansk H, Hellman B. 2012. The neurotransmitter ATP triggers Ca^{2+} responses promoting coordination of pancreatic islet oscillations. *Pancreas* 41:258-263.
18. Dezaki K, Boldbaatar D, Sone H, Dyachok O, Tengholm A, Gylfe E, Kurashina T, Yoshida M, Kakei M, Yada T. 2011. Ghrelin Attenuates cAMP-PKA Signaling to Evoke Insulinostatic Cascade in Islet β -Cells. *Diabetes* 60:2315-2324.
19. Tian G, Sandler S, Gylfe E, Tengholm A, 2011. Glucose- and hormone-induced cAMP oscillations in α - and β -cells within intact islets of Langerhans. *Diabetes*, 60:1535-1543.

Reviews 2011-

20. Gylfe E, Gilon P. 2014. Glucose regulation of glucagon secretion. *Diabetes Res Clin Pract* 103:1-10.
21. Tengholm A. 2012. Cyclic AMP dynamics in the pancreatic β -cell. *Ups J Med Sci* 117:355-69.

Dissertations

Geng Tian: "On the generation of cAMP oscillations and regulation of the Ca^{2+} store-operated pathway in pancreatic α - and β -cells". March 2013

Anne Wuttke: "Lipid signalling in insulin-secreting β -cells". May 2013.

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 β -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

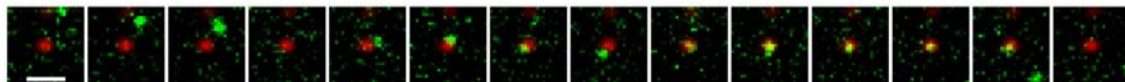
Every β -cell contains thousands of secretory granules that store insulin. When blood glucose is elevated, these granules undergo regulated exocytosis and release the hormone into the blood stream. Before this can happen, granules have to reach the plasma membrane, where

they “dock” and then assemble the exocytosis machinery. When insulin is released, these steps quickly become limiting for how much insulin is released.

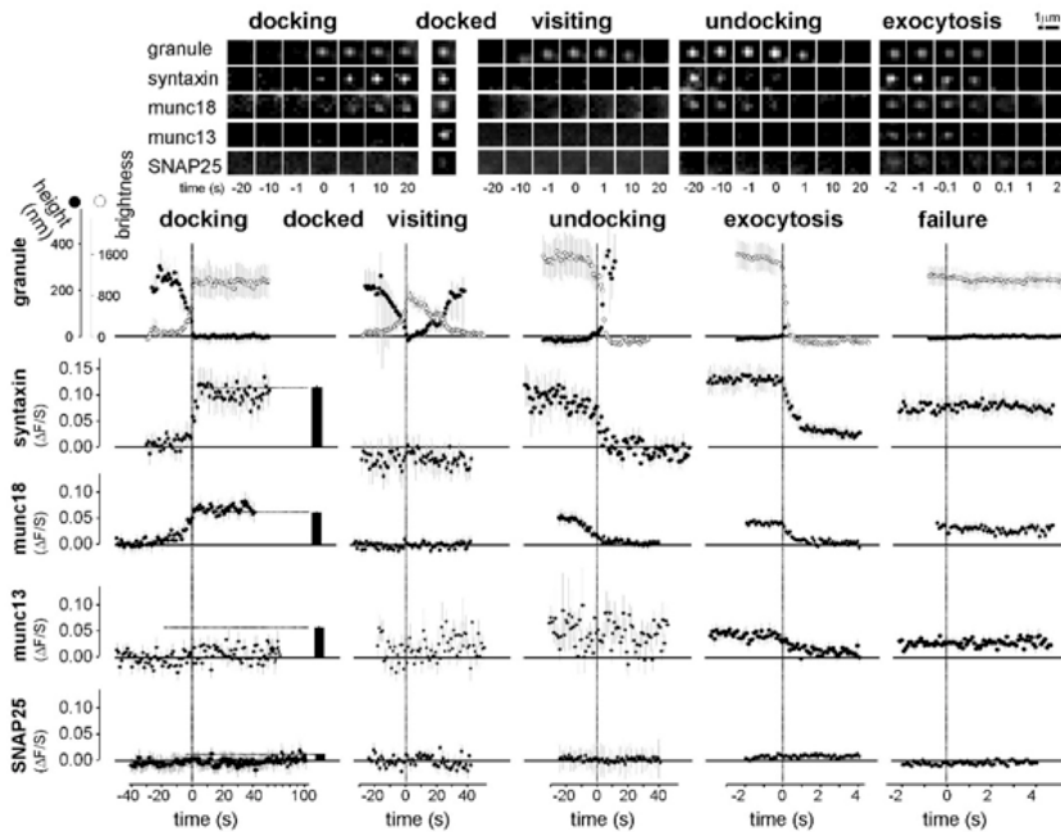
The docking process is not understood in molecular terms, but many of the proteins involved have been identified. One hypothesis that we are currently testing is that some of these proteins (including t-SNAREs) pre-assemble at small hotspots in the plasma membrane. These hotspots, perhaps related to 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, t-SNAREs can be seen to 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.

We have recently provided quantitative measurements of several exocytosis proteins (syntaxin, SNAP25, munc18, munc13, rab3) at 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.



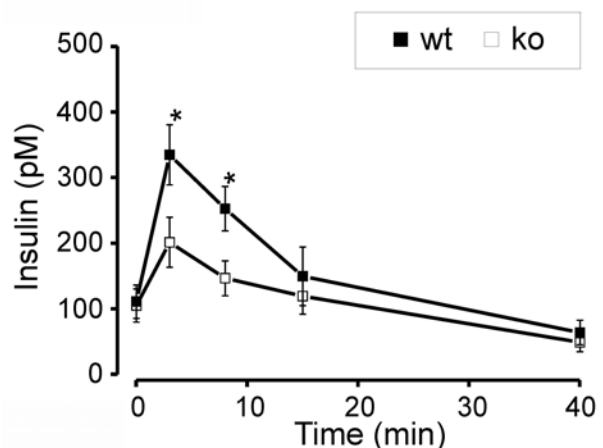
A single molecule of Syx-EGFP (green) binds to a secretory granule (NPY-cherry, red). Scalebar 1 μ m; 20ms per frame.



Quantification of protein affinity during the lifecycle of the docking/release site. (Gandasi and Barg, *Nat Comm* in press).

Secretion of Islet Hormones in Chromogranin-B Deficient Mice

Granins are major constituents of dense-core secretory granules in neuroendocrine cells, but their function is still a matter of debate. Work in cell lines has suggested that the most abundant and ubiquitously expressed granins, chromogranin A and B (CgA and CgB), are involved in granulogenesis and protein sorting. Here we report the generation and characterization of mice lacking chromogranin B (CgB-ko), which were viable and fertile. Unlike neuroendocrine tissues, pancreatic islets of these animals lacked compensatory changes in other granins and were therefore analyzed in detail. Stimulated secretion of insulin, glucagon and somatostatin was reduced in CgB-ko islets, in parallel with somewhat impaired glucose clearance and reduced insulin release, but normal insulin sensitivity *in vivo*. CgB-ko islets lacked specifically the rapid initial phase of stimulated secretion, had elevated basal insulin release, and stored and released twice as much proinsulin as wildtype (wt) islets. Stimulated release of glucagon and somatostatin was reduced as well. Surprisingly, biogenesis, morphology and function of insulin granules were normal, and no differences were found with regard to beta-cell stimulus-secretion coupling. We conclude that CgB is not required for



normal insulin granule biogenesis or maintenance in vivo, but is essential for adequate secretion of islet hormones. Consequentially CgB-ko animals display some, but not all, hallmarks of human type-2 diabetes. However, the molecular mechanisms underlying this defect remain to be determined.

Publications 2011-

1. NR Gandasi and S Barg (2013). Contact-induced clustering of syntaxin and munc18 docks secretory granules at the exocytosis site. *Nature Communications in press*
2. Krus U, King B, Nagaraj, Gandasi NR, Zhang E, Barg S, Blom AM, and Renström E (2013). The complement inhibitor CD59 plays a fundamental role in insulin secretion by controlling recycling of exocytotic fusion proteins. *Cell Metabolism in press*
3. G Zang, G Christoffersson, G Tian, M Harun-Or-Rashid, E Vågesjö, M Phillipson, S Barg, A Tengholm, and M Welsh (2013). Aberrant association between Vascular Endothelial Growth Factor Receptor-2 and VE-cadherin in response to Vascular Endothelial Growth Factor-A in Shb-deficient lung endothelial cells. *Cellular Signalling* 25:85-92
4. Y Jin, S Korol, Z Jin, S Barg and B Birnir (2013). In intact rat islets interstitial GABA activates GABAA channels that generate tonic currents in the α -cells. *PloS ONE* 8:e67228
5. Hoppa MB, Jones E, Karanauskaite J, Ramracheya R, Braun M, Collins SC, Zhang Q, Clark A, Eliasson L, Genoud C, Macdonald PE, Monteith AG, Barg S, Galvanovskis J, Rorsman P. (2011) Multivesicular exocytosis in rat pancreatic beta cells. *Diabetologia*. 55:1001-12
6. Iglesias J, Barg S, Vallois D, Lahiri S, Roger C, Yessoufou A, Pradevand S, McDonald A, Bonal C, Reimann F, Gribble F, Debril MB, Metzger D, Chambon P, Herrera P, Rutter GA, Prentki M, Thorens B, Wahli W. PPAR β/δ affects pancreatic β cell mass and insulin secretion in mice. *J Clin Invest*. 2012, 122:4105-17.
7. Zang G, Christoffersson G, Tian G, Harun-Or-Rashid M, Vågesjö E, Phillipson M, Barg S, Tengholm A, Welsh M. Aberrant association between vascular endothelial growth factor receptor-2 and VE-cadherin in response to vascular endothelial growth factor-a in Shb-deficient lung endothelial cells. *Cell Signal*. 2013, 25:85-92

Members of the group

Sebastian Barg - Docent

Nikhil Gandasi- Graduate student

Yin Peng, Graduate student

Emma Kay, postdoc

Jan Saras, research engineer

Meng Liang, project student

Rutger Schutten, Master thesis student

Swati Arora, Master thesis student

Omar Hmeadi, project student

Kim Vesto, Master thesis student

Agencies that support the work

Diabetes Research Wellness Foundation

Swedish Research Council (Vetenskapsrådet)

Barndiabetesfonden

Novo Nordisk Foundation

European Foundation for the Study of Diabetes/MSD

The Carl Tryggers Foundation

The Göran Gustafsson Foundation

Family Ernfors Foundation

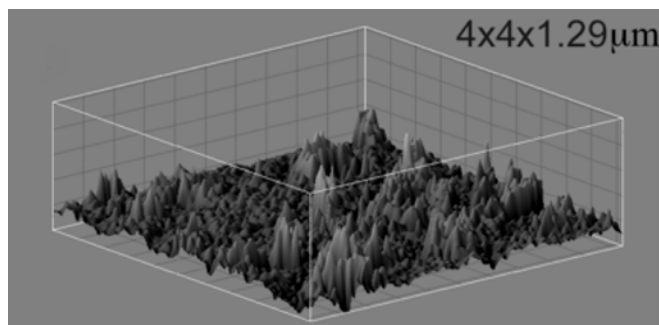
OE och Edla Johanssons stiftelse

PO Zetterlings stiftelse

The functional organisation of the plasma membrane

Ingela Parmryd

The plasma membrane of eukaryotic cells contains ordered nanodomains, commonly referred to as lipid rafts, which are more ordered than the rest of the plasma membrane. The high order has been suggested to be caused by the tight packing of cholesterol and sphingolipids as observed in model membranes. However, we have recently demonstrated that lipid rafts form when actin filaments are attached to the plasma membrane via phosphoinositides (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. The lipid raft aggregation can be achieved by T cell receptor ligation but also by cold stress and changes in plasma membrane cholesterol content. We are investigating what is triggering the formation of ordered plasma membrane domains and to do so we have carefully characterised two environmentally sensitive probes that can determine the proportion of ordered lipid domains in the membrane. Focus areas are the individual order of the two plasma membrane leaflets and the role of phosphatidylinositol (4,5)-bisphosphate and actin dynamics in plasma membrane order.



High resolution hopping ion conductance microscopy image of part of a live FRSK cell. The figure shows that cell topography is an important factor when determining the diffusion coefficients of membrane molecules.

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 develop image analysis software to get quantitative and objective answers to our questions. We have developed a method where image noise, which is unavoidable and leads to the underestimation of the underlying correlation, can be eliminated from the correlation measurement. We have performed a detailed studies on coefficients currently used in colocalisation analyses revealing that several are not fit for their purpose. We advocate that colocalisation analysis should be divided into the two subgroups co-occurrence and correlation (Adler & Parmryd, 2013).

$\gamma 9\delta 2$ 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 $\gamma 9\delta 2$ T cell proliferation (Lindberg et al., 2013) suggesting that phosphoantigens are produced in these cells. We will now address at which parasite stage this production occurs and what metabolic pathway is responsible for the production.

Members of the group

Ingela Parmryd, associate professor

Warunika Aluthgedara, project assistant

Parham Ashrafzadeh, graduate student

Chenxiao Liu, graduate student

Jan Saras, research engineer

Lijun Zhao, laboratory assistant

Publications 2011-

1. Dinic J, Biverstahl H, Mäler L, Parmryd I. (2011) Laurdan and di-4-ANEPPDHQ do not respond to membrane-inserted peptides and are good probes for lipid packing. *Biochim. Biophys. Acta.* 1808, 298-306
2. Daly CJ, Parmryd I, McGrath JC. (2012) Techniques for the visualisation and analysis of vascular receptors using confocal laser scanning microscopy and fluorescent ligands. *Methods Mol. Biol.* 897, 95-107
3. Adler, J, Parmryd, I. (2013) Colocalization analysis in fluorescence microscopy. *Methods Mol. Biol.* 931, 97-109
4. Dinic J, Ashrafzadeh P, Parmryd I. (2013) Actin filaments at the plasma membrane in live cells cause the formation of ordered lipid domains. *Biochim. Biophys. Acta.* 1828, 1102-1111
5. Parmryd I, Önfelt B. (2013) Consequences of membrane topography. *FEBS J.* 280, 2775-2784
6. Lindberg B, Merritt EA, Rayl M, Liu C, Parmryd I, Olofsson B, Faye I. (2013) Immunogenic and antioxidant effects of a pathogen-associated prenyl pyrophosphate in *Anopheles gambiae*. *PLoS One* 8, e73868
7. Mahammad S, Parmryd I. Cholesterol depletion using methyl- β -cyclodextrin. *Methods Mol. Biol.* In press.
8. Mahammad S, Parmryd I. What can different methods tell us about membrane nanodomains in cells? *Essays Biochem.* In press.

