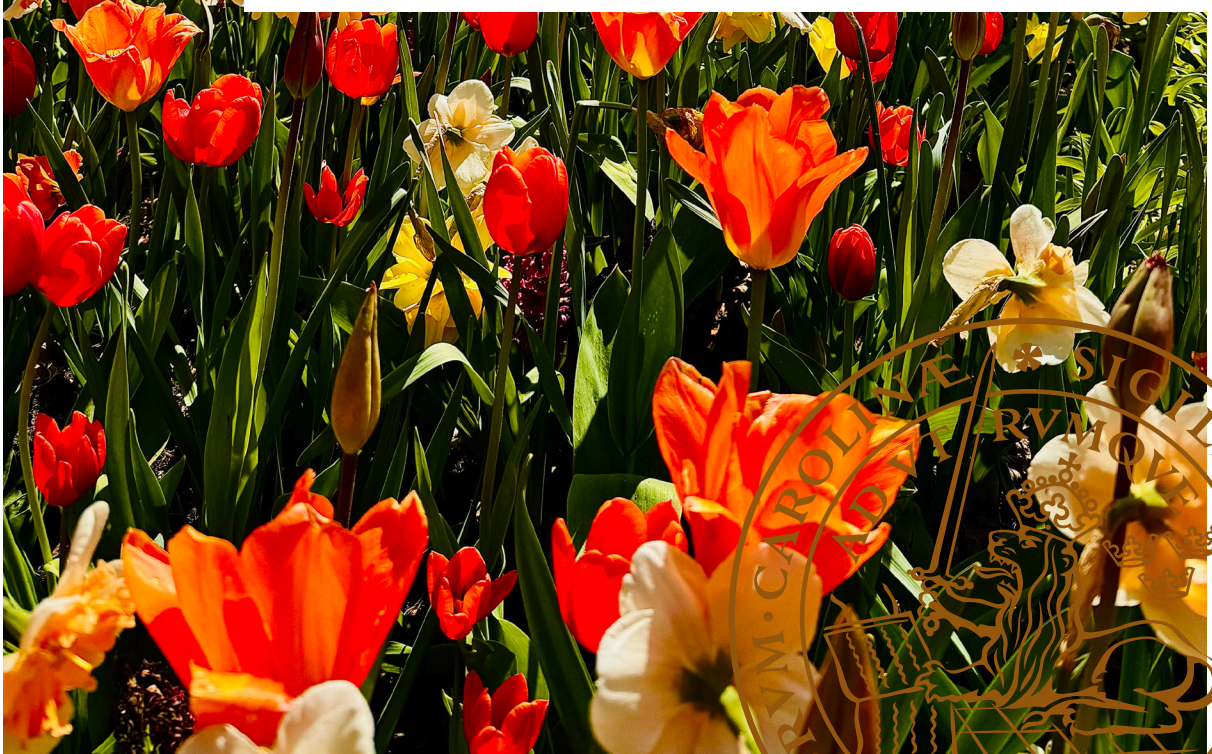
European Islet Study Group 2025

JUNE 11-13, 2025 I HOTEL SCANDIC TRIANGELN I MALMÖ, SWEDEN
LUND UNIVERSITY DIABETES CENTRE



European Islet Study Group 2025

Conference Program

This is the version of the booklet for print use.
Full interactive booklet can be found at:
`http://eisg2025.se/`

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Contents

About

About EISG 2025

The European Islet Study Group is a conference for Islet biology in diabetes research. Organized annually since 1991, EISG gathers the leading European and international islet researchers in the field of diabetes.

The EISG conference is hosted by the Lund University Diabetes Centre and EXODIAB, and takes place in Malmö, Sweden on June 11-13, 2025.

The objective of EISG is to facilitate the exchange and development of ideas that could advance ground-breaking research related to diabetes diagnosis, pathology, and treatment, and the role that islet cells play in the disease.

The European Islet Study Group

The European Islet Study Group arose from discussions of participants of the islet meeting held at Coleraine 15-18 September 1991. Since this time, the Group has held meetings annually in close association with the main EASD Congress. In February 2022, the group became independent from the EASD but with no change to research interests and group members.

EISG 2025 Organizing committee

Charlotte Ling	Lena Eliasson	Hindrik Mulder
Nils Wierup	Philipp Kaldis	Malin Fex
Isabella Artner	Ulrika Blom-Nilsson	Luis Serratos

EISG Steering committee

Guy Rutter	*Chair*
Raphael Scharfmann	*Treasurer*
Anna L. Gloyn	*Member*
Heiko Lickert	*Member*
Charlotte Ling	*Member*

About the hosts

Lund University Diabetes Centre (LUDC) is a vibrant research environment at Lund University with the long-term goal of preventing and curing diabetes, as well as improving the treatment of the disease and its complications. treatment of the disease and its complications. At LUDC, over 300 people conduct cutting-edge diabetes research spanning basic, molecular and genomic mechanisms to studies of people's lifestyles, diets and genetic predisposition of disease, as well as carrying out clinical trials.

LUDC conducts advanced research in islet biology, addressing fundamental aspects of both type 1 and type 2 diabetes. This includes in-depth studies of islet cell exocytosis and physiology to understand the precise cellular mechanisms that regulate hormone secretion. Metabolic pathways within islet cells and autoimmune responses are examined as are the genomic and epigenetic landscapes of islet cells, allowing identification of genetic determinants and molecular signatures underpinning diabetes. These cutting-edge studies aim to unravel complex biological processes, paving the way for novel therapeutic strategies and a potential cure for diabetes. For more information, visit our website at: `www.ludc.lu.se`

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EXODIAB: Excellence of Diabetes Research in Sweden, is a national Strategic Research Area and collaborative platform between Lund University and Uppsala University. Its overarching aim is to reduce the burden of diabetes through world-leading research that integrates experimental science with clinical innovation and population-based studies. EXODIAB brings together a multidisciplinary network of researchers focused on understanding the causes and progression of diabetes and developing more effective methods for diagnosis, prevention, and treatment.

Research within EXODIAB spans the full translational spectrum, from detailed investigations into beta cell function, insulin resistance, and immune system dysregulation, to large-scale genomic and biomarker studies in human populations. The collaboration facilitates access to extensive biobanks and patient cohorts, enabling precision medicine approaches and accelerating the translation of basic findings into clinical practice. By combining technological expertise with strong clinical foundations, EXODIAB fosters innovation and drives impactful advances in diabetes care.

LUND UNIVERSITY

LUDC DIABETES CENTRE

exodiab
Excellence of Diabetes Research in Sweden

Useful Information

Information about the conference

Sessions will be held in the **Ballroom** of Hotel Scandic Triangeln. It is situated on the third floor of the hotel and can be accessed via elevators.

Coffee breaks and lunches will be offered in the breakout hall area surrounding the Ballroom.

The **poster session** will be held on Wednesday and Thursday on the breakout hall area surrounding the Ballroom.

Complimentary **Wi-Fi** will be available during the conference.

The **conference dinner** for Wednesday will be held at the Restaurant "*Mötesplats CRC*" located in the premises of the Clinical Research Centre, Lund University. The address is Jan Waldenströms gata 35, and it's around 900 m walking distance from the conference venue.

On Wednesday, prior to the dinner, our hosts (Lund University Diabetes Centre and EXODIAB) warmly welcome you to a **pre-dinner reception** on their premises on floor 12 (3rd floor up from ground level), building 91, of the Clinical Research Centre (Jan Waldenströms gata 35, Malmö).

On Thursday, our **dinner and social program** will take place at the venue "Luftkastellet" which offers amazing views over the Öresund Bay and of the iconic Öresund Bridge connecting Denmark and Sweden. Bus transportation will be provided to and from the venue. Please refer to the timetable for details.

How to get to the venue: Hotel Scandic Triangeln

Scandic Triangeln is one of Malmö's most iconic hotels, centrally located along the city's vibrant Triangeln area. The hotel is **next to station *Malmö Triangeln***, that serves both trains and buses. If you're coming from Copenhagen Airport Kastrup, just take the Öresund Train (*Öresundståg*) to Sweden. The **Malmö Triangeln** train station is the second stop you'll make in Sweden ($\approx$ 22 minutes trip).