Agencies that support the work

The Swedish Research Council

AFA Insurance

Signhild Engkvist's Foundation

The Clas Groschinsky Memory Foundation

The O. E. and Edla Johansson's Foundation

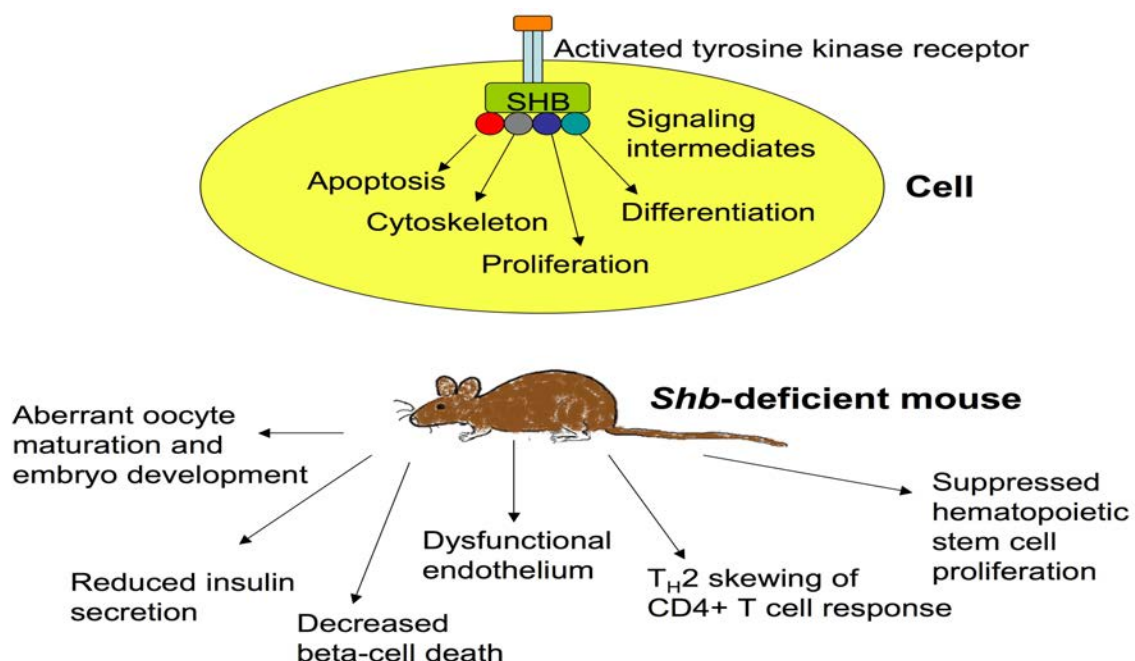
Magnus Bergvall's Foundation Foundation

Sigurd and Elsa Golje's Foundation

Importance of Shb-dependent signaling for glucose homeostasis, angiogenesis, hematopoiesis and reproduction

Michael Welsh

Shb is an SH2-domain adapter protein operating downstream of tyrosine kinase receptors such as the 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. The mechanisms of these effects on β -cells are currently being explored.

Shb-knockout mice also display reduced angiogenesis and this causes diminished tumor expansion (subcutaneously injected tumor cells or inheritable RIP-Tag insulinomas). An important aspect that has not yet been determined is whether tumor metastasis is affected or not by the absence of *Shb* and this will be studied. *Shb* deficient endothelial cells have an 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. The underlying signaling event(s) responsible for these aberrations are currently being elucidated and our findings so far suggest that they may reflect altered Rac1-activation.

The absence of *Shb* exerts effects on hematopoiesis and peripheral T lymphocyte function. The blood profile demonstrates fewer macrophages this appears to result from decreased proliferative capacity of hematopoietic stem cells. Such an effect may have consequences for the progression of leukemia/myeloproliferative disease. 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 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. Since *Shb* is only highly conserved among mammals with a true placenta, our intention is to assess the role of *Shb* in placenta formation.

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

Michael Welsh - Professor

Maryam Nikpour-Post-Doc

Björn Åkerblom-Post-Doc

Karin Gustafsson - PhD-student

Maria Jamalpour – PhD-student

Publications 2011-

1. Gustafsson, K., Calounova, G, Hjelm, F., Kriz, V., Heyman, B., Grönvik, K.-O., Mostoslavsky, G., Welsh, M. *Shb* deficient mice display an augmented T_H2 response in peripheral CD4⁺ T cells. *BMC Immunology*, 13:3, 1-10, 2011

2. Åkerblom, B., Zang, G., Zhuang, Z. W., Calounova, G., Simons, M., Welsh, M. Heterogeneity among RIP-Tag2 insulinomas allows Vascular Endothelial Growth Factor-A independent tumor expansion as revealed by studies in *Shb*-mutant mice: implications for tumor angiogenesis. *Mol. Oncol.*, 6, 333-346, 2012
3. Christoffersson, G., Zang, G., Zhuang, Z. W., Vågesjö, E., Simons, M., Phillipson, M., Welsh, M. Vascular adaptation to a dysfunctional endothelium as a consequence of *Shb* deficiency. *Angiogenesis*, 15, 469-480, 2012
4. Zang, G., Christoffersson, G., Tian, G., Harun-Or-Rashid, M., Vågesjö, E., Phillipson, E., Barg, S., Tengholm, A., Welsh, M. Isolated lung endothelial cells from *Shb* knockout mice show aberrant association between Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) and VE-cadherin in response to VEGF-A. *Cell. Signal.*, 25, 85-92, 2013
5. Gustafsson, K., Heffner, G., Wenzel, P.L., Curran, M., Grawé, J., McKinney-Freeman, S.L., Daley, G.Q. and Welsh, M. The Src homology 2 protein *Shb* promotes cell cycle progression in murine hematopoietic stem cells by regulation of focal adhesion kinase activity. *Exp Cell Res*, 319, 1852-1864, 2013

Reviews 2011-

6. Welsh, M. The platelet-derived growth factor (PDGF) family of tyrosine kinase receptors: a Kit to fix the beta cell. *Diabetologia*, 55, 2092-2095, 2012
7. Claesson-Welsh, L. and Welsh, M. VEGFA and tumor angiogenesis. *J. Int. Med.*, 273, 114-127, 2013

Agencies that support the work

The Swedish Research Council

The Swedish Cancer Foundation

The Swedish Diabetes Association

Stiftelsen Familjen Ernfors fond

Complications in pregnancy

Ulf Eriksson, Parri Wentzel

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

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

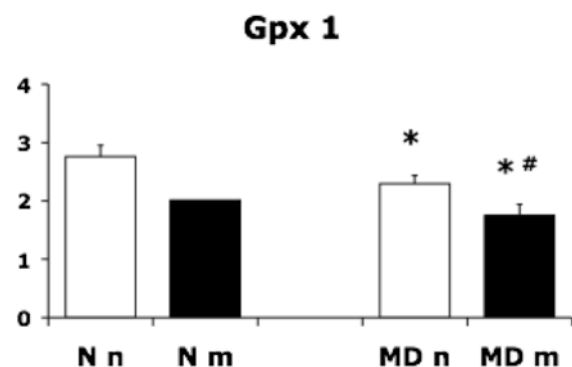
We have identified one gene, Glutathione Peroxidase-1, which is underexpressed in malformed offspring of diabetic rats (compared with non-malformed offspring of same litter), and its gene product, the antioxidative enzyme Gpx-1, is less distributed in the embryonic tissues, and its enzymatic activity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

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 pursue 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



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



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

pregnancy, as well as attempting to affect embryonic development *in vitro* by subjecting the embryos and embryonic cells to fatty acids and other lipid compounds.

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.



Rat fetus lacking tail, from obese mother

Members of the group

Ulf Eriksson, professor

Parri Wentzel, associate professor

Andreas Ejdesjö, postdoc

Collaborator

Peter Nawroth, professor



Rat fetus with only 2 ossified vertebrae, from obese mother

Publications 2011-

1. Genetic and environmental influence on diabetic rat embryopathy. Ejdesjö A, Wentzel P & Eriksson UJ. *Am J Physiol Endocrinol Metab* 300: E454-E467, 2011.
2. Altered gene expression in rat neural crest cells exposed to a teratogenic glucose concentration *in vitro*: paradoxical downregulation of antioxidative defense genes. Wentzel P & Eriksson UJ. *Birth Defects Res B* 92: 487-497, 2011.
3. Linkage study of congenital malformations in diabetic pregnancy. Nordquist N, Luthman H, Pettersson U & Eriksson UJ. *Reprod Toxicol* 33: 297-307, 2012.
4. Influence of maternal metabolism and parental genetics on fetal maldevelopment in diabetic rat pregnancy. Ejdesjö A, Wentzel P, & Eriksson UJ. *Am J Physiol Endocrinol Metab* 302: E1198-E1209, 2012

Reviews 2011-

1. Diabetic Embryopathy. Eriksson UJ & Wentzel P. *Methods Mol Biol* 889: 425-436, 2012.

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.

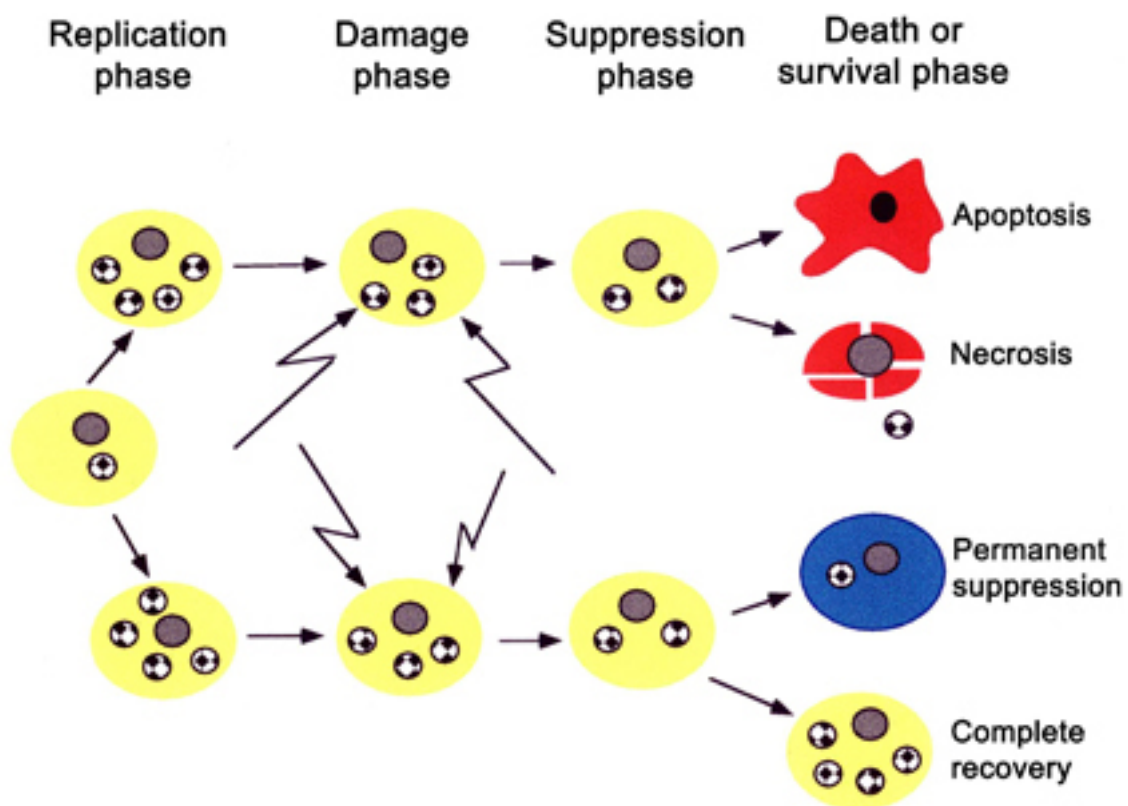


Fig. 1. Schematic view of the β -cell outcome following different immunologic or toxic assaults. In fetal and neonatal life, β -cell replication is increased, but later it becomes restricted. After birth β -cell acquire the full capacity to synthesise and release insulin (speckled symbols) upon appropriate 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. Evaluation of cytokine traps in experimental diabetes
- C. Mitochondrial targeted preconditioning, using K_{ATP} -channel openers (KCO), to rescue β -cells against acute destruction
- D. Exploration of the bank vole as an animal model for human diabetes
- E. Antiviral intervention in NOD mice

Example of findings and hypothesis

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

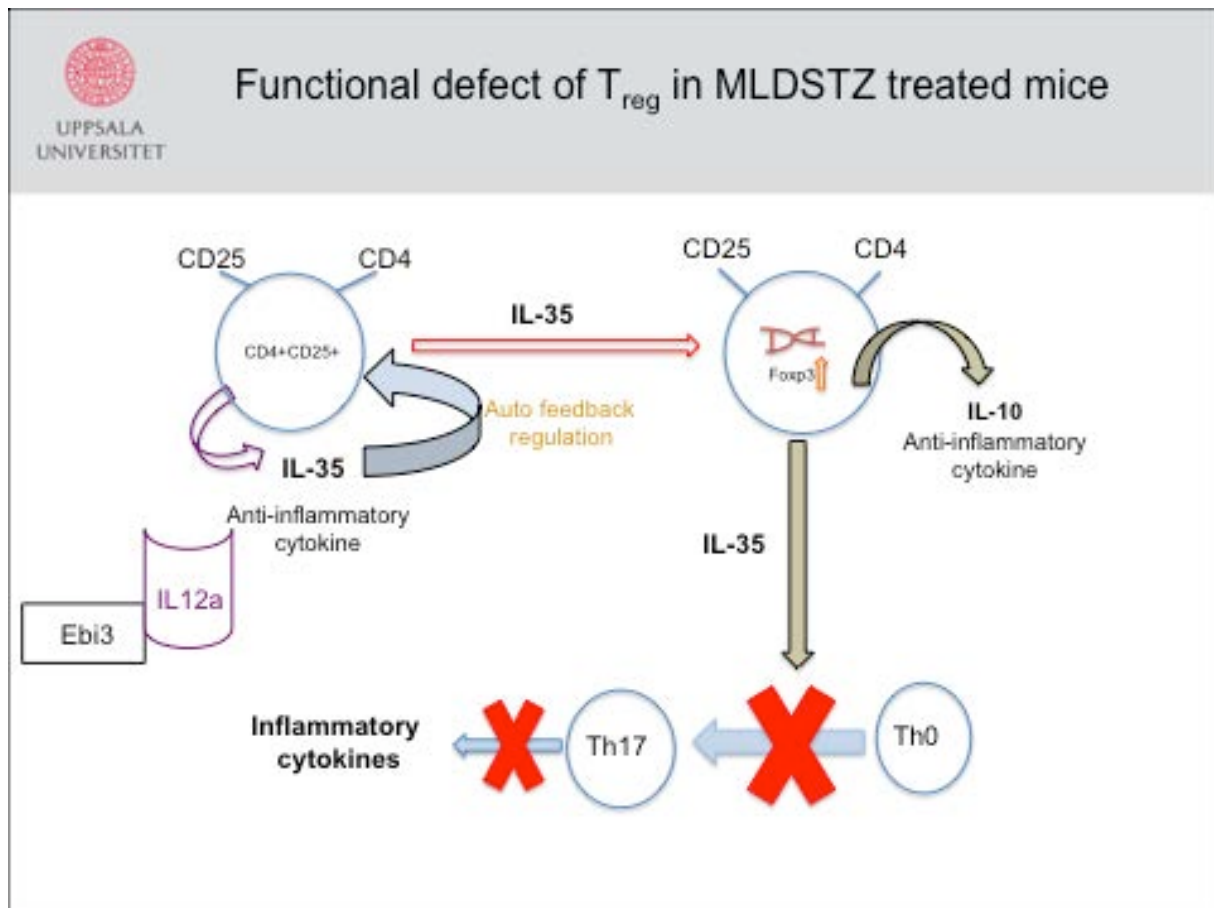


Fig. 2. Based on a number of different experiments we propose that Tregs in the multiple low dose streptozotocin (MLDSTZ) model of T1DM are functionally impaired, since a key cytokine (IL-35) is not being up-regulated in response to the proinflammatory environment induced by MLDSTZ.

Mechanism of mitochondrial K_{ATP} channel opening and β -cell protection, (cf topic B)

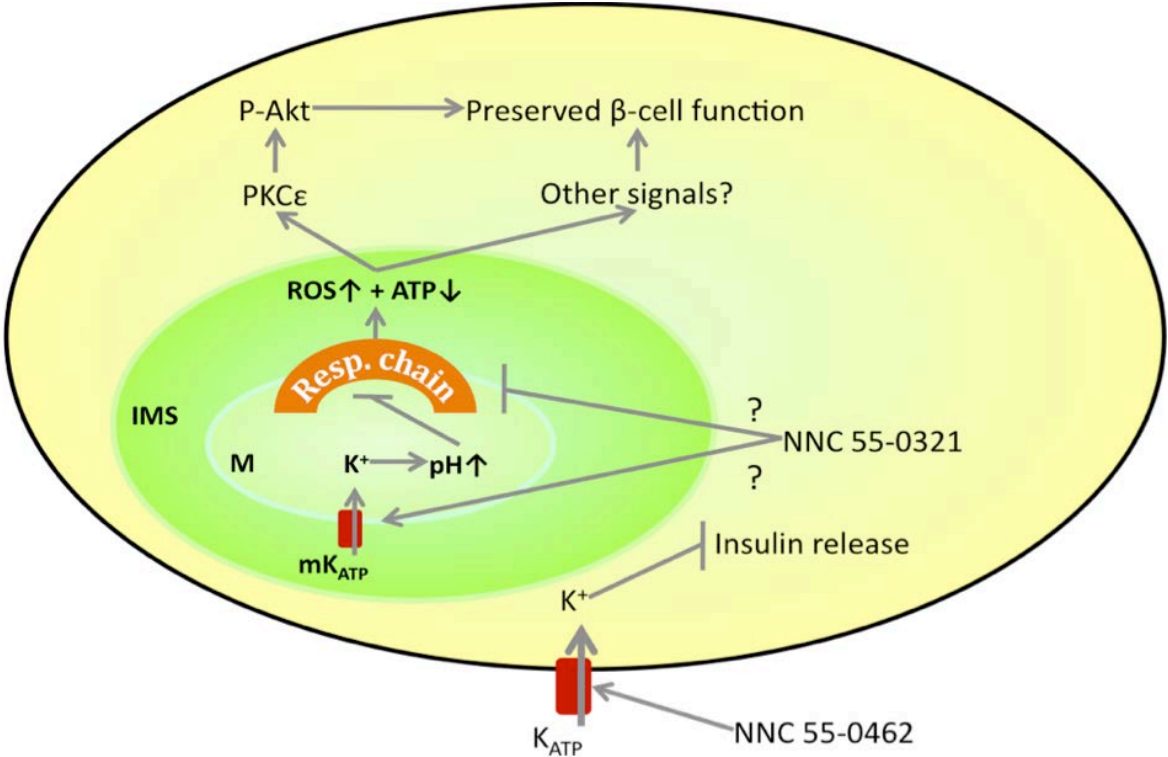


Fig. 3. NNC 55-0321 acutely down-regulates mitochondrial function. A lowered respiratory chain activity is accompanied by increased ROS production, PKCε activation and phosphorylation of the survival promoting kinase Akt. Inhibition of mitochondrial function by NNC 55-0321 may be caused by opening of a mitochondrial potassium channel (mK_{ATP}), which promotes K^+ entry from the intermembrane space (IMS) into the mitochondrial matrix (M), thereby increasing pH and inhibiting the respiratory chain (I). Alternatively, NNC 55-0321 can directly inhibit mitochondrial respiration independently of the presence of and conductance in an mK_{ATP} (II). NNC 55-0462 primarily acts on the plasma membrane bound K_{ATP} channel and causes inhibition of insulin secretion by preventing depolarization of the plasma membrane, but this does not provide protection against β -cell damage (cf aim C above).

Pancreatic islet in a diabetic bank vole

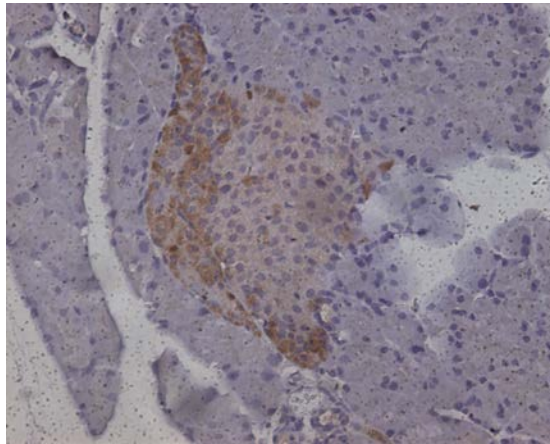


Fig. 4. Pancreatic islet of a female colonized bank vole 18 weeks of age. The bank vole was hyperglycemic (17.4 mM) 120 min after the IPGTT and serum insulin was elevated (2.34 mg/ml). The section was IHC stained with an Ljungan virus-specific antibody (brown colour) showing strong staining in some areas 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.

Publications 2011-

1. Börjesson A, Rønn SG, Karlsen AE, Billestrup N and Sandler S. b-cell specific overexpression of suppressor of cytokine signalling-3 does not protect against multiple low dose streptozotocin induced type 1 diabetes in mice. *Immunol Lett* 136: 74-79, 2011
2. Tian G, Sandler S, Gylfe E and Tengholm A. Glucose and hormone-induced CAMP oscillations in a- and b-cells within pancreatic islets. *Diabetes*: 60: 1535-1543, 2011.
3. Lindgren S, Brännström T, Hanse E, Ledin T, Nilsson G, Sandler S, Tidefelt U, and Donnér J. Medical education in Sweden. *Medical Teacher* 33: 798-803, 2011.
4. Ludvigsen E, Stridsberg M, Janson ET and Sandler S. Altered expression of somatostatin receptors in pancreatic islets from NOD mice cultured at different glucose concentrations in vitro and in islets transplanted to diabetic NOD mice in vivo. *Exp Diabetes Res* 2011 (623472): 1-8, 2011. doi:10.1155/2011/623472.

5. Saksida T, Stosic-Grujicic S, Timotijevic G, Sandler S and Stojanovic I. Macrophage migration inhibitory factor deficiency protects pancreatic islets from palmitic acid-induced apoptosis *Immunol Cell Biol* 90: 688-698, 2011.
6. Rydgren T, Börjesson A, Carlsson A, and Sandler S. Elevated glucagon-like peptide-1 plasma levels, as a possible adaptive response, in diabetic NOD mice. *Biochem Biophys Res Commun* 423: 583-587, 2012.
7. Stojanovic I, Saksida T, Timotijevic G, Sandler S and Stosic-Grujivic S. Macrophage migration inhibitory factor (MIF) enhances palmitic acid- and glucose-induced murine beta-cell dysfunction and destruction in vitro. *Growth Factors* 30: 385-393, 2012.
8. Blixt M, Sandler S and Niklasson B. Ljungan virus and diabetes. *In: Diabetes and Viruses*, Eds. Taylor K, Hyöty H, Toniolo A and Zuckerman AJ. Springer Science, New York. pp. 81-86, 2013 (ISBN 978-1-4614-4051-2)
9. Rydgren T, Öster E, Sandberg M and Sandler S. Administration of IL-1 Trap prolongs survival of transplanted pancreatic islets to type 1 diabetic NOD mice. *Cytokine* 63: 123-129, 2013
10. Chowdhury A, Dyachok O, Tengholm A, Sandler S and Bergsten S. Functional changes in aggregated insulin producing cells. *Diabetologia* 56: 1557-1568, 2013
11. Sreiber O, Petersson J, Waldén T, Ahl D, Sandler S, Philipson M and Holm L. iNOS-dependent increase in colonic mucus thickness in DSS-colitic rats. *PLOS One* 8: e71483, 2013
12. Singh K, Hjort M, Thorvaldson L and Sandler S. Concomitant analysis of Helios and Neuroligin-1 as marker to detect thymic derived regulatory T cells in naive mice. (Submitted)
13. Blixt M, Niklasson B and Sandler S. Analysis of pancreatic islet morphology of diabetic bank voles revealed alterations seen in type 2 diabetes. (Submitted)
14. Singh K, Kadesjö E, Lindroos J, Hjort M, Lundberg M, Sandler S* and Thorvaldson L*. (Shared authorship). Central role of impaired IL-35 production in beta-cell destruction during early development of murine Type 1 diabetes. (Submitted)
15. Ludvigsen E, Carlsson C, Janson ET, Sandler S and Stridsberg M. Somatostatin receptor 1-5; expression profiles during rat development. (Submitted)

Members of the research group

The following colleagues are engaged in the projects described above:

PhD Martin Blixt (guest lecturer, part-time research)

PhD Tobias Rydgren (post-doc stipend)

PhD (Lina Thorvalson (part time post-doc)

Laboratory technician IngBritt Hallgen (part-time)

Adjunct Prof Bo Niklasson

PhD Student Kailash Singh

PhD student Gutaf 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

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).

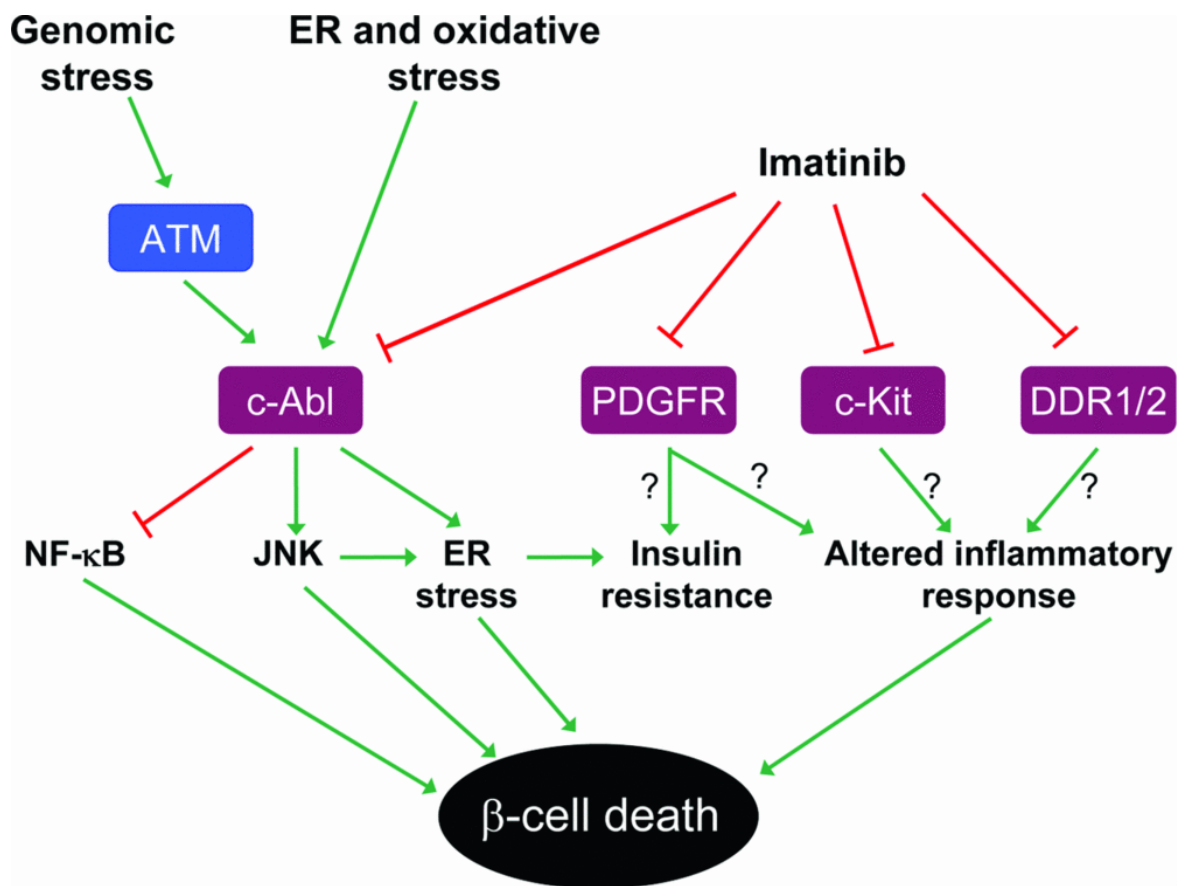


Figure 1 Possible mechanisms for the anti-diabetic effects of imatinib

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- κ B. 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 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.