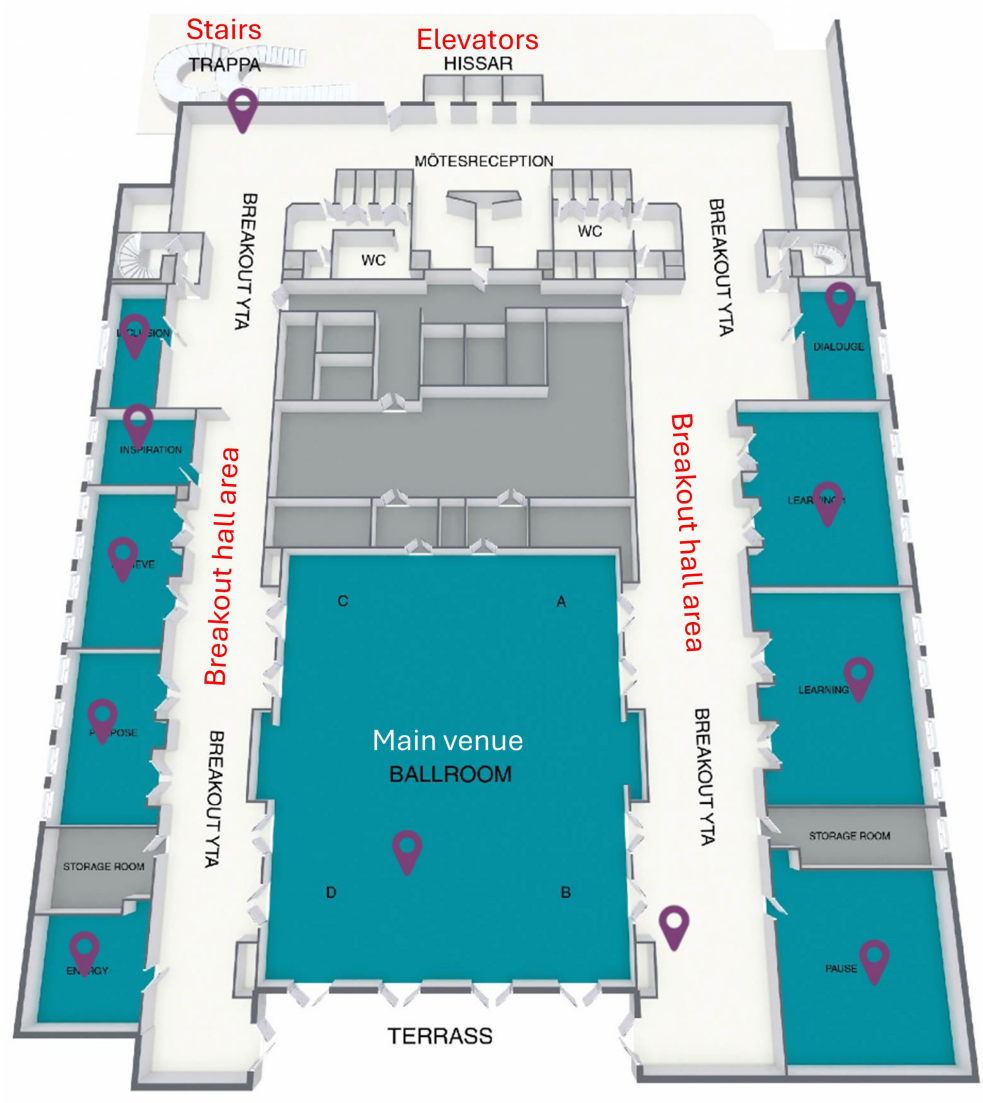
If you're coming **from the centre of Malmö**, green bus lines 2, 7, and 8 transit directly from *Malmö C* to *Malmö Triangeln*.

The easiest way to use public transportation is by downloading the Skånetrafiken app, available on both the App Store and Play Store.

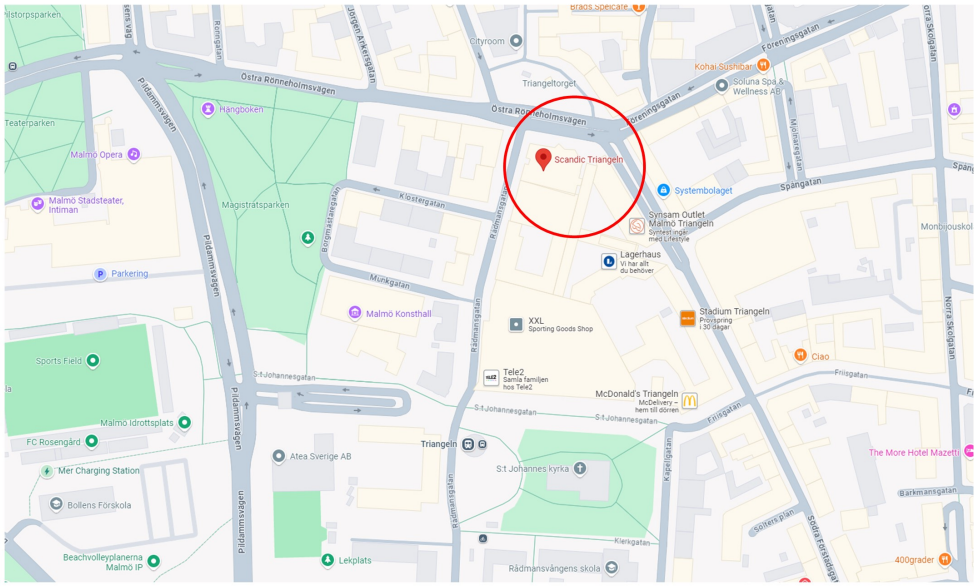
However, green city buses offer the possibility to buy a ticket omboard via contactless payment. Please note that **trains do not sell tickets on board** and these need to be acquired in the app or at machines at the airport prior to boarding. You can consult more info on rules and timetables on Skånetrafiken's website: `www.skanetrafiken.se`.

Maps of interest

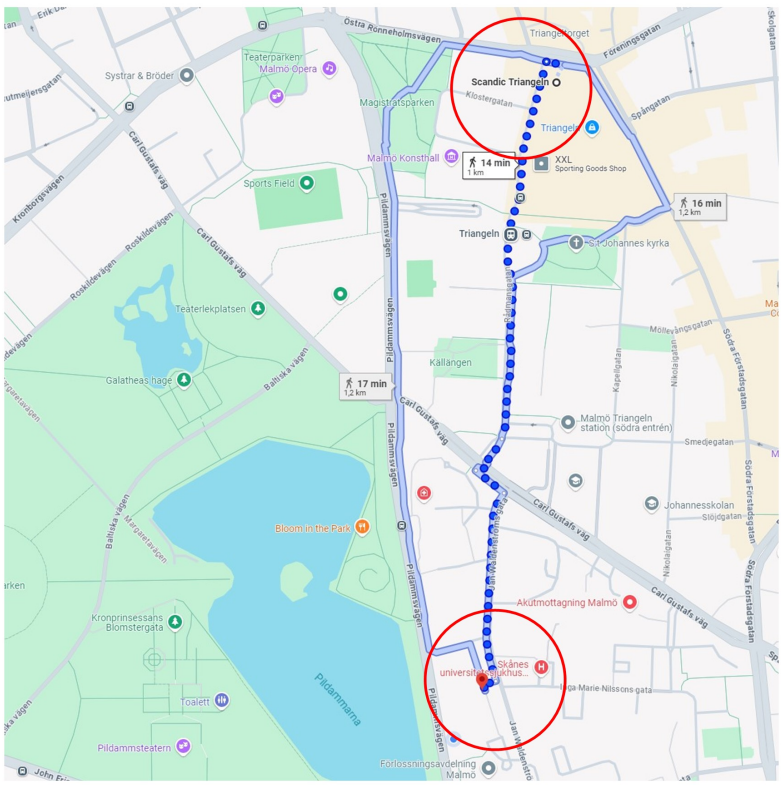
Layout of the conference venue: Hotel Scandic Triangeln 3rd floor



Conference Venue (**Hotel Scandic Triangeln**) and surrounding areas



From the conference venue to dinner venue Wednesday (**Clinical Research Centre, Lund University**)



Timetable

KS: Keynote Speaker IS: Invited Speaker CT: Contributed Talk ST: Sponsored Talk

Wednesday, 11 of June, 2025

11:00–11:50	**Registration**		
11:50–12:00	**Opening words & welcome remarks**		
	Session 1: Genomics of islet cells Chair: James Johnson University of British Columbia		
12:00–12:30	IS	**Amélie Bonnefond** University of Lille	Functional genetics of rare diabetes-associated variants illuminates islet pathophysiology
12:30–13:00	IS	**Rohit Kulkarni** Harvard University	The role of Epitranscriptomics in regulating metabolism
13:00–13:30	IS	**Torben Hansen** University of Copenhagen	Uncovering Genetic and Molecular Drivers of Insulin Secretion and Diabetes: Insights from Arctic Populations to Early Childhood
13:30–13:45	CT	**Sara Bsharat** Lund University	Identification of novel MAFB target genes in endocrine progenitor cells
13:45–14:00	CT	**Benoit Hastoy** University of Oxford	Artificial Intelligence (AI) identified Type 2 Diabetes targets Semaphorin 3E (SEMA3E) and Neuropilin-1 (NRP1) alter pancreatic islet secretory function
14:00–14:30	**Coffee**		
	Session 2: Metabolism in islet cells Chair: Guy Rutter University of Montreal		
14:30–15:00	IS	**David Hodson** University of Oxford	The belt and braces of glucose metabolism in pancreatic β cells
15:00–15:30	IS	**Jakob Grunnet Knudsen** University of Copenhagen	Glucose and Glucagon – is it all about metabolism?
15:30–16:00	IS	**Hindrik Mulder** Lund University	Acyl-CoA synthetase (*ACSL1*) is key regulator of insulin secretion and a pathogenetic factor in Type 2 Diabetes
16:00–16:15	CT	**Emil Z. Skovhøj** University of Copenhagen	Diabetes risk gene *KCNQ1* modulates pancreatic beta-cell sensitivity to age and metabolic stress
16:15–16:30	CT	**Matthew Lloyd** University of Oxford	Chronic glucokinase activation impairs pancreatic beta cell function under euglycaemic conditions
16:30–16:40	ST	**Hilda Ahnstedt**	Breakthrough T1D
16:40–16:55	**Coffee**		
16:55–17:50	**Poster session 1** Chair: Gustavo Santos Federal University of Santa Catarina		

17:50–18:45	**Poster session 2** Chair: Rashmi Prasad Lund University
18:45–19:00	**Walk to dinner venue**
19:00-19:30	**Pre-dinner reception** *Venue:* Clinical Research Centre, Building 91, Floor 12 **Address**: Jan Waldenströms gata 35, Malmö ≈ 900 m walking distance from Conference hotel
19:30-22:00	**Dinner** *Dinner venue:* Restaurant Mötesplats CRC Clinical Research Centre, Ground Floor Same address as pre-dinner reception

Thursday, 12 of June, 2025

09:00–09:45	KS	**Prof. Lori Sussel** University of Colorado **Chair: Charlotte Ling** Lund University	Unraveling the Regulation of Islet Cell Fates
09:45–10:00		**Coffee**	
		Session 3: The islet cell in Type-2 Diabetes and treatment strategies Chair: Malin Fex Lund University	
10:00–10:30	IS	**Mariana Igoillo-Esteve** Université Libre de Bruxelles	Exploring the impact of altered tRNA fragmentation in pancreatic β-cell
10:30–11:00	IS	**Ulf Ahlgren** Umeå University	Illuminating the pancreas – a new view on islet cellularity and β-cell distribution in health and disease
11:00–11:30	IS	**Lena Eliasson** Lund University	Influence of microRNAs on human islet insulin secretion in type 2 diabetes
11:30–11:45	CT	**Marie Gasser** University of Montreal	Roles of zinc depletion in the protective effects of *SLC30A8* variants in human beta-like cells
11:45–12:00	CT	**Cristina Cosentino** University of Lausanne	5'tRNA-derived fragments modulate β-cell homeostasis and islet macrophage activation in type 2 diabetes.
12:00–13:00		**Lunch**	
		Session 4: The islet cell in Type-1 Diabetes and treatment strategies Chair: Raphael Scharfmann INSERM Cochin Institute	
13:00–13:30	IS	**Carmella Evans-Molina** Indiana University	Organelle calcium gradients at the nexus of type 1 diabetes pathogenesis

13:30–14:00	IS	**Roberto Mallone** INSERM Cochin Institute	Title TBD
14:00–14:30	IS	**Teresa Rodriguez-Calvo** Helmholtz Munich	Unmasking islet profiles: the link between *HLA-I* expression, immune infiltration and insulin dynamics in type 1 diabetes
14:30–14:45	CT	**Jon Vergara Ucin** University of Copenhagen	Urocortin-3 regulates glucagon secretion through an inhibitory β- to δ- to α-cell axis
14:45–15:00	CT	**Theodore dos Santos** University of Alberta	Mechanisms underlying islet cell dysfunction in human type 1 diabetes
15:00–15:15	**Coffee**		
	Session 5: Modelling human islet cell development Chair: Isabella Artner Lund University		
15:15–15:45	IS	**Francesca Spagnoli** King's College London	Progenitor niches in the developing pancreas: regulation of beta cell fate and beyond
15:45–16:15	IS	**Diego Balboa** University of Helsinki	Modelling human islet development and function with stem cell models
16:15–16:45	IS	**Limor Landsman** Tel Aviv University	Immune and Vascular Crosstalk Modulates β Cell Development and Function
16:45–17:00	CT	**Simona Chera** University of Bergen	HNF1A is essential for GLI3 processing by primary cilia in the posterior foregut, its dysfunction favoring duodenal cell fate and altering lineage choices
17:00–17:15	CT	**Chieh Min Jamie Chu** University of British Columbia	Characterization of *INS* gene activity states in human primary and stem-cell derived β-cells
17:15–17:30	CT	**Valéria Fabríciová** Institute of Biotechnology of the Czech Academy of Sciences	Sex differences in diabetic mouse model of conditional knockout of transcriptional repressor and epigenetic modulator ISL1
17:30–17:40	ST	**Houston Barenholtz**	Biorep Technologies
17:40–18:40	**Poster session 3** Chair: Gustavo Santos Federal University of Santa Catarina		
18:45	**Bus transportation to dinner venue** Meet in hotel lobby to board buses		
19:15-22:00	**Dinner & Social Program** *Dinner venue:* Luftkastellet *Address:* Utsiktsvägen 10, Limhamn, Sweden Bus transportation from and to the Conference hotel will be provided		

Friday, 13 of June, 2025

09:00–09:45	KS	**Klaus H. Kaestner** University of Pennsylvania **Chair: Charlotte Ling** Lund University	The human islet in diabetes – lessons learned from the Human Pancreas Analysis Program (HPAP)
09:45–10:00	**Coffee**		
	Session 6: Signaling and crosstalk in the islet Chair: Nils Wierup Lund University		
10:00–10:30	IS	**Mark Huising** University of California, Davis	Dynamic crosstalk within the islet shapes insulin release
10:30–11:00	IS	**Cécile Jacovetti** University of Lausanne	The mitochondrial tRNA-derived fragment, *mt-tRF-Leu*^{TAA}, modulates both pancreatic insulin secretion and muscle insulin sensitivity
11:00–11:30	IS	**Andraž Stožer** University of Maribor	Calcium Messages Within and Between Beta Cells: Recent Contributions to their Understanding from Functional Multicellular Calcium Imaging
11:30–11:45	CT	**Marta Perez-Frances** University of Geneva	Appropriate insulin secretion and glucose homeostasis in absence of α-cells
11:45–12:00	CT	**Haiqiang Dou** University of Gothenburg	Optogenetic interrogation of metabolic and paracrine heterogeneity of pancreatic α-cells
12:00–13:00	**Lunch**		
13:00–14:00	**Debate: Are alpha cells and glucagon needed for islet function and glucose homoeostasis?** **Chair:** **Mark Huising**, University of California, Davis **For:** **Patrik Rorsman**, University of Oxford; **Caroline Bonner**, Institute Pasteur de Lille **Against:** **Sarah Richardson**, University of Exeter; **Pedro Herrera**, University of Geneva		
	Session 7: Beta & non-beta cells Chair: Philipp Kaldis Lund University		
14:00–14:30	IS	**Charna Dibner** University of Geneva	Time zones of α- and β-cells: circadian orchestration of the islet cell function
14:30–15:00	IS	**Meritxell Rovira** University of Barcelona	Shedding light on pancreas regeneration and PDAC cell of origin with single cell sequencing?
15:00–15:30	IS	**Sebastian Barg** Uppsala University	Somatostatin resistance in human α- and δ-cells
15:30–15:45	CT	**Isaline Louvet** Université de Lille	Heterozygous Hnf1a Mutation in Mice Reveals Multiple Pathogenic Mechanisms of HNF1A-MODY
15:45–16:00	CT	**Jin Feng** Helmholtz Zentrum München	eGLP1/GSK3i Enhances β-Cell Function and Proliferation in Diabetes Models
16:00–16:15	CT	**Marta Gironella-Torrent** University of Gothenburg	Single-cell quantification of β-cell secretory capacity in T2D
16:15–16:30	**Closing of EISG 2025**		

List of Abstracts – Talks

KS: Keynote Speaker IS: Invited Speaker CT: Contributed Talk

Wednesday, 11 of June, 2025

IS Functional genetics of rare diabetes-associated variants illuminates islet pathophysiology.