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

Rikard Fred - Post-doc doc

Camilla Kappe - Post-doc

Kyryl Turpaev - Post-doc

Xuan Wang - PhD-student

Ebrahim Anvari – Licentiate-student

Publications 2011-

1. Olerud J, Mokhtari D, Johansson M, Christoffersson G, Lawler, J, Welsh N, Carlsson P-O. Thrombospondin-1: an islet endothelial cell signal of importance for β -cell function. *Diabetes* 2011 Jul;60(7):1946-54.
2. Fred RG, Sandberg M, Pelletier J, Welsh N. The human insulin mRNA is partly translated via a cap- and eIF4A-independent mechanism. *Biochem Biophys Res Commun*. 2011 412:693-698.
3. Dariush Mokhtari, Tao Lu, Nils Welsh Effects of Imatinib Mesylate (Gleevec) on human islet NF-kappaB activation and chemokine production in vitro. *PLoS One*. 2011;6(9):e24831. Epub 2011 Sep 14.
4. Hindlycke H, Lu T, Welsh N. Cytokine-induced human islet cell death in vitro correlates with a persistently high phosphorylation of STAT-1, but not with NF- κ B activation. *Biochem Biophys Res Commun*. 2012 Feb 3. [Epub ahead of print]
5. Anongnad, Kyryl Turpaev, Nils Welsh, Elena Kozlova. Co-culture of insulin producing RINm5F cells with neural crest stem cells protects partially against cytokine-induced cell death. *Pancreas*. 2012 Apr;41(3):490-2.
6. F. Allagnat, M. Fukaya, T.C. Nogueira, N. Welsh, P. Marchetti, D.L. Eizirik, A.K. Cardozo. C/EBP Homologous Protein (CHOP) Contributes to Cytokine-induced Pro-Inflammatory Responses and Apoptosis in Beta Cells. *Cell Death Differ*. 2012 Jun 1. doi: 10.1038/cdd.2012.67.
7. D Mokhtari, A Al-Amin, K Turpaev, T Li, O Idevall-Hagren, J Li, A Wuttke, RG Fred, P Ravassard, R Scharfmann, A Tengholm, N Welsh. Imatinib mesylate-induced phosphatidylinositol-3-kinase signaling and improved survival in insulin-producing cells: Role of Src homology 2-containing inositol 5'-phosphatase interaction with c-Abl *Diabetologia*. 2013 Jun;56(6):1327-38
8. Anongnad Ngamjariyawat, Kyryl Turpaev, Svitlana Vasylovska, Elena N. Kozlova, Nils Welsh. Co-culture of neural crest stem cells (NCSC) and insulin producing beta-TC6 cells results in cadherin junctions and protection against cytokine-induced beta-cell death. *PLoS One* 2013 Apr 17;8(4):e61828. doi: 10.1371/journal.pone.0061828
9. Ebrahim Anvari, Nils Welsh. The H1-receptor antagonist cetirizine protects partially against cytokine- and hydrogen peroxide-induced beta-TC6 cell death in vitro. *Pancreas*, in press.

10. Xuan Wang, Lin Jiang, Ulla Engström, Adam Ameer, Ola Wallerman, Yu Qi, Leif Andersson and Nils Welsh The transcription factor ZBED6 exists in beta-cells as nuclear and cytoplasmic forms and affects gene expression, proliferation and cell death. PNAS, 2013 Oct 1;110(40):15997-6002. doi: 10.1073/pnas.1303625110
11. Lopes M, Kutlu B, Miani M, Bang-Berthelsen CH, Størling J, Pociot F, Goodman N, Hood L, **Welsh N**, Bontempi G, Eizirik DL. Temporal profiling of cytokine-induced genes in pancreatic β -cells by meta-analysis and network inference. Genomics. 2014 Jan 23. pii: S0888-7543(14)00006-8. doi: 10.1016/j.ygeno.2013.12.007.
12. Welsh N. Does the small tyrosine kinase inhibitor imatinib mesylate counteract diabetes by affecting pancreatic islet amyloidosis and fibrosis? Expert Opinion on Investigational Drugs. 2012, 21(11):1743-50

Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

Novo Nordic Foundation

Barndiabetesfonden

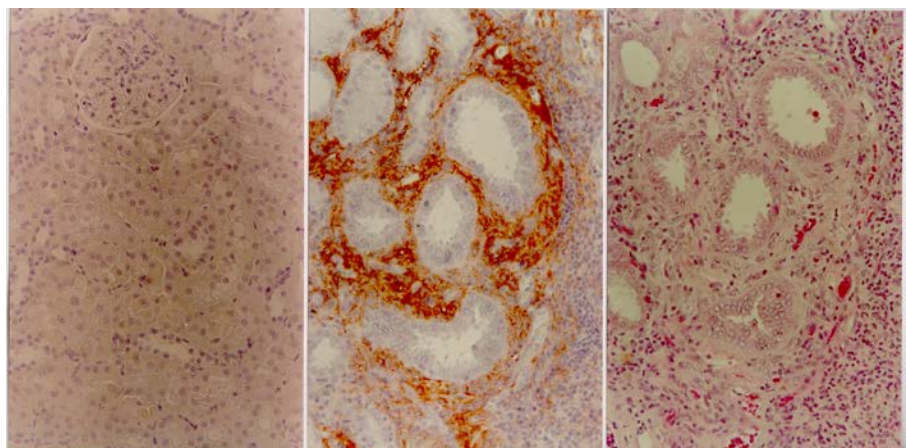
Stiftelsen Familjen Ernfors Fond

UU-Innovation

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 proinflammatory property of HA is also evaluated in



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 infiltrating immune competent cells, as seen by parallel staining with haematoxylin-eosin (right).

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

Sara Stridh - Graduate Student

Angelica Fasching - Laboratory Engineer

Fredrik Palm – Professor

Per Liss – Assoc Professor

Publications 2011-

1. Kidney Hypoxia, Attributable to Increased Oxygen Consumption, Induces Nephropathy Independently of Hyperglycemia and Oxidative Stress. Friederich-Persson M, Thörn E, Hansell P, Nangaku M, Levin M & Palm F. Hypertension 2013 Nov;62(5):914-9.
2. Determinants of kidney oxygen consumption and their relation to tissue oxygen tension in diabetes and hypertension. Hansell P, Welch WJ, Blantz RC & Palm F. Clin Exp Physiol Pharmacol 2013; 40: 123-137.
3. Editorial: NADPH-oxidase driven oxidative stress during experimental diabetes offsets NO-mediated regulation of renal medullary sodium transport. A potential treatment modality during type 1 diabetes? Hansell P. Acta Physiol (Oxf) 2013 Oct;209(2):94.

4. Hypoxia in the diabetic kidney is independent of advanced glycation end-products. Nordquist L, Liss P, Fasching A, Hansell P & Palm F. *Adv Exp Biol Med* 2013;765:185-193.
5. Adenosine A2 receptor-mediated regulation of renal hemodynamics and glomerular filtration rate is abolished in diabetes. Persson P, Hansell P & Palm F. *Adv Exp Biol Med* 2013;765:225-230.
6. Increased kidney metabolism as a pathway to kidney tissue hypoxia and damage: effects of triiodothyronine and dinitrophenol in normoglycemic rats. Friederich-Persson M, Persson P, Fasching A, Hansell P, Nordquist L, Palm F. *Adv Exp Med Biol.* 2013;789:9-14.
7. Inhibition of hyaluronan synthesis in rats reduces renal ability to excrete fluid and electrolytes during acute hydration. Stridh S, Palm F & Hansell P. *UJMS* 2013 Nov;118(4):217-221.
8. Intravoxel incoherent motion MR imaging of the kidney. Preliminary results. Eckerbom P, Hansell P, Bjerner T, Palm F, Weis J, Liss P. *Adv Exp Biol Med* 2013;765:55-58.
9. NADPH oxidase inhibition reduces tubular sodium transport and improves kidney oxygenation in diabetes. Persson P, Hansell P & Palm F. *Am J Physiol- Regulatory* 2012;302:R1443-R1449
10. Renal interstitial hyaluronan – functional aspects during normal and pathological conditions. Stridh S, Palm F & Hansell P. *Am J Physiol – Regulatory* 2012;302 R1235-R1249
11. Insulin induces the correlation between renal blood flow and glomerular filtration rate in diabetes – Implications for mechanisms causing hyperfiltration. Pihl L, Persson P, Fasching A, Hansell P, Dibona Gf & Palm F. *Am J Physiol- Regulatory* 2012; 303: R39-R47.
12. Coenzyme Q10 prevents GDP-sensitive mitochondria uncoupling, glomerular hyperfiltration and proteinuria in kidneys from db/db-mice as a model of type 2 diabetes. Friederich Persson M, Franzèn S, Catrina S-B, Dallner G, Hansell P, Brismar K & Palm F. *Diabetologia* 2012;55:1535-1543
13. Angiotensin converting enzyme inhibition blocks interstitial hyaluronan dissipation in the neonatal rat kidney via hyaluronan synthase 2 and hyaluronidase 1. Stridh S, Kerjaschki D, Chen Y Rügheimer L, Åstrand A, Johnsson C, Friberg P, Olerud J, Takahashi T, Ikegami-Kawai M, Palm F & Hansell P. *Matrix Biol.* 2011 Jan;30(1):62-69
14. Oxidative Stress and hypoxia in the Pathogenesis of Diabetic Nephropathy. Palm F, Nordquist L, Wilcox CS & Hansell P. In: *Oxidative Stress in Applied Basic Research and Clinical Practice.* Eds: T. Miyata, K-U Eckhardt, M Nangaku. Humana Press/Springer Science. ISBN 978-1-60761-856-0. Chapter 29, pp 559-586, 2011.

Book chapter

15. Oxidative Stress and hypoxia in the Pathogenesis of Diabetic Nephropathy. Palm F, Nordquist L, Wilcox CS & Hansell P. In: *Oxidative Stress in Applied Basic Research and Clinical Practice.* Eds: T. Miyata, K-U Eckhardt, M Nangaku. Humana Press/Springer Science. ISBN 978-1-60761-856-0. Chapter 29, pp 559-586, 2011.

Doctoral thesis (main supervisor)

Regulation of hyaluronan in water handling. Studies *in vivo* and *in vitro*. Sara Stridh. Acta Universitatis Upsaliensis. 2013. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine No 951. ISBN 978-91-554-8800-0.

Agencies that support the work

The Swedish Research Council

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 sense 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.

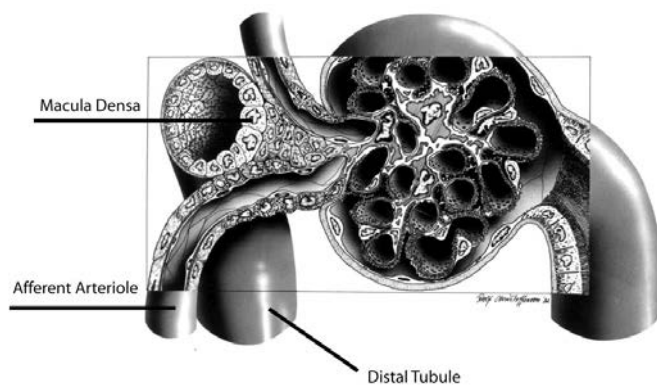


Figure 1. Schematic illustration of the juxtaglomerular apparatus (JGA) with the macula densa cells in wall of the distal tubule and the glomerular arterioles.

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 cardiovascular disease.

Members of the group

A. Erik Persson - Professor emeritus

Mattias Carlström - Researcher

Johan Sällström - Post doc

Mauricio Sendeski - Guest researcher

Andreas Patzak - Guest researcher

Gau Xian - Graduate student

Ammar Farman - Graduate student

Peter Flacker- Graduate student

Zheng Bing Zhuge-Graduate student

Publications 2011-

1. Carlström M., Persson AE., Larsson E., Hezel ., Scheffer PG., Teerlink T., Weitzberg E., Lundberg JO. :Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injureis and reduces blood pressure in saltinduced hypertemnsion. Cardiovascular Research 2011, 15;891, 574.
2. Wolgast M., Persson AE : The gel hypothesis applied to the rat renal capillary membranes :a review. Acta Physiologica 2011, 202. 617-28
3. Brown RD., Turner AJ., Carlström M., Persson AE, Gibson KJ: Tubuloglomerular feedback response in the prenataland postnatal ovine kidney. Am. J Physiol 2011 300, F1368-74.
4. Lai EY., Wang Y., Persson AE., Manning RG., Liu R., Pressure induces calcium changes in juxtaglomerular cells in perfused afferent arterioles. Hypertension Res. 2011, 34, 942-8.
5. Gao X., Patzak A., Sendeski M., Scheffer, PG., Teerlink T., Sällström, J., Fredholm BB., Persson AE., Carlström M : Adenosine A1receptor defieciency diminishes afferent arteriolar and blood pressure responses during nitric oxide inhibition and angiotensin II treatment. Am. J. Physiol. Regul. 2011, 301, R1669-81.