Amélie Bonnefond, Ph.D.

CNRS / Université de Lille 2 / Institut Pasteur de Lille, France

Type 2 diabetes (T2D), which represents the vast majority of diabetes cases globally, is a complex metabolic disorder that poses a major burden on public health and healthcare systems. While lifestyle factors such as obesity and physical inactivity are central to its pathogenesis, a strong genetic component underpins susceptibility to the disease, evident from heritability estimates exceeding 40% in familial and twin studies.

Beyond the classical dichotomy of monogenic versus polygenic diabetes, emerging research reveals a more nuanced continuum that includes monogenic, oligogenic, and polygenic factors, each playing a complementary role in T2D pathophysiology. The search for oligogenic variants is particularly informative, offering unique windows into the molecular pathways disrupted in T2D. In this talk, I will present how human functional genomics (particularly the study of rare variants) has been instrumental in elucidating T2D pathophysiology, particularly in pancreatic islets. By integrating next-generation sequencing approaches with *in vitro* and *in vivo* functional validation, we have uncovered novel disease-contributing genes and mechanisms. I will showcase several key examples of genes where rare loss- or gain-of-function coding and non-coding variants lead to altered insulin secretion, β-cell dysfunction, or dysregulation of central metabolic pathways. These discoveries do not only deepen our understanding of disease biology, but also hold translational potential, by identifying new therapeutic targets and enabling more precise, genotype-guided patient care.

IS The role of Epitranscriptomics in regulating metabolism

Rohit N. Kulkarni, M.D, Ph.D.

Joslin Diabetes Center, Harvard Medical School, United States

While changes in DNA and proteins have been topics of intense epigenetics research in metabolism for several decades the role of RNA modifications (epitranscriptome) is only recently being recognized. For example, N^6 -methyladenosine (m^6A) and pseudouridylation (ψ) are among the most abundant chemical modifications in RNA, and play important roles in mammalian stem cell pluripotency maintenance and differentiation. We have recently reported, for the first time, the role of m^6A in the postnatal control of insulinsecreting β-cell function in physiological states and to modulate key transcripts that are involved in innate immunity. mRNA modifications have also been shown to modulate brown and white fat biology that are directly relevant to the pathophysiology of metabolic diseases. This presentation will

focus on these emerging concepts on the ability of mRNA modifications to regulate metabolic tissue function and speculate on identifying targets that can be harnessed for treating metabolic disease.

IS Uncovering Genetic and Molecular Drivers of Insulin Secretion and Diabetes: Insights from Arctic Populations to Early Childhood

Torben Hansen, M.D., Ph.D.

University of Copenhagen, Denmark

In this talk, I will explore how integrative genomic and proteomic approaches are advancing our understanding of insulin secretion and diabetes susceptibility across diverse populations. Drawing on recent work at the University of Copenhagen, I will first discuss key genetic determinants of glucose-stimulated insulin secretion, shedding light on pathways that govern pancreatic β-cell function. I will then turn to the Greenlandic population, where unique genetic architecture—shaped by Arctic adaptation—has revealed high-impact variants that significantly influence diabetes risk. Finally, I will share emerging data from proteomic studies in children, highlighting early molecular signatures linked to metabolic health trajectories. By integrating genetic and protein-level insights across age groups and ancestries, this work underscores the importance of diversity in precision medicine.

CT Identification of novel MAFB target genes in endocrine progenitor cells.

Sara Bsharat, Kavya Achanta, Ludivine Bertonnier-Brouty, Isabella Artner

Endocrine Cell Differentiation and Function Group, Department of Clinical Sciences Malmö, Lund University Diabetes Centre, Sweden

Background and Aims Diabetes results from beta cell loss or dysfunction and/or insulin resistance, leading to impaired glucose regulation. Generating transplantable β-cells for diabetes therapy requires understanding the molecular mechanisms governing β-cell differentiation. *MafB*, a transcription factor expressed in differentiating α- and β-cells and a subset of *Neurog*$^{3+}$ endocrine progenitors, plays a role in islet formation. This study investigates the impact of *MafB* deficiency on endocrine cell differentiation during fetal development.
Materials and Methods We used *MafB*-deficient mouse pancreata, where GFP replaces *MafB*, to investigate its impact on endocrine differentiation *in utero*. Single-cell analysis was performed on the 10x Genomics platform and sequenced using NovaSeq.
Results Loss of *MafB* significantly altered gene expression in *Neurog*$^{3+}$ progenitors, representing early stages of endocrine differentiation. Over 200 genes involved in transcriptional regulation were impacted. *MafB*-deficient β-like cells derived from human ES cells showed reduced expression of key islet transcription factors. Additionally, we identified a novel cluster of islet transcription factors, including *AUTS2*, *ETV1*, *MEIS2*, *RUNX1T1*, and *TOX3*. Most of these factors are regulated by *MafB* and are highly expressed in human endocrine progenitor cells, highlighting their role in pancreatic development. Type 2 Diabetes-associated risk alleles were found in the *AUTS2* and *ETV1* enhancer regions, potentially altering transcription factor binding.
Conclusion Our study highlights *MafB*'s critical role in early endocrine progenitor cells and identifies novel transcription factors, including *AUTS2* and *ETV1*, which have Type 2 diabetes risk alleles in their regions. These findings provide insights into pancreatic development and diabetes susceptibility mechanisms.

CT Artificial Intelligence (AI) identified Type 2 Diabetes targets Semaphorin 3E (SEMA3E) and Neuropilin-1 (NRP1) alter pancreatic islet secretory function.

Samuel Acreman[1,2,3], ***Sameena Nawaz***[1,2,3], ***Upamanyu Ghose***[1,4], ***Jing Yu***[5], ***Hadeel Abozendah***[1], ***Tryfon Zarganis-Tzitzikas***[1,6], ***Paul Brennan***[1], ***Quan Zhang***[2,3,7], ***Edith Yvonne Jones***[5], ***Cornelia van Duijn***[1,8], ***Patrik Rorsman***[2,3,9], ***Alejo Nevado-Holgado***[1,4], ***Benoit Hastoy***[1,2,3]

[1] Centre for Artificial Intelligence in Precision Medicine, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford, United Kingdom
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[4] Department of Psychiatry, University of Oxford, United Kingdom
[5] Division of Structural Biology, Centre for Human Genetics, University of Oxford, United Kingdom
[6] ARUK - Oxford Drug Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford
[7] Center for Neuroscience and Cell Biology, Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Portugal
[8] Nuffield Department of Population Health, University of Oxford, United Kingdom
[9] Institute of Neuroscience and Physiology, Department of Physiology, University of Gothenburg, Sweden

Introduction. Identifying gene targets for Type 2 Diabetes (T2D) drug development remains a critical challenge in personalized medicine. Our AI-driven Neural Network (NN) analysis of UKBiobank revealed novel associations between T2D-risk and *SEMA3E* and *NRP1* genes. The resulting proteins interact with PLXD1 receptor encoded by *PLXND1*, a gene linked to T2D-risk by GWAS. These suggest *SEMA3E-PLXND1-NRP1* signalling axis may influence T2D pathogenesis, though its mechanisms remain unclear.
Aims. We characterise the roles of *SEMA3E* and *NRP1* on pancreatic islet secretory activity.
Methods. We tested *SEMA3E* peptide and *NRP1*-inhibitor (*NRP1*-i) on EndoC-βH1, rodent and human isolated islets using live-cell confocal calcium imaging (Ca^{2+}-spiking), hormone secretion assays and immunofluorescence.
Results. Echoing single-cell RNA sequencing data, *PLXD1* and *NRP1* are expressed in β-cells, but not *SEMA3E*. *SEMA3E* incubation inhibited significantly Glucose-Stimulated Insulin Secretion (GSIS) from EndoC-βH1 ($p < 0.0001$), mouse ($p < 0.0001$) and human ($p = 0.0001$) islets by 60%, 49%, and 23% respectively. In mouse β-cells, this effect is supported by significant reduction Ca^{2+}-spiking frequency and AUC (50%). *SEMA3E* reduced low glucose-induced glucagon secretion by 25% ($p < 0.05$) in rodent and 40% ($p < 0.001$) in human. While *NRP1*-i alone also reduced glucagon secretion by 60% ($p < 0.001$), it was without effect on GSIS in rodent and human. However, its preincubation protected islet GSIS from *SEMA3E* inhibition.
Conclusion. Although underlying mechanisms require further dissection, these data validate our NN approach and show the importance of *SEMA3E-PLXND1-NRP1* on islet function. As increased plasma *SEMA3E* levels were observed in patients with diabetes, antagonising *NRP1* activity has potential for preserving islet function in T2D.

IS The belt and braces of glucose metabolism in pancreatic beta cells

David Hodson, Ph.D., FRCVS

University of Oxford, United Kingdom

To link fluctuations in blood glucose with appropriate insulin release, pancreatic beta cells possess a number of adaptations to their glucose-sensing machinery including expression of low affinity glucose transporter and glucokinase. Glucose that enters the beta cell undergoes glycolysis to produce pyruvate, which feeds the tricarboxylic acid (TCA) cycle and oxidative phosphorylation. The ensuing increases in ATP/ADP ratio lead to KATP channel closure and insulin release, further assisted by anaplerotic flux through pyruvate carboxylase and the phosphoenolpyruvate (PEP) cycle. Alternative fates for pyruvate (i.e. lactate conversion) are suppressed by low expression of lactate dehydrogenase

A. Likewise, pyruvate management is prioritized through suppression of fatty acid oxidation. During type 2 diabetes, both these mechanisms begin to fail such that beta cells become more dependent on fatty acid as a fuel source, and relatively less pyruvate flux is available for the TCA cycle, oxidative phosphorylation and the PEP cycle due to conversion into lactate. We recently identified lactate dehydrogenase B and prolyl hydroxylase 3 as key enzymatic pathways involved in pyruvate fate/management, as well as fatty acid oxidation. The current presentation will detail the central contribution of these two enzymes to beta cell glucose-sensing and function, before discussing the changes that occur during type 2 diabetes.

IS Glucose and Glucagon – is it all about metabolism?

Jakob G Knudsen, Ph.D.

Section for cell biology and physiology, Department of Biology, University of Copenhagen, Denmark

Alpha cells secrete glucagon when circulating glucose levels are low to increase glucose production from the liver, which is an essential part of the counterregulatory response. However, alpha cells also seem to play an important role in the development of diabetes, where dysregulated glucagon secretion leads to impaired regulation of hepatic metabolism and is thought to contribute to the elevated circulating glucose levels. Despite this there is no consensus model for the regulation of glucagon secretion by glucose.
We have recently suggested that alpha cells utilise fatty acids as substrate for ATP production and that elevations in extra cellular glucose leads inhibition of fatty acid oxidation and reduced intracellular ATP. This hypothesis aligns with previous data suggesting that glucose oxidation in alpha cells is low. Interestingly, while alpha cell oxidises low amounts of glucose, glucose utilisation is the same as in beta cells. We have therefore explored the role of glucose metabolism in the regulation of glucagon secretion.

IS Acyl-CoA synthetase (*ACSL1*) is key regulator of insulin secretion and a pathogenetic factor in Type 2 Diabetes

Hindrik Mulder, M.D., Ph.D.

Lund University Diabetes Centre, Sweden

A key role of lipids in beta-cell stimulus-secretion coupling has long been postulated and, to some extent, proven. Genetic evidence, however, underpinning this role has been lacking. I will share a series of studies that fills this gap in knowledge identifying synthesis of acyl-CoA in beta-cells as a key regulatory mechanism. Using eight different indices reflecting insulin secretion during an OGTT in ~ 26,000 individuals, we discovered 55 independent genetic associations at 44 loci. Among these, rs4862423 at the *ACSL1* locus, encoding acyl-CoA synthetase long chain family member 1, was found to interact with islet enhancers and islet-specific transcription factors, as inferred from data on islet 3D-chromatin interactions, eQTL and single cell RNA sequencing. Its T-allele associated with decreased insulin secretion, hyperglycaemia and risk of T2D. We have taken these genomic insights further to a functional level. Silencing of *ACSL1* in clonal beta-cells and rat and human islets impaired insulin secretion provoked by glucose, while *ACSL1* overexpression in clonal beta-cells enhanced insulin secretion. Concurrently, *ACSL1*-deficiency reduced respiration and hindered the glucose-provoked hyperpolarisation of the inner mitochondrial membrane. In parallel, rises in the ATP/ADP ratio and calcium influx were abrogated, and oxidation of fatty acids in mitochondria was inhibited by *ACSL1*-deficiency. Metabolomics analysis of clonal beta-cells revealed impaired formation of pyruvate, citrate, 2-oxoglutarate and glutamate upon silencing of *ACSL1*. The lipidome exhibited a perfect separation by *ACSL1*-deficiency, seemingly driven by reduced levels of oleate and palmitate. In vivo studies in *ACSL1* null mice are ongoing. We conclude that the *ACSL1* risk allele is associated with impaired insulin secretion traits and T2D. This appears to be caused by impaired formation of acyl-CoA, fatty acid oxidation and mitochondrial metabolism. These observations provide a genetic basis

for the long presumed, but not proven, functional and pathogenetic roles of lipid metabolism in beta-cells.

CT Diabetes risk gene *KCNQ1* modulates pancreatic beta-cell sensitivity to age and metabolic stress

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Background: Loss-of-function (LoF) mutations in *KCNQ1*, encoding K^+ channel $K_v7.1$, cause Long QT Syndrome 1 (LQT1), as well as hyperinsulinemia. Paradoxically, LQT patients have an increased burden of type 2 diabetes (T2D) and *KCNQ1* is a T2D susceptibility gene. We previously developed a *Kcnq1* LoF mouse model, revealing an age-dependent transition from hyper- to hypoinsulinemia.
Aim/Methods: We hypothesized that *Kcnq1* LoF sensitizes islets to age- and metabolic stress-induced β-cell dysfunction. To investigate this and its mechanisms, mice were aged ($>$70 weeks), or received a high-fat-high-sucrose (HFHS) diet, and β-cell function was assessed.
Results: In islets of young *Kcnq1* LoF mice, hypersecretion coincided with increased glucose-induced Ca^{2+} influx, in line with reduced repolarization. However, islets of aged *Kcnq1* LoF mice demonstrated decreased glucose-induced insulin secretion ($p = 0.0099$), exocytosis (dispersed β-cells; $p = 0.0063$), and Ca^{2+} influx ($p = 0.0002$), despite comparable insulin granule count and intact KCl-induced responses, indicating upstream defects in stimulus-secretion coupling. Metabolic stress accelerated β-cell dysfunction. On a HFHS diet, wild-type mice maintained blood glucose ($p = 0.55$) and increased insulin secretion by 69$\pm$12% compared to a control diet, while in *Kcnq1* LoF mice, blood glucose rose ($p = 0.0003$) and insulin secretion reduced by 15$\pm$9% ($p = 0.001$ LoF vs WT). Notably, endoplasmic reticulum stress was not involved in this deterioration. Reanalysis of LQT1 data suggests an accelerated age-dependent decline in insulin secretion in human KCNQ1 LoF as well.
Conclusion: *Kcnq1* LoF initially enhances insulin secretion, but predisposes β-cells to age- and metabolic stress-induced dysfunction of stimulus-secretion coupling, providing insights into the paradoxical link to hyper- and hypoinsulinemia.

CT Chronic glucokinase activation impairs pancreatic beta cell function under euglycaemic conditions

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Introduction and aims: Glucokinase activators (GKAs) are being developed as anti-hyperglycaemic drugs, aiming to increase glucose-stimulated insulin secretion (GSIS). However, several GKAs have failed to show long-term efficacy in clinical trials. Previously, we have shown that partial glucokinase inhibition can prevent the decline in GSIS caused by diabetes/chronic hyperglycaemia. Here, we investigated whether chronic glucokinase activation by GKAs MK-0941 and dorzagliatin can impair beta-cell function in euglycaemia.
Methods: Microtissues reaggregated from dispersed primary non-diabetic human islets (3 donors) were cultured at 5.5mM or 16.7mM glucose $\pm$ 10μM MK-0941 for 14 days. INS-1 832/13 cells were cultured at 5mM or 25mM glucose $\pm$ 10μM MK-0941 or 1μM dorzagliatin for 48h. Insulin was quantified by ELISA, gene expression by RT-qPCR, and

mitochondrial respirometry using Seahorse XFe24 Extracellular Flux Analyser.
Results: In euglycaemic human islet microtissues, MK-0941 increased basal secretion but abolished GSIS and reduced insulin content 10-fold ($p = 0.012$), equivalent to changes at 16.7mM glucose. GSIS was abolished and insulin content reduced by both GKAs in euglycaemic INS-1 cells ($p < 0.01, n = 3$), equivalent to changes at 25mM glucose. MK-0941 also markedly reduced mRNA expression of insulin and associated transcription factors Mafa and Pdx1 (all $p < 0.0001, n = 3$) and altered expression of many metabolic enzymes. MK-0941 increased basal oxygen consumption rate (OCR) and reduced glucose-stimulated OCR.
Conclusion: Chronic glucokinase activation had an equivalent impact to chronic hyperglycaemia on beta-cell function in both models. Metabolic alterations including impaired mitochondrial respiration likely underlie the loss of GSIS. These results could explain the failure of GKAs to maintain glycaemic control long-term after initially reducing HbA1c.