6. Perlewitz A., Persson AE., Patzak A., :The juxtaglomerular apparatus. *Acta Physiologica* 2012, 205, 1748, 6-8.
7. Carlström M, Brown RD, Yang T, Hezel M, Larsson E, Scheffer PG, Teerlink T, Lundberg JO, Persson AE; L-arginine or tempol supplementation improves renal and cardiovascular function in rats with reduced renal mass and chronic high salt intake. *Acta Physiol (Oxf)*. 2013 Apr;207(4):732-41. doi: 10.1111/apha.12079. Epub 2013 Feb 25.
8. Sällström J, Friden M; Simultaneous determination of renal plasma flow and glomerular filtration in conscious mice using dual bolus injection. *J Pharmacol Toxicol Methods J Pharmacol Toxicol Methods*. 2013 Jan 30;67(3):187-193.
9. Sällström J, Peuckert C, Gao X, Larsson E, Nilsson A, Jensen BL, Onozato ML, Persson AE, Kullander K, Carlstrom M. Impaired EphA4 signaling leads to congenital hydronephrosis, renal injury and hypertension. *Am J Physiol Renal Physiol*. 2013,1:305, F71-9.
10. Sällström J, Engström T, Fredholm BB, Persson AEG, Palm F. Inhibition of sodiumlinked glucose reabsorption in the kidney normalizes diabetes-induced glomerular hyper-filtration in conscious adenosine A1-receptor-deficient mice. *Acta Physiologica* 2013,
11. Persson AE, Lai EY, Gao X, Carlström M, Patzak A. Interactions between adenosine, angiotensin II and nitric oxide on the afferent arteriole. *Front Physiol* 2013, 18;4:187.
12. Carlström M, Liu M, Yang T, Zollbrecht C, Huang L, Peleli M, Borniquel S, Kishikawa H, Hezel M, Persson AE, Weitzberg E, Lundberg JO. Cross-talk beteen Nitrate-nitrite and NO synthase pathways in the control of vascular NO homeostasis. *Antioxid Redox Signal*, 2014, Feb 6.
13. Gao X, JansonL, Persson AE, Sandberg . Short-term glucosamine infusion increases islet blood flow in anaesthetized rats. *Islets* 2013, Nov 25

Licentiate examination

Xiang Gao: "Adenosine influences vascular reactivity in the afferent arteriole to the glomerulus and the pancreatic islet" March 2013

Agencies that support the work

The Swedish Research Council

Hjärt-Lungfonden

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

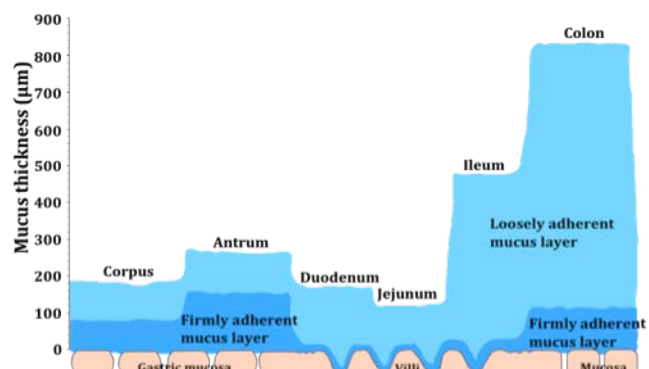


Fig 1. The mucus layers in different parts of the GI tract

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

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 (4,5). 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 (1,5,6,7). 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 (3). 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 (3).

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 (2). Leukocyte recruitment in response to proinflammatory chemokine and NSAID was decreased. Despite attenuation of the acute immune response, the overall ability to clear a bacterial infection was not suppressed.

Members of the group

Lena Holm, professor

David Ahl, PhD student

Annika Jägare, laboratory engineer

Shokoufeh Karimi, PhD student*

Haoyu Liu, PhD, post doc

Sanna-Nilsson-Hellgren, undergraduate student

Mona Qundos, undergraduate student

Richard Shore, PhD student**

Tomas Waldén, PhD, post doc

* Shared affiliation with the Department of Microbiology, SLU, Uppsala

** Shared affiliation with the Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm

Publications 2011-

1. J. Petersson, O. Schreiber, G. C. Hansson, S. J. Gendler, A. Velcich, J. O. Lundberg, S. Roos, **L. Holm**, & M. Phillipson. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2011;300 G327-G333.
2. C Jädert, J Petersson, S Massena, D Ahl, L Grapensparr, L Holm, JO Lundberg, M Phillipson. Decreased leukocyte recruitment by inorganic nitrate and nitrite in microvascular inflammation and NSAID-induced intestinal injury. *Free Radic Biol Med*. 2012 Feb 1;52(3):683-92. Epub 2011 Nov 30. PMID: 22178413 [PubMed - in process]
3. J Dicksved*, O Schreiber*, B Willing^a, J Petersson, S Rang, M Phillipson, L Holm, S Roos * These authors contributed equally to this work. Lactobacillus reuteri Maintains a Functional Mucosal Barrier during DSS Treatment Despite Mucus Layer Dysfunction. *PLoS One*. 2012;7(9):e46399. doi: 10.1371/journal.pone.0046399. Epub 2012 Sep 27. PMID: 23029509
4. C Jädert, G Jakobsdottir, L Holm, M E. Nyman. Propionic and butyric acids, formed in caecum of rats fed highly fermentable dietary fibre, are reflected in portal and aortic serum. <http://dx.doi.org/10.1017/S0007114513000809>, Published online: 26 March 2013
5. O. Schreiber, J. Petersson, T. Waldén, D Ahl, S. Sandler, M. Phillipson and L. Holm. iNOS-dependent increase in colonic mucus thickness in DSS-colitic rats. *PLoS ONE* 2013 8(8): e71843. doi:10.1371/journal.pone.0071843
6. C Jädert, M Phillipson, L Holm, JO Lundberg, S Borniquel. Preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease. *Redox Biol*. 2013 Dec 24;2:73-81. doi: 10.1016/j.redox.2013.12.012. eCollection 2014. PMID: 24494186 [PubMed]
7. Kober, D Ahl, L Holm, SR Carding, N Juge. $\gamma\delta$ T-cell-deficient mice show alterations in mucin expression, glycosylation and goblet cells but maintain an intact mucus layer. *Am J Physiol Gastrointest Liver Physiol*. 2014 Feb 6. [Epub ahead of print]

Book chapters

8. L Holm, M Phillipson. Assessment of Mucus Thickness and Production In Situ. Michael A. McGuckin and David J. Thornton (eds.), *Mucins: Methods and Protocols*, Methods in Molecular Biology, vol. 842, DOI 10.1007/978-1-61779-513-8_12, © Springer Science+Business Media, LLC 2012

Agencies that support the work

The Swedish Research Council

Formas (The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning)

BioGaia AB

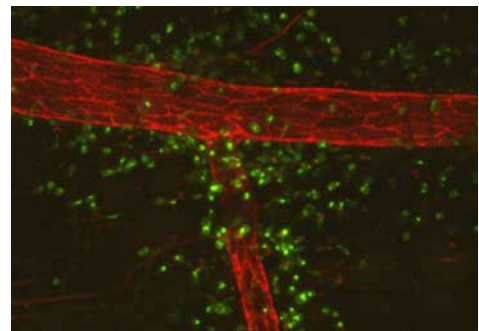
Leukocyte recruitment during inflammation and angiogenesis

Mia Phillipson

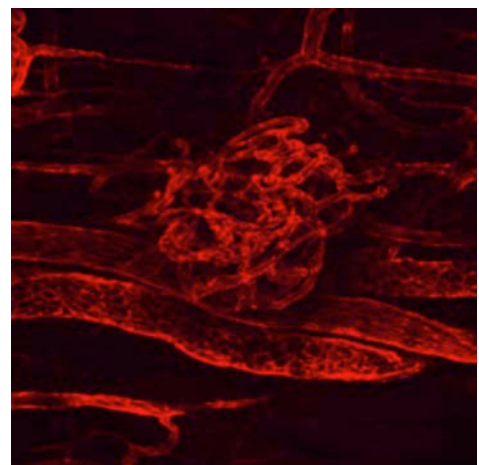
The capacity of circulating leukocytes to arrest on the surface of inflamed endothelium, transmigrate, and penetrate into the underlying tissue are key steps in response to infections as well as other inflammatory processes, and the importance of these events also during angiogenesis has recently been suggested. Detrimental inflammation is involved in the pathology of the majority of diseases, and increasing the knowledge of the mechanisms that regulate leukocyte recruitment is very important to be able to control and eventually limit inflammatory response, tumor growth and tissue damage.

Our overall aim is to study how different subsets of leukocytes are recruited from the circulation during inflammation or hypoxia, as well as their specific roles under the different settings. 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 study how chemokines are transported into the inflamed venules, and recently found that chemokines sequestered on endothelial heparan sulphate direct crawling leukocytes towards optimal sites for transmigration (Massena et al., *Blood*, 2010).

Most of our studies are conducted in vivo in unique mouse models, which enable registration of leukocyte-endothelial interactions using bright-field or spinning disk confocal microscopy. Inflammation is induced either by administration of one or more specific chemokines (applied protein, or through plasmid DNA delivery) or bacterial infection. Alteration of immune responses during diabetes or nitrate rich diet is investigated. Hypoxia is induced either by ligation of muscle arteries, or by transplantation of isolated insulin-producing pancreatic islets to the muscle. By using the latter model, 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, the organ traditionally used for islet transplantation (Christoffersson et al., *Diabetes*, 2010). We have also identified a specific neutrophil subtype in the circulation with pro-angiogenic features that are recruited to sites of hypoxia by Vascular Endothelial Growth Factor A (VEGF-A) (Christoffersson et al., *Blood*, 2012).



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



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

Members of the group

Mia Phillipson - Associate Professor

Gustaf Christoffersson – Post Doc

Antoine Giraud – Research Engineer

Jalal Haft – Master Student

Cecilia Jädert – PhD student*

Sara Massena Santos – PhD student

John Sedin – Post Doc

Cédric Seigneiz – Post Doc

Magnus Söderling – Researcher

Evelina Vågesjö – PhD student

Tomas Waldén – Post Doc

* Shared affiliation with the Department of Physiology and Pharmacology, Karolinska Institute, Stockholm

Publications 2011-

1. LC Dieterich, P Schiller, S Massena, **M Phillipson**, A Dimberg. Regulation of alphaB-crystallin in tumor angiogenesis and regulation of the tumor microenvironment. *Angiogenesis*, 16: 975-83, 2013.
2. C Jädert, **M Phillipson**, L Holm, JO Lundberg, S Borniquel. Preventive and therapeutic effects of nitrite supplementation in experimental inflammatory bowel disease. *Redox Biol*, 2: 73-81, 2013.
3. O Schreiber, J Petersson, A Jägare, S Sandler, JO Lundberg, **M Phillipson**, L Holm. iNOS-dependent increase in colonic mucus thickness in DSS-colitic rats. *PLoS One*, 8:e71843, 2013.
4. C Benedict, J Cedernaes, V Giedraitis, E Nilsson, PS Hogenkamp, E Vågesjö, US Pettersson, S Massena, G Christoffersson, **M Phillipson**, JE Broman, L Lannfelt, H Zetterberg, HB Schiöth. Acute sleep deprivation increases serum levels of neuron-specific enolase (NSE) and S-100 calcium binding protein B (S100B) in healthy young men. *Sleep*, 37:195-8, 2013.
5. Z Sun^{*}, X Li^{*}, S Massena^{*}, S Kutchera, N Padhan, L Gualandi, V Sunvold Gjerstad, K Gustafsson, WW Choy, G Zang, M Quach, L Jansson, M Phillipson, MR Abid, A Spurkland, L Claesson-Welsh. VEGFR2 – TAd - Src signaling regulates vascular permeability in vivo. *J Exp Med* 209:1363-77, 2012.
6. G Christoffersson^{*}, G Zang^{*}, Zw Zhuang, E Vågesjö, M Simons, M Phillipson, M Welsh. Vascular adaptation to a dysfunctional endothelium as a consequence of Shb deficiency. *Angiogenesis* 15, 469-80, 2012.
7. US Pettersson, TB Walden, PO Carlsson, L Jansson, M Phillipson. Female C57Bl/6 Mice are Protected against High Fat Diet-Induced Metabolic Syndrome due to Anti-inflammatory Actions in Visceral Adipose Tissue. *PLoS One*, 7, e46057, 2012.

8. J Dicksved, O Schreiber, B Willing, J Petersson, S Rang, M Phillipson, L Holm, S Roos. Lactobacillus reuteri counteract translocation of bacteria but does not affect changes of the microbiota seen in a DSS colitis model. PLoS One, 7, e46399, 2012.
9. G Christoffersson, E Vågesjö, J Vandooren, M Liden, S Massena, Rb Reinert, M Brissova, Ac Powers, G Opdenakker, M Phillipson. VEGF-A recruits a proangiogenic MMP-9-delivering neutrophil subset that induces angiogenesis in transplanted hypoxic tissue. Blood, 120: 4653-4662, 2012.
10. G Zang, G Christoffersson, G Tian, M Harun-Or-Rashid, E Vågesjö, M Phillipson, S Barg, A Tengholm, M Welsh. Aberrant association between Vascular Endothelial Growth Factor Receptor-2 and VE-cadherin in response to Vascular Endothelial Growth Factor-A in Shb-deficient lung endothelial cells. Cell Signal, 25:85-92, 2012.
11. Jädert C, Petersson J, Massena S, Ahl D, Holm L, Lundberg J, Phillipson M. Inhibition of leukocyte recruitment by inorganic nitrate and nitrite. Free Radic Biol Med. 52:683-692, 2012.
12. Pettersson US, Christoffersson G, Massena S, Ahl D, Jansson L, Henriksnäs J, Phillipson M. Increased recruitment but impaired function of leukocytes during inflammation in mouse models of type 1 and type 2 diabetes. PLoS One, 6, e22480, 2011.
13. Petersson J, Schreiber O, Hansson GC, Gendler SJ, Velcich A, Lundberg JO, Roos S, Holm L, Phillipson M. Importance and regulation of the colonic mucus barrier in a mouse model of colitis. Am J Physiol Gastrointest Liver Physiol. 300: G327-333, 2011.
14. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, Squadrito ML, Segura I, Li X, Knevels E, Costa S, Vinckier S, Dresselaer T, Åkerud P, De Mol M, Salomäki H, Phillipson M, Wyns S, Larsson E, Buyschaert I, Botling J, Himmelreich U, Van Ginderachter JA, De Palma M, Dewerchin M, Claesson-Welsh L, Carmeliet P. HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. Cancer Cell 19: 31-44, 2011.
15. Petri B, Kaur J, Long EM, Li H, Parsons SA, Butz S, Phillipson M, Vestweber D, Patel KD, Robbins SM, Kubes P. Endothelial LSP1 is involved in endothelial dome formation, minimizing vascular permeability changes during neutrophil transmigration in vivo. Blood 117: 942-52, 2011.