Thursday, 12 of June, 2025

KS Unraveling the Regulation of Islet Cell Fates

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Transcription factors are indispensable for maintaining pancreatic islet cell identity through the regulation of cell-specific gene expression. Specification of the different islet cell populations is predominantly regulated by shared transcription factors; yet the mechanisms that enable a single transcription factor to regulate cell-specific targets are poorly characterized. We have previously demonstrated that the transcription factor NKX2.2 is critical for the specification, identity and function of islet beta cells. More recently we have characterized the role of NKX2.2 in maintaining the identity of pancreatic islet alpha cells. These studies have demonstrated that NKX2.2 exerts its cell specific regulatory functions through differential genomic binding that is facilitated by novel protein-protein interactions. These studies are beginning to shed light on the complex gene regulatory processes that underlie the development and maintenance of pancreatic islet cell fates and provide insight into how islet cell identity is disrupted during disease.

IS Exploring the impact of altered tRNA fragmentation in pancreatic β-cell

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Transfer RNAs (tRNAs) are very important for preserving β-cell function and survival. Alterations in tRNAs or tRNA-modifying enzymes, affecting tRNA modifications and tRNA fragmentation, may cause β-cell failure. tRNA-derived fragments are a new class of small non-coding RNAs that may modulate multiple cellular processes by different mechanisms. Changes in tRNA fragment homeostasis are associated with human pathologies including diabetes. We have demonstrated that inactivating mutations in the *TRMT10A* gene cause insulin-dependent diabetes, microcephaly and intellectual disability.
TRMT10A is a tRNA methyltransferase that methylates cytosolic tRNAs at guanosine in position 9. *TRMT10A*-deficient β-cells are dysfunctional, sensitive to stress-induced apoptosis, and exhibit multiple dysregulated transcripts associated with ion transport, viral response, inflammation and lipid metabolism. Moreover, *TRMT10A*-silenced β-cells have increased levels of various tRNA-derived fragments.
While *in silico* predictions indicate that some of these fragments may modulate the expression of particular genes dysregulated under *TRMT10A* deficiency; RNA pulldown experiments followed by mass spectrometry, showed that other fragments can interact with protein targets potentially regulating their expression or activity. Interestingly, we found that insults associated with type 1 diabetes (e.g. IFNα, double stranded RNA or Coxsackie virus infections) reduce *TRMT10A* expression and enhance tRNA fragmentation suggesting that *TRMT10A* deficiency is not limited to a rare monogenic form of diabetes. This talk will highlight our ongoing and previous work, with particular attention to the implications of increased levels of specific tRNA-derived fragments on the functionality and gene expression profile of pancreatic β-cells.

IS Illuminating the pancreas – a new view on islet cellularity and β-cell distribution in health and disease

Ulf Ahlgren, Ph.D.

Department of Medical and Translational Medicine, Umeå University, Sweden

We have developed a method enabling specifically labelled cells to be studied throughout the volume of the entire human pancreas, in 3D, and at a microscopic resolution. Herewith, we have generated a complete account of the pancreatic β-cell distribution and identified previously unrecognized heterogeneities in the cellularity of the islets of Langerhans. Most significantly, 50% of the insulin expressing islets are in fact devoid of glucagon expressing cells, two hormones suggested to be required to constitute a fully functional islet unit. Our data now point to a range of pronounced heterogeneities in islet cellularities also with regards to the other endocrine cell-types, which may may re-define how we see the islets and their functionality in health and disease. Prompted by these results, we show that significant regional heterogeneities in islet cellularity also applies to the mouse pancreas, emphasizing the importance of spatial context when interprating results using this model for diabetes research.

Using the same imaging approach on a whole human late onset – T1D pancreas, we show that remaining β-cell mass may be heterogenously distributed across the organ. β-cell density was significantly higher in the pancreatic head and as much as 85% of the residual β-cells were not associated to islet structures. Instead, they reside in the pancreas as single scattered cells or as "punctuated" β-cell clusters that are not overlapping with any other endocrine cell type. As residual β-cell function may have positive effects on diabetes regulation and complications, unravelling the nature of these β-cells may be of importance for potential therapeutic developments.

IS Influence of microRNAs on human islet insulin secretion in type 2 diabetes

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Lund University Diabetes Centre, Sweden

Background and aim: Insulin resistance in target tissues leads to a compensatory increase in insulin secretion from pancreatic β-cells; failure to do so results in type 2 diabetes (T2D). We hypothesize that differential expression of miRNAs in islets from T2D individuals either compensates for β-cell insulin secretion or directly regulates processes leading to reduced insulin secretion. Therefore, we aim to determine the differential expression of human islet miRNAs in T2D and investigate their association with insulin secretion. In addition, we aim to explore the potential of using LNA-inhibitors to enhance insulin secretion.
Methods and results: Using small RNA-sequencing we show differential expression of miRNAs in islets from human T2D individuals. In our data we identified several upregulated miRNAs associated with first and second-phase insulin secretion. We confirmed previous differentially expressed miRNAs and identified one novel miRNA of particular interest, which we believe is upregulated to improve insulin secretion compensatorily. Additionally, some islet miRNAs are upregulated and instead reduce insulin secretion, contributing to T2D development. One such example is miR-200c, which we have shown to target ETV5. LNA-inhibitors represent a potential method to improve insulin secretion, and we have demonstrated that LNA-200c can improve glucose stimulated insulin secretion in human islets.
Conclusion: We propose that a group of differentially expressed miRNAs in human islets associated with insulin secretion participate in T2D pathogenesis and advocate for the use of LNA-inhibitors to improve impaired insulin secretion.

CT Roles of zinc depletion in the protective effects of *SLC30A8* variants in human beta-like cells

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Loss of function mutations in *SLC30A8*, encoding the ZnT8 zinc transporter in pancreatic beta cells, are associated with protection against apoptosis induced by zinc depletion, a condition associated with T2D development. The mechanisms underlying this protection remain unclear.
This study examined whether forced changes in intracellular Zn^{2+} using the zinc chelator Tetrakis-(2-pyridylmethl) ethylenediamine (TPEN) affect cell survival and function in human pancreatic beta-like cells. The interaction with a protective inactivating allele of *SLC30A8* was also explored. INS(GFP/w) human embryonic stem cells (MEL1), or a CRISPR/Cas9-engineered *SLC30A8* R138X subline were differentiated *in vitro* into pancreatic beta-like cells. TPEN (1 μM for 48h) was used to deplete intracellular zinc. Apoptosis, mRNA/protein levels and glucose-stimulated intracellular Ca^{2+} dynamics were assessed. Results are from three independent differentiations.
TPEN treatment in wild type beta-like cells induced apoptosis (TUNEL staining), reduced apparent insulin protein and decreased *NKX6.1* and *somatostatin* mRNA levels. *SLC30A8* immunoreactivity showed a strong tendency to decrease. These changes were associated with increased glucose-induced Ca^{2+} dynamics and increased oscillation frequency. In the absence of TPEN, R138X cells demonstrated reduced Ca^{2+} dynamics and the protective effects of TPEN were exaggerated in R138X cells versus variant controls.
These findings demonstrate that intracellular zinc depletion impairs human beta cell identity and function, with earlier observations highlighting that protective inactivating variants of *SLC30A8* display increased cytosolic Zn^{2+} levels, likely reflecting impaired Zn^{2+} uptake into the secretory granule. Lowered Zn^{2+} levels in common variant carriers may contribute to poorer beta cell survival and function versus carriers of inactivating *SLC30A8* variants.

CT 5'tRNA-derived fragments modulate β-cell homeostasis and islet macrophage activation in type 2 diabetes.

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During obesity, pancreatic β-cells experience nutritional stress, while islet-resident macrophages (iMACs) undergo metabolic reprogramming that contributes to β-cell dysfunction. Stress-induced cleavage of transfer RNAs (tRNAs) generates regulatory fragments (tRFs), whose role in this context remains poorly understood. We aimed to characterize tRF profiles and functions in pancreatic islets during obesity and diabetes.
β-cells and iMACs were isolated by FACS from islets of db/db and wild-type mice. Human islets were obtained via laser capture microdissection from pancreatic cryosections of individuals with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or type 2 diabetes (T2D). Small RNA sequencing revealed increased levels of $5'tRF^{Glu(CTC)}$ and $5'tRF^{Gly(GCC)}$ in β-cells and iMACs from db/db mice, and in islets from T2D versus NGT patients. Moreover, $5'tRF^{Glu(CTC)}$ levels negatively correlated with insulin secretion rates. In vitro, palmitate (0.5 mM) exposure induced these tRFs in both mouse and human islets.
Inhibition of $5'tRF^{Glu(CTC)}$ with an antisense oligonucleotide in mouse islets altered gene expression, prevented palmitate-induced β-cell apoptosis, and partially restored glucose-stimulated insulin secretion. Pull-down coupled with mass spectrometry identified several RNA-binding proteins as interactors of $5'tRF^{Glu(CTC)}$.

iMAC-like cells, generated by co-culture of bone marrow-derived macrophages with MIN6 cells, showed mixed polarization and high IL-1β expression, as previously reported for iMACs. Palmitate treatment induced anti-inflammatory markers and reduced IL-1β. Blocking $5'tRF^{Glu(CTC)}$ attenuated this phenotypic shift and partially rescued insulin secretion in co-cultured β-cells.
These findings suggest that $5'tRF^{Glu(CTC)}$ contributes to β-cell dysfunction through both intrinsic effects and modulation of iMAC activation.

IS Organelle calcium gradients at the nexus of type 1 diabetes pathogenesis

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The evolution of type 1 diabetes (T1D) involves a complex dialogue that is established between immune cells that invade the islet and immunogenic signals released by injured or dying β cells. Our research program is focused on understanding how altered calcium (Ca^{2+}) signaling in the β cell influences this dialogue, and much of this research has centered on defining how impaired Ca^{2+} uptake into the endoplasmic reticulum (ER) by the sarcoendoplasmic reticulum Ca^{2+} ATPase2 (*SERCA2*) pump orchestrates T1D pathogenesis. Our interest in this area is driven by three observations: (1) individuals with Darier White Disease (loss-of-function mutations in the *SERCA2* gene) are at increased risk of T1D, suggesting a connection between *SERCA2* deficiency and diabetes pathogenesis; (2) we have identified marked reductions in β cell *SERCA2* activity and expression in models of diabetes, driven in part by pro-inflammatory cytokine stress; and (3) we have found that loss of *SERCA2* activity leads to alterations in ER Ca^{2+}, impaired β cell function and survival, and increased β cell immunogenicity. In this talk, we will describe the mechanistic underpinnings linking these observations, leveraging data from non-obese diabetic (NOD) mice with *SERCA2* haploinsufficiency (NOD-S2$^{+/-}$), human model systems, and human islets from donors with T1D.

IS Unmasking islet profiles: the link between *HLA-I* expression, immune infiltration and insulin dynamics in type 1 diabetes.

Teresa Rodriguez-Calvo, D.V.M., Ph.D.

Institute of Diabetes Research, Helmholtz Munich, Germany

High Human Leukocyte Antigen I (*HLA-I*) expression is considered one of the hallmarks of Type 1 Diabetes (T1D). Previous studies aiming to decipher its role showed that it is strongly associated with the presence of β cells in islets from T1D donors, and that interferons are powerful drivers of its expression (*in vitro*). In this study we aimed to determine: 1) the magnitude of *HLA-I* expression 2) the timeline of *HLA-I* expression, immune infiltration and β cell loss and 3) the dynamics of *HLA-I* upregulation and its correlation with β cell function.
Pancreatic sections of 41 donors with and without T1D were obtained through nPOD and stained for different islet and immune markers, and *HLA-I*. By analyzing more than 9500 islets we found that high *HLA-I* expression affects, on average, 15% of the islets in T1D donors, and that recent-onset T1D donors show high islet *HLA-I* expression on 39% of them. Immune infiltration densities are highest in islets with high *HLA-I* expression of all donor groups; on average, 83% of *HLA-I* high islets in T1D donors are infiltrated by at least 1 $CD3^+$ T-cell, compared to 63% in islets with low expression. *HLA-I* Low islets in T1D are predominantly insulin-deficient islets or seriously depleted of β cells.
To determine the impact of *HLA-I* expression on β cell function, stem-cell (SC) derived islets were treated with IFN-α and IFN-γ. Staining for HLA-I, insulin and glucagon, as well as glucose-stimulated insulin secretion (GSIS) were performed. SC-islets treated with IFNs showed increased *HLA-I* expression at the cell membrane. An analysis of intracellular insulin levels and clustering showed no significant changes between treated and not treated islets, but the GSIS revealed an increase in basal insulin secretion in low glucose conditions and a decreased response to high glucose. Based

on these results, we hypothesize that strategies aiming at decreasing *HLA-I* expression may delay or avoid disease progression and preserve β cell mass and function, alone, or in combination with strategies focusing on eliminating immune-mediated β cell destruction.

CT Urocortin-3 regulates glucagon secretion through an inhibitory β- to δ- to α-cell axis

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Background. In type 1 diabetes (T1D), the glucagon response to hypoglycemia is lacking. During the pathogenesis of T1D, β-cells are lost, which can lead to a defective paracrine regulation of glucagon secretion. We recently identified that urocortin-3 (UCN-3), from the β-cells, inhibits the secretion of glucagon.
Aim. Unravel the physiological role and the mechanism of action of UCN-3 in the pancreas.
Methods. The secretion of pancreatic hormones was investigated using the isolated mouse pancreas perfusion in response to exogenous UCN-3, and/or specific SST-receptor and Corticotropin Releasing Hormone Receptor 2 (CRHR2) antagonists in a healthy and a beta-cell deficient model (streptozotocin (STZ) treated mice).
Results. UCN-3 potently stimulates SST and inhibits glucagon secretion in the mouse pancreas. The inhibitory actions of UCN-3 on glucagon secretion can be mitigated by antagonizing SST receptors, while the stimulatory actions on SST can be prevented by co-infusing UCN-3 with a CRHR2 antagonist. Changing glucose conditions from hypoglycemia (3.5mM) to hyperglycemia (10mM) while blocking the actions of endogenous UCN-3 reduced the inhibitory and stimulatory potency of glucose on glucagon and SST secretion, respectively. This glucose-dependent regulatory paracrine pathway is lost in the STZ-treated mouse.
Conclusion. This study supports the existence of a β- to δ- to α-cell axis driven by UCN-3. Here we show that endogenous UCN-3, secreted in response to glucose, activates CRHR2 in δ-cells to stimulate somatostatin secretion which in turn inhibits glucagon secretion through SSTR activation. Moreover, we show that this paracrine pathway is disrupted in a T1D mouse model.

CT Mechanisms underlying islet cell dysfunction in human type 1 diabetes

Theodore dos Santos [1,2], ***Xiao-Qing Dai*** [1,2], ***Robert C Jones*** [3,4,5], ***Aliya F Spigelman*** [1,2], ***Hannah M Mummey*** [6], ***Jessica D Ewald*** [7], ***Cara E Ellis*** [1,2], ***James G Lyon*** [2], ***Nancy Smith*** [1,2], ***Austin Bautista*** [2], ***Jocelyn E Manning Fox*** [1,2], ***Norm F. Neff*** [4], ***Angela Detweiler*** [4], ***Michelle Tan*** [4], ***Rafael Arrojo E Drigo*** [8,9], ***Jianguo Xia*** [10], ***Joan Camunas-Soler*** [11,12], ***Kyle J Gaulton*** [13], ***Stephen Quake*** [3,4,5], ***Patrick E MacDonald*** [1,2,14]

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Introduction: In type 1 diabetes (T1D) surviving β-cells may behave abnormally, while α-cells secrete excess glucagon at normoglycemia but insufficient glucagon during hypoglycemia. Yet, the mechanisms underlying these dysfunctions remain elusive.
Aim: Identify and validate mechanisms of islet cell dysfunction in T1D through linked electrical and transcriptomic profiling.
Methods: Islets from donors with T1D (9 donors) or matched controls (ND; 17 donors) were attained from the Alberta Diabetes Institute IsletCore. We generated electrical and transcriptomic data (patch-seq) from single α- and β-cells which we analyzed using electrophysiological fingerprinting, differential expression analyses, gene set enrichments, genome wide association studies (GWAS) cross-validation, and correlative approaches. We validated pathways via protein-protein interaction analysis (PPI), STELLARIS imaging of pancreatic biopsies, and dynamic perfusions.
Results: T1D α- and β-cells show hypersecretory electrical behaviour at normoglycemia and transcriptomic alterations when compared to ND. Notably, T1D α-cells fail to suppress electrical behaviour at high glucose and show enrichment for antigen presentation and immaturity markers. GWAS enrichment, correlations, and PPI revealed MHC class I, immaturity, and abrogated mTORC1 assembly linked to T1D α-cell dysfunction. Imaging corroborated these findings as T1D α-cells show altered glucagon and MHC class I localization, loss in nuclear accessibility to key transcription factors, and lysosomal disorder. Last, mTOR inhibition increases glucagon secretion at normoglycemia.
Conclusions: We reveal significant functional and molecular differences in T1D α- and β-cells. Further, we provide mechanistic links between transcriptional, electrophysiological, and functional disruptions in T1D α-cells, including links with T1D GWAS, highlighting their potential contribution to disease pathophysiology.