Reviews 2011-

16. M Phillipson M, Claesson-Welsh L, Welsh M. VEGFA-induced vascular permeability and leukocyte extravasation. Invited review in *Vascular Signaling in Health and Disease*, Drs Mirko HH Schmidt and Stefan Liebner, Springer, 2013.
17. Phillipson M, Kubes P. The neutrophil in vascular inflammation. Nature Medicine, 17:1381-90, 2011.
18. Massena S, Phillipson M. Intravascular leukocyte chemotaxis: The rules of attraction. Chapter in the book Hematology, ISBN 979-953-307-516-6, edited by Charles H. Lawrie, In press
19. Holm L, Phillipson M. Assessment of Mucus Thickness and Production In Situ. Methods Mol. Biol. 842:217-227, 2012.
20. Christoffersson G, Carlsson PO, Phillipson M. Intramuscular Islet Transplantation Promotes Restored Islet Vascularity. Islets 3: 69-71, 2011

Dissertations

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

Ulrika S Pettersson "Blood Flow Regulation and Inflammatory Response in Experimental Models of Diabetes" PhD February 2012

Agencies that support the work

Swedish Research Council

Knut and Alice Wallenberg foundation

Ragnar Söderberg Foundation

Swedish Foundation for Strategic Research

The Novo Nordic Foundation

The Swedish Diabetes Foundation

The Ernfors family foundation

The Diabetes Wellness Foundation

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

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., *Diabetologia* 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. Therefore, we are the first to show that increased oxygen

utilization is enough to cause kidney hypoxia and nephropathy. This is a major breakthrough since previous studies always have been associated with confounding factors, such as hyperglycemia, increased oxidative stress and altered tubular transport.

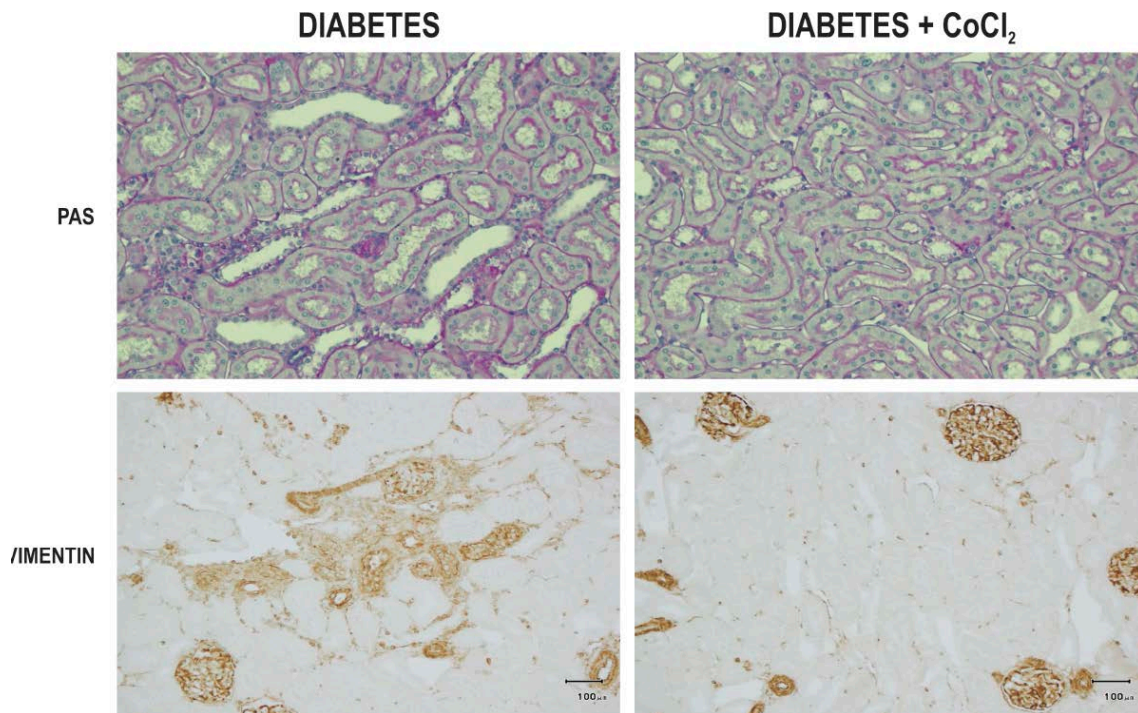


Figure 1. Activation of the hypoxic gene response by chronic CoCl₂ treatment prevented the diabetes-induced kidney hypoxia and the clinical signs of diabetic nephropathy, such as tubulointerstitial fibrosis, proteinuria and glomerular hyperfiltration.

We have also approached the problem with kidney hypoxia from another angle by chronically treating diabetic animals with CoCl₂, 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.

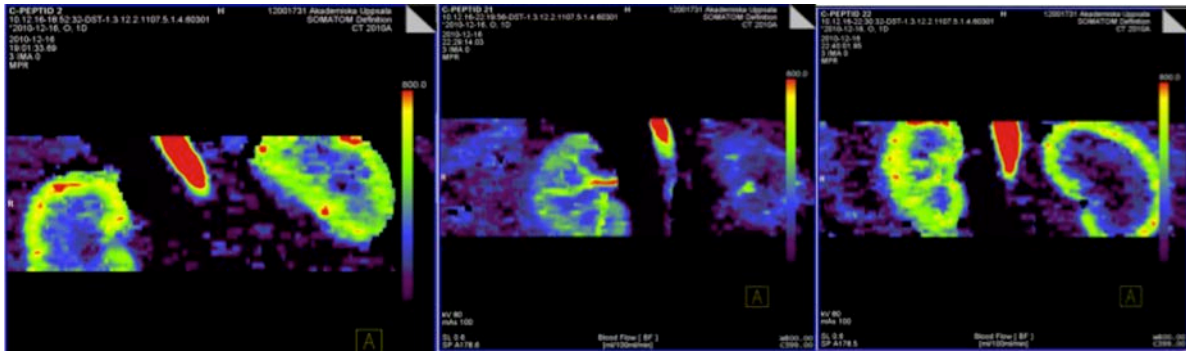


Figure 2. Thirty minutes of warm ischemia to the left kidney (right kidney on the images) did not alter kidney function or the intrarenal blood flow (images above measured by computed tomography) in control rats four weeks after the ischemic insult. However, the same ischemic insult caused markedly reduced kidney function (glomerular filtration rate about 10% of normal), atrophy and 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.

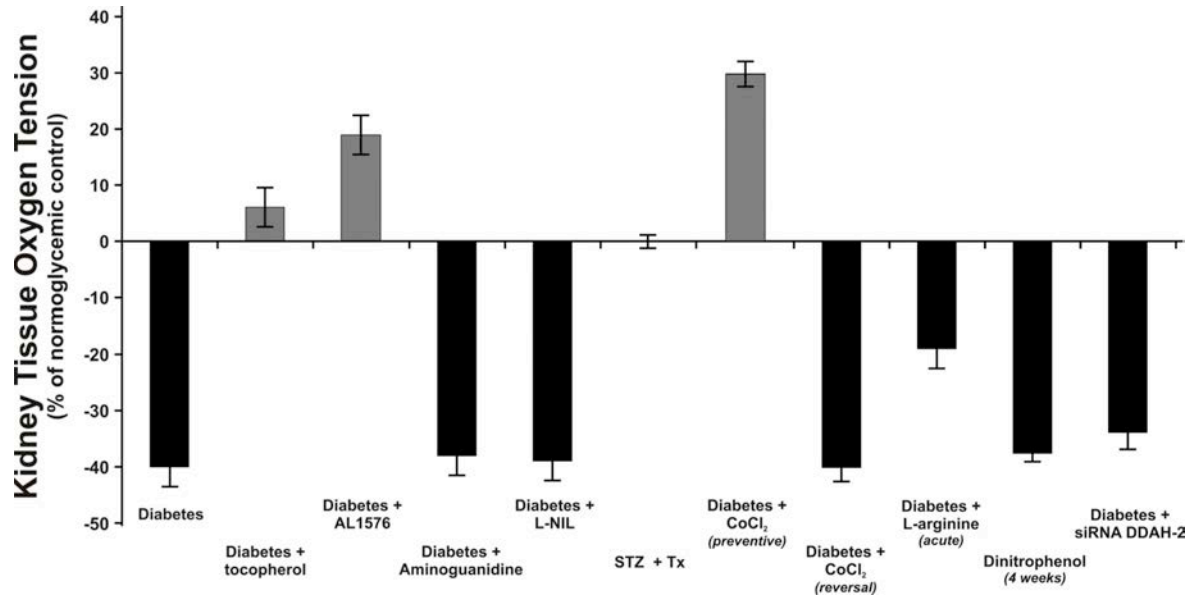


Figure 3. Summary of all investigated mechanisms affecting intrarenal oxygenation in diabetes. Tocopherol (vitamin E) is an antioxidant, AL1576 inhibits aldose reductase and presents activation of the polyol pathway, aminoguanidine inhibits AGE formation, L-NIL inhibits iNOS, STZ + Tx denotes animals administered streptozotocin (to induce diabetes) and 24h thereafter received enough islets of Langerhan's to reverse the hyperglycemia, CoCl₂ 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. Interestingly, 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 finding 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

Swedish Heart and Lung Foundation

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.

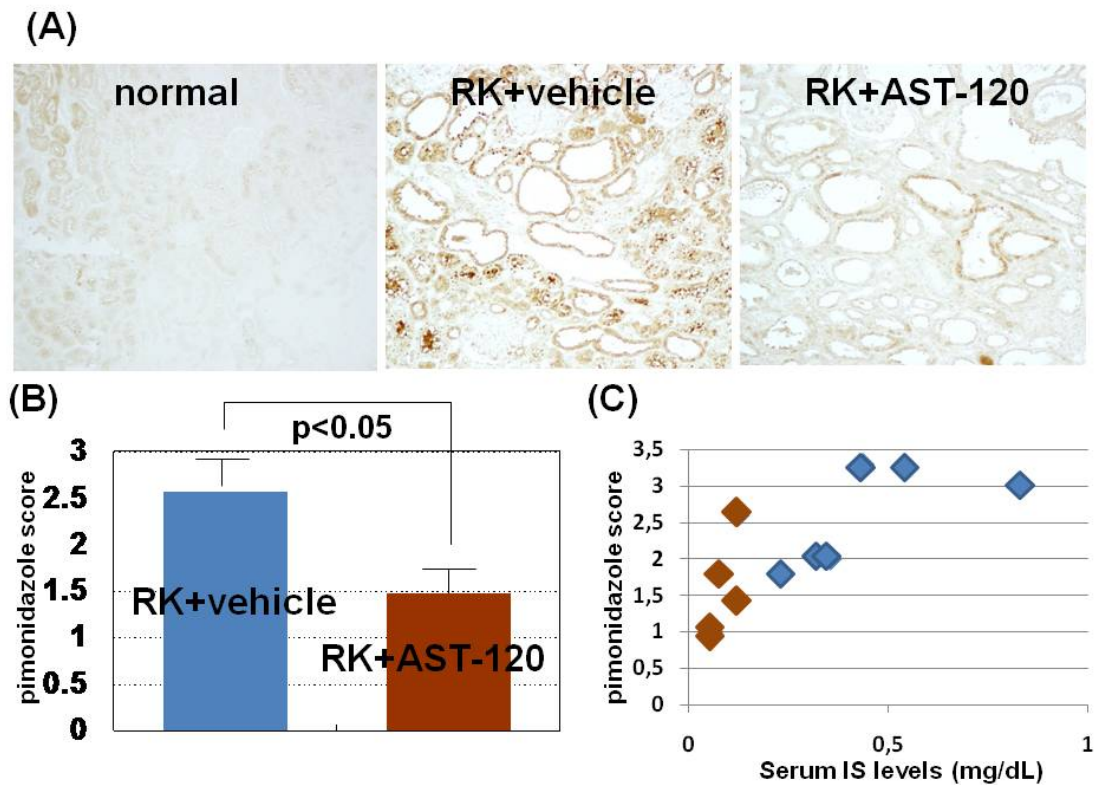


Figure 4. Improvement of oxygenation of the remnant kidney by reduction of uremic toxins. (A) Immunohistochemical staining of pimonidazole accumulation in the cortex showed improvement of oxygenation of the remnant kidney in animals treated with the oral absorbent AST-120 that reduces plasma levels of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

Swedish Society for Medical Research

Lars Hierta Foundation

Members of the group

Fredrik Palm, Ph.D.

Lina Nordquist, Ph.D., core director

Per Liss, MD, Ph.D., Associate professor

Angelica Fasching - research engineer

Malou Friederich – Ph.D. Post Doc

Patrik Persson, Ph.D. Post Doc

Ebba Sivertsson, Ph.D.-student

Per Eckerbom, Ph.D.-student

Publications 2011-

1. Sara Stridh, David Kerjaschaki, Yun Chen, Louise Rügheimer, Ann-Britt Åstrand, Cecilia Johnsson, Peter Friberg, Johan Olerud, Fredrik Palm, Tomoko Takahashi, Mayumi Ikegami-Kawai and Peter Hansell. Angiotensin converting enzyme inhibition blocks interstitial hyaluronan dissipation in the neonatal rat kidney via hyaluronan synthase 2 and hyaluronidase 1. *Matrix Biol* 2011, 30:62-69
2. Emma Lindahl, Lina Nordquist, Patrick Müller, Eli El Agha, Malou Friederich, Karin Dahlman-Wright, Fredrik Palm and Hans Jörnvall. Early transcriptional regulation by C-peptide in freshly isolated rat proximal tubular cells. *Diab Metab Res Rev* 2011, 27:697-704
3. Malou Friederich Persson, Stephanie Franzén, Sergiu Catrina, Gustav Dallner, Peter Hansell, Kerstin Brismar and Fredrik Palm. Coenzyme Q10 prevents GDP-sensitive mitochondrial uncoupling, glomerular hyperfiltration and proteinuria in kidneys from db/db mice as a model of type 2 diabetes. *Diabetologia* 2012, 55(5):1535-1543
4. Patrik Persson, Peter Hansell and Fredrik Palm. NADPH oxidase inhibition reduces tubular sodium transport and improves kidney oxygenation in diabetic rat. *Am J Physiol Regul Integr Comp Physiol* 2012, 302:R1443-1449
5. Mariska Davids, Eliane Swieringa, Fredrik Palm, Desirée E. Smith, Yvo M. Smulders, Peter G. Scheffer, Henk J. Blom and Tom Teerlink. Simultaneous determination of asymmetric and symmetric dimethylarginine, L-monomethylarginine, L-arginine, and L-homoarginine in biological samples using stable isotope dilution liquid chromatography tandem mass spectrometry. *J Chromatogr B* 2012, 900:38-47
6. Liselotte Pihl, Patrik Persson, Angelica Fasching, Peter Hansell, Gerald F. DiBona and Fredrik Palm. Insulin induces the correlation between renal blood flow and glomerular filtration rate in diabetes - Implications for mechanisms causing hyperfiltration. *Am J Physiol Regul Integr Comp* 2012, 303:R39-47
7. En Yin Lai, Zaiming Luo, Maristela L. Onozato, Earl H. Rudolph, Glenn Solis, Pedro A. Jose, Anton Wellstein, Shakil Aslam, Mark T. Quinn, Kathy K. Griendling, Thu H. Le, Ping Li, Fredrik Palm, William J. Welch and Christopher S. Wilcox. Effects of the antioxidant drug tempol on renal oxygenation in mice with reduced renal mass. *Am J Physiol Renal Physiol* 2012, 303:F64-74
8. Malou Friederich Persson, Shakil Aslam, William J. Welch, Lina Nordquist, Christopher S. Wilcox and Fredrik Palm. Acute knockdown of uncoupling protein-2 increases mitochondria uncoupling via the adenine nucleotide transporter and decreases oxidative stress in the diabetic kidney. *PLoS ONE* 2012, 7(7):e39635
9. Per Eckerbom, Peter Hansell, Tomas Bjerner, Fredrik Palm, Jan Weis and Per Liss. Intravoxel incoherent motion MR imaging of the kidney: Pilot study. *Adv Exp Med Biol* 2013, 765:55-58
10. Lina Nordquist, Per Liss, Angelica Fasching, Peter Hansell and Fredrik Palm. Hypoxia in the diabetic kidney is independent of advanced glycation end-products. *Adv Exp Med Biol* 2013, 765: 185-193
11. Malou Friederich Persson, William J. Welch, Christopher S. Wilcox and Fredrik Palm. Kidney function after in vivo gene silencing of Uncoupling Protein-2 in streptozotocin-induced diabetic rats. *Adv Exp Med Biol* 2013, 765:217-223
12. Patrik Persson, Peter Hansell and Fredrik Palm. Adenosine A2 receptor-mediated regulation of renal hemodynamics and glomerular filtration rate is abolished in diabetes. *Adv Exp Med Biol* 2013, 765:225-230

13. Christopher S. Wilcox, Fredrik Palm and William J. Welch. Renal oxygenation and function of the rat kidney: effects of inspired oxygen and preglomerular oxygen shunting. *Adv Exp Med Biol* 2013, 765:329-334
14. Daniela Patinha, Angelica Fasching, Dora Pinho, António Albino-Teixeira, Manuela Morato and Fredrik Palm. Angiotensin II contributes to glomerular hyperfiltration in diabetic rats independent of adenosine type 1 receptors. *Am J Physiol Renal Physiol* 2013, 304:F614-622.
15. Malou Friederich-Persson, Patrik Persson, Angelica Fasching, Peter Hansell, Lina Nordquist and Fredrik Palm. Increased kidney metabolism as a pathway to kidney tissue hypoxia: effects of triiodothyronine and dinitrophenol in normoglycemic rats. *Adv Exp Med Biol* 2013, 789:9-14.
16. Frank Helle, Trude Skogstrand, Idit F. Schwartz, Doron Schwartz, Bjarne M. Iversen, Fredrik Palm and Michael Hultström. Nitric oxide in afferent arterioles after uninephrectomy depends on extracellular L-arginine. *Am J Physiol Renal Physiol* 2013, 304:F1088-1098.
17. Peter Stenvinkel, Ole Frøbert, Björn Anderstam, Fredrik Palm, Monica Eriksson, Ann-Christin Bragfors-Helin, Abdel Rashid Qureshi, Tobias Larsson, Andrea Friebe, Andreas Zedrosser, Johan Josefsson, My Svensson, Berolla Sahdo, Lise Bankir and Richard J Johnson. Metabolic changes in summer active and anuric hibernating free-ranging Brown bears (*Ursus arctos*). *PLoS One* 2013, 8 (9):e72934.
18. Sara Stridh, Fredrik Palm and Peter Hansell. Inhibition of hyaluronan synthesis reduces renal ability to excrete fluid and electrolytes during acute hydration. *Upsala J Med Sci* 2013, 118:217-221.
19. Malou Friederich-Persson, Erik Thörn, Peter Hansell, Masaomi Nangaku, Max Levin and Fredrik Palm. Kidney hypoxia, attributable to increased oxygen consumption, induces nephropathy independently of hyperglycemia and oxidative stress. *Hypertension* 2013, 62(5):914-919.
20. Johan Sällström, Terese Engström, Bertil B. Fredholm, A. Erik G. Persson and Fredrik Palm. Inhibition of sodium-linked glucose reabsorption normalizes diabetes-induced glomerular hyperfiltration in conscious adenosine A1-receptor deficient mice. *Acta Physiol*, 2014, 210:440-445.
21. Christoffer Laustsen, Sara Lycke, Fredrik Palm, Jakob Appel Østergaard, Bo Martin Bibby, Rikke Nørregaard, Allan Flyvbjerg, Michael Pedersen, Jan Henrik Ardenkjær-Larsen. High altitude may alter oxygen availability and renal metabolism in diabetics as measured by hyperpolarized [1-13C]pyruvate magnetic resonance imaging. *Kidney Int*, available online.
22. Stephanie Franzén, Liselotte Pihl, Nadeem Khan, Fredrik Palm and Håkan Gustavsson. Repetitive measurements of intrarenal oxygenation in vivo using L band Electron Paramagnetic Resonance. *Adv Exp Med Biol*, accepted.
23. Malou Friederich-Persson, William J. Welch, Zaiming, Luo, Fredrik Palm and Lina Nordquist. Angiotensin II reduces transport-dependent oxygen consumption, but increases transport-independent oxygen consumption in immortalized mouse proximal tubular cells. *Adv Exp Med Biol*, accepted.
24. Stephanie Franzén, Masaomi Nangaku, Peter Hansell and Fredrik Palm. Differences in susceptibility to develop parameters of diabetic nephropathy among four mouse models of type 1 diabetes. *Am J Physiol – Renal Physiol*, accepted.
25. Patrik Persson, Angelica Fasching, Tom Teerlink, Peter Hansell and Fredrik Palm. L-citrulline, but not L-arginine, prevents diabetes-induced glomerular hyperfiltration and proteinuria in rats. *Hypertension*, accepted.

Reviews 2011-

1. Fredrik Palm, Lina Nordquist, Christopher S. Wilcox and Peter Hansell. Oxidative stress and hypoxia in the pathogenesis of diabetic nephropathy. Chapter 26 (pp. 559-586) in the textbook "Studies on Renal Disorders", editors Toshio Miyata, Kai-Uwe Eckardt and Masaomi Nangaku. 1st ed, 2011, ISBN 978-1-60761-856-0.
2. Fredrik Palm and Lina Nordquist. Renal Tubulointerstitial hypoxia: Cause and consequence of kidney dysfunction. Clin Exp Pharmacol Physiol 2011, 38:424-430
3. Fredrik Palm and Lina Nordquist. Renal oxidative stress, oxygenation and hypertension. Am J Physiol - Regul Integr Comp 2011, 301:R1229-1241
4. Lina Nordquist, Åsa Kallas, Sara Stridh, Fredrik Palm and John Wahren. Renoprotective effects of C-peptide on type 1 diabetes. Chapter 7 (pp. 67-78) in the textbook "Diabetes and C-peptide", ed Anders A.F. Sima. 2011, ISBN 978-1-61779-390-5
5. Sara Stridh, Fredrik Palm and Peter Hansell. Structural and functional aspects of hyaluronan in the kidney during normal and pathophysiological conditions. Am J Physiol Regul Integr Comp 2012, 302(11):R1235-1249. Editor's pick. Illustration selected for front cover.
6. Peter Hansell, William J. Welch, Roland C. Blantz and Fredrik Palm. Determinants of kidney oxygen consumption and its relation to tissue oxygen tension in diabetes and hypertension. Clin Exp Pharmacol Physiol 2013, 40(2):123-137
7. Costas Tsioufis, Iraklis Tatsis, Costas Thomopoulos, Christopher S. Wilcox, Fredrik Palm, Athanasios Kordalis, Niki Katsiki, Vasilios Papademetriou and Christodoulos Stefanadis. Effects of hypertension, diabetes mellitus, obesity and other factors on kidney hemodynamics. Curr Vasc Pharmacol, 2012 available online.
8. Fredrik Palm and Lina Nordquist. Diabetesnefropati. Chapter 8 in textbook "Njurmedicin", 3rd edition. Eds Aurell and Samuelsson. 2014. Liber, ISBN .

Dissertations

- Malou Friederich-Persson "The Role of Mitochondrial Uncoupling in the Development of Diabetic Nephropathy" PhD March 2012.
- 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.

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 fibrillar aggregate where beta strands of protein monomers are assembled perpendicularly to the fibrillar 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 A β protein. A β 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. Further studies are conducted in collaboration with Irina Alafuzoff (IGP) and Martin Ingelsson (pubcare).

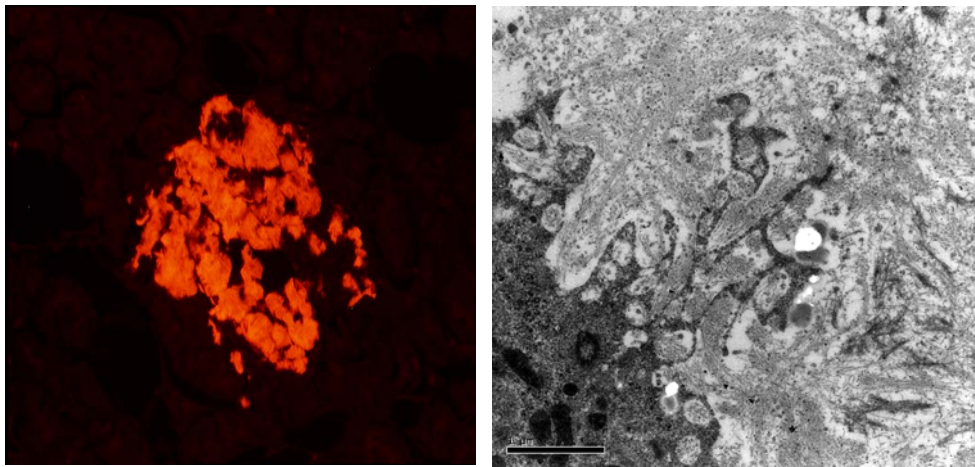
We have established a new model in *Drosophila melanogaster* for studies of proIAPP/IAPP amyloid formation. In transgenic flies expression of human proIAPP or IAPP amyloid is detected already in 20 days old flies. As expected, amyloid does not develop in control flies expressing non-amyloid-forming mouse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



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 A β -transgenic mice, human brain tissue and A β 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 fibrillar structures can prime for AA-amyloidosis. This finding is interesting and points to a possible environmental component in the pathogenesis of the disease. To reduce the numbers of mice used for our transmission studies we explore the possibility to establish a model for AA amyloidosis in *C. elegans*. This work is ongoing and we have now transgenic worms that express human protein AA, and in these develops amyloid. Feeding worms on OP50 bacteria mixed with amyloid fibrils leads to disturbance in mobility and is indicative for transmission.



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

Members of the group

Gunilla T Westermark, PI

Sara Bohman, Post Doc

Camilla Krappe, Post Doc

Marie Oskarsson, PhD Student

Gu Xiaohong, PhD student

Ye Wang, Ph.D.

Renman Wail, Master Student

Marianne Ljungkvist, Laboratory engineer

Jan Sara, Laboratory engineer

Agencies that support the work

The Swedish Research Council

The Swedish Diabetes Association

Family Ernfors Foundation

Publications 2011-

1. Westermark GT, Westermark P. Localized amyloids important in diseases outside the brain--lessons from the islets of Langerhans and the thoracic aorta. *FEBS J.* 2011;278:3918-3929 Review
2. Westermark P, Andersson A, Westermark GT. Islet amyloid polypeptide, islet amyloid, and diabetes mellitus. *Physiol Rev.* 2011;91:795-826 Review
3. Schultz SW, Nilsson KP, Westermark GT. *Drosophila melanogaster* as a model system for studies of islet amyloid polypeptide aggregation. *PLoS One.* 2011;6(6):e20221
5. Jurgens CA, Toukatly MN, Fligner CL, Udayasankar J, Subramanian SL, Zraika S, Aston-Mourney K, Carr DB, Westermark P, Westermark GT, Kahn SE, Hull RL. β -cell

- loss and β -cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol.* 2011;178:2632-2640
6. Lord A, Philipson O, Klingstedt T, Westermark G, Hammarström P, Nilsson KP, Nilsson LN. Observations in APP bitransgenic mice suggest that diffuse and compact plaques form via independent processes in Alzheimer's disease. *Am J Pathol.* 2011;178:2286-2298
 7. Kieninger B, Gioeva Z, Krüger S, Westermark GT, Friedrich RP, Fändrich M, Röcken C. PTAA and B10: new approaches to amyloid detection in tissue-evaluation of amyloid detection in tissue with a conjugated polyelectrolyte and a fibril-specific antibody fragment. *Amyloid.* 2011;18:47-52
 8. Nyström SN, Westermark GT. AA-Amyloid is cleared by endogenous immunological mechanisms. *Amyloid*, 19:138, 2012
 9. Bohman S, Westermark GT. Extensive amyloid formation in transplanted microencapsulated mouse and human islets. *Amyloid*, 19:87, 2012
 10. Sjölander J, Westermark GT, Renström E, Blom AM. Islet amyloid polypeptide triggers limited complement activation and binds complement inhibitor C4b-binding protein, which enhances fibril formation. *J Biol Chem.*, 287:10824, 2012
 11. Dahlqvist J, Westermark GT, Vahlquist A, Dahl N. Ichthyin/NIPAL4 localizes to keratins and desmosomes in epidermis and Ichthyin mutations affect epidermal lipid metabolism. *Arch Dermatol Res.*, 304:377, 2012
 12. Westermark GT, Davalli AM, Secchi A, Folli F, Kin T, Toso C, Shapiro AM, Korsgren O, Tufveson G, Andersson A, Westermark P. Further evidence for amyloid deposition in clinical pancreatic islet grafts. *Transplantation*, 93:219, 2012
 13. Rönnbäck A, Sagelius H, Bergstedt KD, Näslund J, Westermark GT, Winblad B, Graff C. Amyloid neuropathology in the single Arctic APP transgenic model affects interconnected brain regions. *Neurobiol Aging*, 33:831.e11, 2012
 14. 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.* 2013 Nov 13;8(11):e79104.
 15. Westermark GT, Westermark P. Islet amyloid polypeptide and diabetes. *Curr Protein Pept Sci.*, 14:330, 2013
 16. 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, 2013

Dissertations 2013

- Gustaf Christoffesson:** Leucocytes in angiogenesis. Learning from transplanted pancreatic islets.
- Karin Gustafsson:** Consequences of *Shb* deficiency on hematopoietic cell function.
- Patrik Persson:** Aspects of regulation of GFR and tubular function in the diabetic kidney. Roles of adenosine, nitric oxide and oxidative stress.
- Sara Stridh:** Regulation of hyaluronan i water handling. Studies *in vivo* and *in vitro*.
- Geng Tian:** On the generation of cAMP oscillations and regulation of the Ca^{2+} store-operated pathway in pancreatic islet α - and β -cells.
- Anne Wuttke:** Lipid signalling dynamics in insulin-secreting β -cells.

Licentiate theses 2013

- Nikhil Gandasi:** Quantitative analysis of proteins involved in insulin granule docking and exocytosis
- Xiang Gao:** Adenosine influences vascular reactivity in the afferent arteriole to the glomerulus and the pancreatic islet

Economy

(kSEK)

	2012	2013
Undergraduate Education appropriations	30 780	35 103
Faculty appropriations	23 738	20 665
External Grants	22 944	35 878
Contract research	595	149
Total	78 057	91 795

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.

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 biological 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 program 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.

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

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

Basic medical physiology

Summer research school

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 studies in parallel with a graduate research project. After finishing medical studies the MD/PhD students 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

BMC Electron Microscopy Unit

Since the Biomedical centre (BMC) was founded in 1968, a single organization has been responsible for the administration and service of the facilities electron microscopes. This organization, BMC - EM, is currently the responsibility of the Department of Medical Cell

Biology, but other researchers take part in its activities. Any microscopist in Uppsala can utilize the equipment. All equipment is connected to our computer central and to Internet.

For information about the various electron microscopes available at the BMC, and some practical details concerning the microscopic work, please visit our web site. We hope that this information will make you aware of the resources for electron microscopy that are available at BMC and encourage you to exploit these resources in your own research. In addition, qualified and experienced staff is available to help you with any problems connected to specimen preparation and imaging. BMC - EM welcomes you at the electron microscopy centre.

Responsible scientist: Professor Gunilla Westermark, 018 471 4169

For technical information and booking, please contact

Marianne Ljungkvist, Technician

Marianne.Ljungkvist@mcb.uu.se 018 471 4967

Jan Saras, research engineer

Jan.Saras@mcb.uu.se

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 Ca²⁺, 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 Ca²⁺, IP₃, DAG, PIP₂, PIP₃ 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 microscope 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 isolate 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, Joey Lau, 018 471 4397).

Gel imaging

The department has 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) technique.

Other equipment

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

Flow cytometry and cell sorting (BD FACS Calibur, 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 absorbance (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)

Prizes and awards 2013

Gustaf Christoffersson: Rolf Luft-stipendiat

Gustaf Christoffersson: Diabetesfondens jubileumsstipendium

Michael Hultström: Svenska Sällskapet för Medicinsk Forsknings stora anslag

E-mail address list

Department of Medical Cell Biology

www.mcb.uu.se

Address: Uppsala University, Biomedical Center, Box 571, 751 23 Uppsala, Sweden

Office: Fax +46 18 471 4059, Phone +46 18 471 4328, +46 18 471 4431

AHL DAVID	David.Ahl@mcb.uu.se
AHOOGALANDARI PARVIN	Parvin.Ahoogalandari@mcb.uu.se
ALUTHGEDARA WARUNIKA	Warunika.Aluthgedara@mcb.uu.se
ANDERSSON ARNE	Arne.Andersson@mcb.uu.se
ANVARI EBRAHIM	Ebrahim.Anvari@mcb.uu.se
ARBRANDT GUSTAV	Gustav.Arbrandt@mcb.uu.se
ASHRAFZADEH PARHAM	Parham.Ashrafzadeh@mcb.uu.se
BARBU ANDREEA	Andreea.Barbu@mcb.uu.se
BARG SEBASTIAN	Sebastian.Barg@mcb.uu.se
BERGSTEN PETER	Peter.Bergsten@mcb.uu.se
BLIXT MARTIN	Martin.Blixt@mcb.uu.se
BOHMAN SARA	Sara.Bohman@mcb.uu.se
BORG HÅKAN	Hakan.Borg@mcb.uu.se
CARLSSON PER-OLA	Per-Ola.Carlsson@mcb.uu.se
CHOWDHURY AZAZUL ISLAM	Azazul.Chowdhury@mcb.uu.se
CHRISTOFFERSSON GUSTAF	Gustaf.Christoffersson@mcb.uu.se
DROTT CARL JOHAN	Carl.Drott.5483@student.uu.se
DYACHOK OLEG	Oleg.Dyachok@mcb.uu.se
EJDESJÖ ANDREAS	Andreas.Ejdesjo@mcb.uu.se
ERIKSSON ULF	Ulf.Eriksson@mcb.uu.se
ESPE DANIEL	Daniel.Espes@mcb.uu.se
FASCHING ANGELICA	Angelica.Fasching@mcb.uu.se
FRANZÉN PETRA	Petra.Franzen@mcb.uu.se
FRED RIKARD	Rikard.Fred@mcb.uu.se
FRIEDERICH PERSSON MALOU	Malou.Friederich@mcb.uu.se

GANDASI NIKHIL	Nikhil.Gandasi@mcb.uu.se
GAO XIANG	Gao.Xiang@mcb.uu.se
GIRAUD ANTOINE	Antoine.Giraud@mcb.uu.se
GRAPENGIESSER EVA	Eva.Grapengiesser@mcb.uu.se
GRAPENSPARR LIZA	Liza.Grapensparr@mcb.uu.se
GU XIAOHONG	Xiaohong.Gu@mcb.uu.se
GUSTAFSSON KARIN	Karin.Gustafsson@mcb.uu.se
GYLFE ERIK	Erik.Gylfe@mcb.uu.se
HANSELL PETER	Peter.Hansell@mcb.uu.se
HELLMAN BO	Bo.Hellman@mcb.uu.se
HJORTBERG MATS	Mats.Hjortberg@mcb.uu.se
HOLM LENA	Lena.Holm@mcb.uu.se
HULTSTRÖM MICHAEL	Michael.Hultstrom@mcb.uu.se
IDEVALL-HAGREN OLOF	Olof.Idevall@mcb.uu.se
JAKOBSSON IDA	Ida.Jakobsson@mcb.uu.se
JANSSON LEIF	Leif.Jansson@mcb.uu.se
JÄGARE ANNIKA	Annika.Jagare@mcb.uu.se
KAY EMMA	Emma.Kay@mcb.uu.se
KOROL TETYANA	
KRISTINSSON HJALTI	Hjalti.Kristinsson@mcb.uu.se
KULLMAN LISEN	Lisen.Kullman@mcb.uu.se
KÄLLSKOG ÖRJAN	Orjan.Kallskog@mcb.uu.se
LAU JOEY	Joey.Lau@mcb.uu.se
LI JIA	Jia.li@mcb.uu.se
LI ZHANCHUN	Zhanchun.Li@mcb.uu.se
LILJEBÄCK HANNA	Hanna.Liljeback@mcb.uu.se
LIU CHENXIAO	Chenxiao.Liu@mcb.uu.se
LIU HAoyu	Haoyu.Liu@mcb.uu.se
LJUNGKVIST MARIANNE	Marianne.Ljungkvist@mcb.uu.se
LOMEI JALAL	Jalal.Lomei@mcb.uu.se
MANUKYAN LEVON	Levon.Manukyan@mcb.uu.se
MASENA SARA	Sara.Masena@mcb.uu.se
NIKLASSON BO	Bo.Niklasson@mcb.uu.se
NIKPOUR MARYAM	Maryam.Nikpour@mcb.uu.se
NILSSON OVE	Ove.Nilsson@mcb.uu.se
NORDQUIST LINA	Lina.Nordquist@mcb.uu.se
OHLSSON HANNES	Hannes.Ohlsson@mcb.uu.se
OSKARSSON MARIE	Marie.Oskarsson@mcb.uu.se
PALM FREDRIK	Fredrik.Palm@mcb.uu.se
PAN SHUMIN	Shumin.Pan@mcb.uu.se
PARMRYD INGELA	Ingela.Parmryd@mcb.uu.se
PERSSON ERIK	Erik.Persson@mcb.uu.se
PERSSON PATRIK	Patrik.Persson@mcb.uu.se
PETTERSSON ULRIKA	Ulrika.Pettersson@mcb.uu.se
PHILLIPSON MIA	Mia.Phillipson@mcb.uu.se

QUACH MY	My.Quach@mcb.uu.se
RYDGREN TOBIAS	Tobias.Rydgren@mcb.uu.se
SAGULIN LISBETH	Lisbeth.Sagulin@mcb.uu.se
SANDBERG MONICA	Monica.Sandberg@mcb.uu.se
SANDIN ERIK	Erik.Sandin@mcb.uu.se
SANDLER STELLAN	Stellan.Sandler@mcb.uu.se
SARAS JAN	Jan.Saras@mcb.uu.se
SARGSYAN ERNEST	Ernest.Sargsyan@mcb.uu.se
SEDIN JOHN	John.Sedin@mcb.uu.se
SEIGNEZ CEDRIK	Cedric.Seigneur@mcb.uu.se
SHUAI HONGYAN	Hongyan.Shuai@mcb.uu.se
SING KAILASH	Kailash.Singh@mcb.uu.se
SIVERTSSON EBBA	Ebba.Sivertsson@mcb.uu.se
STAAF JOHAN	Johan.Staaf.4137@student.uu.se
STRIDH SARA	Sara.Stridh@mcb.uu.se
STÅHL GÖRAN	Goran.Stahl@mcb.uu.se
SÄLLSTRÖM JOHAN	Johan.Sallstrom@mcb.uu.se
SÄVMARKER CAMILLA	Camilla.Savmarker@mcb.uu.se
SÖDERLING MAGNUS	Magnus.Soderling@mcb.uu.se
TENGHOLM ANDERS	Anders.Tengholm@mcb.uu.se
THORVALDSON LINA	Lina.Thorvaldson@mcb.uu.se
TIAN GENG	Geng.Tian@mcb.uu.se
ULLSTEN SARA	Sara.Ullsten@mcb.uu.se
VASYLOVSKA SVITLANA	Svitlana.Vasylovska@mcb.uu.se
VÅGESJÖ EVELINA	Evelina.Vagesjo@mcb.uu.se
WALDÉN TOMAS	Tomas.Walden@mcb.uu.se
WALL JACOB	
WANG XUAN	Xuan.Wang@mcb.uu.se
WELSH MICHAEL	Michael.Welsh@mcb.uu.se
WELSH NILS	Nils.Welsh@mcb.uu.se
WENTZEL PARRI	Parri.Wentzel@mcb.uu.se
WESTERMARK GUNILLA	Gunilla.Westermark@mcb.uu.se
WESTMAN JAN	Jan.Westman@mcb.uu.se
WOLGAST MATS	Mats.Wolgast@mcb.uu.se
WUTTKE ANNE	Anne.Wuttke@mcb.uu.se
XU YUNJIAN	Yunjian.Xu@mcb.uu.se
YIN PENG	Peng.Yin@mcb.uu.se
YU QIAN	Qian.Yu@mcb.uu.se
ZHAO LIJUN	Lijun.Zhao@mcb.uu.se
ZHUGE ZHENG BING	Zhengbing.Zhuge@mcb.uu.se
WANG YE	Ye.Wang@mcb.uu.se
ÅKERBLOM BJÖRN	Bjorn.Akerblom@mcb.uu.se
ÖSTER ELIN	