IS Calcium Messages Within and Between Beta Cells: Recent Contributions to their Understanding from Functional Multicellular Calcium Imaging

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Cell identities are assigned by the interplay of extrinsic signals and intrinsic determinants. My research interests focus on the mechanisms regulating cell identity and plasticity in metabolic organs, such as the pancreas. We use multimodal approaches in mouse embryos and human models to elucidate how distinct cell types arise from common progenitors and crosstalk to each other and surrounding microenvironment to acquire specialized shapes and form functional organs. Is the establishment of distinct cellular identities and morphogenetic programs interdependent? How plastic are these cellular states? Can we harness cell plasticity towards novel regenerative therapies for diabetes? Ultimately, the long-term goal of our research is to translate these concepts into a better understanding of the pathogenesis as well as treatment options of diabetes.

IS Modelling human islet development and function with stem cell models

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University of Helsinki, Finland

Human pancreatic islet development is challenging to investigate, primarily due to the limited availability of fetal tissue for research. Consequently, much of our current understanding of the signaling pathways and regulatory mechanisms guiding islet formation is derived from studies in rodent models. However, important differences exist between mouse and human pancreatic development, particularly in the regulatory programs that govern these processes.
Advances in the directed differentiation of human pluripotent stem cells have made possible the generation of functional pancreatic islets *in vitro*, constituting a powerful tool to advance diabetes disease modelling and cellular therapies. Indeed, these efforts have led to promising clinical trials where stem cell-derived islets are used to treat individuals with diabetes.
We are using genome-engineered stem cell-derived pancreatic cell models to understand the precise regulatory mechanisms specific to human islet development. By integrating single-cell omics, with chemical and genetic perturbation approaches, we are dissecting how the disruption of human-specific transcriptional regulatory mechanisms can lead to diabetes.

IS Immune and Vascular Crosstalk Modulates β Cell Development and Function

Limor Landsman, Ph.D.

Tel Aviv University, Israel

The development and function of pancreatic beta cells are regulated by intrinsic cellular programs and extrinsic signals from the islet microenvironment, including vascular and immune components. Within this niche, pericytes and endothelial cells are critical for supporting beta cell development and function. Additionally, under physiological conditions, islet-resident macrophages support beta cell activity and glucose homeostasis, yet under metabolic stress and diabetic conditions, macrophages may adopt a harmful phenotype contributing to beta cell dysfunction. The identity of factors produced by immune and vascular cells within the islet microenvironment that regulate glucose homeostasis is only beginning to be uncovered.
Our research identifies pericytes as key regulators of beta cell maturation and function through direct and indirect mechanisms. We found that pericyte-derived growth factors, including BMP4 and HGF, are essential for beta cell development and maturation by promoting the expression of critical functional genes and enhancing insulin production and secretion. Additionally, pericytes secrete cytokines, such as CCL2 and CXCL1, that promote the beneficial phenotype of islet macrophages, further supporting beta cell function and glucose regulation.
These findings reveal a dual role for pericytes in coordinating beta cell maturation and immune signaling within the islet microenvironment. Targeting pericyte-mediated pathways may provide new therapeutic avenues for improving glucose control and generating fully functional stem cell-derived beta cells for diabetes treatment.

CT HNF1A is essential for GLI3 processing by primary cilia in the posterior foregut, its dysfunction favoring duodenal cell fate and altering lineage choices

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Introduction The inhibition of Hedgehog signaling pathway (Hh-signaling) is compulsory in early pancreas development. Ectopic activation of Hh-signaling leads to increased duodenal cell fate specification at the expense of the pancreatic lineage, while the inhibition of Hh-signaling leads to the expansion of the pancreatic expression domain. Despite its proven importance, the molecular mechanisms by which HH signaling is controlling lineage segregation in the posterior foregut are underdefined.
Aims This study investigates the developmental regulatory networks focusing on the *HNF1A* and Hh-signaling interplay.
Methods We used differentiating human induced pluripotent stem cells and transgenic mice analyzed by bulk and single-cell transcriptomics, physiology and lineage tracing.
Results At posterior foregut stage *HNF1A* and Hh-signaling are involved in a regulatory loop necessary for elevating *HNF1A* expression. The *HNF1A*^{P291fsinC} mutation impedes this circuit, leading to disrupted *GLI3* processing by cilia proteins, therefore abolishing the *HNF1A* upregulation in response to Hh-signaling inhibition. Consequently, *HHEX* fails to increase its expression, while *CDX2*, essential for adopting the intestinal lineage but also for glucagon expression, maintains its levels. The persistent reinforcement of the differentiation program in vitro combined with cellular robustness partially rescues the islet cell fate, yet displaying a significant tilt towards glucagon-producing cell identity. In mice, the endocrine-restricted *Hnf1a* mutation leads to increased alpha-cell population in the islet and significant elongation of the small intestine. The intestine elongation is mediated by the glucagon/GLP expressing cells, as the *Hnf1a* mutation specific in these cells replicating the phenotype and significantly increasing their numbers.
Conclusion We describe an essential autoregulatory loop between Hedgehog signaling and *HNF1A* responsible for cell fate selection in foregut.

CT Characterization of *INS* gene activity states in human primary and stem-cell derived β-cells

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Introduction: Murine insulin-producing pancreatic β-cells can exist in, and move between, states of low and high *INS* gene activity. *INS* gene activity states have not been thoroughly characterized in primary or stem cell-derived human β-cells.
Aims: Characterize insulin gene activity states in human primary and stem cell derived β-cells (SCβ).
Methods: We performed fluorescence-activated cell sorting (FACS), transcriptomics, proteomics, and live-cell imaging to interrogate *INS* gene activity in an *INS*-EGFP knock-in stem cell line and cells from primary human donor islets infected with a RIP-GFP adenovirus.
Results: We found that *INS* gene activity in SCβ-cells was bimodal, with high (*INS*(EGFP)^{HIGH}) and low (*INS*(EGFP)^{LOW})

states. 3D live-cell imaging revealed that *INS*(EGFP)^{HIGH} cells tend to reside in the center of SCβ islets. Long-term live-cell imaging revealed that cells can transition between the two states, and showed that *INS*(EGFP)^{HIGH} is a more fragile state. Transcriptomics and proteomics confirmed that *INS*(EGFP)^{HIGH} cells had more insulin mRNA and protein, and revealed increased abundance of maturity markers (*PDX1*, *IAPP*, *ENTPD3*) and ER resident proteins (*HSPA5*, *HSP90B1*). FACS of transduced primary human β-cells also revealed bimodality, while scRNAseq analyses on primary human β-cells with high *INS* mRNA from 69 donors revealed distinct transcript expression and transcription factor activity profiles, with coordinated β-cell regulon activation absent in low *INS* cells.
Conclusions: These data provide a comprehensive comparison between low and high *INS* states in human β-cells.

CT Sex differences in diabetic mouse model of conditional knockout of transcriptional repressor and epigenetic modulator ISL1

***Valéria Fabríciová*, Laura Lebrón Mora, Romana Bohuslavová, Věra Slaninová, Zuzana Berková, Pavel Abaffy, Gabriela Pavlínková**

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ISL1 is a critical transcription factor, epigenetic modulator, and repressor of progenitor programs during pancreatic endocrine lineage development. To investigate its role, we generated a novel diabetic mouse model by conditionally deleting Isl1 in endocrine progenitors (Neurod1-Cre; Isl1$^{f/f}$). Isl1CKO mice exhibit a failure to produce δ and PP lineages, retain predominantly immature β cells, and display an aberrant α-cell lineage, but survive up to one month. To elucidate the molecular mechanisms by which ISL1 orchestrates lineage-specific transcriptional programs and identify pathways supporting survival in Isl1CKO mice, we performed single-cell RNA sequencing (scRNA-seq) on FACS-sorted endocrine cells from one-month-old mice. Intriguingly, scRNA-seq revealed sex-dependent clustering, prompting further exploration of compensatory mechanisms. While Isl1CKO females showed normal weight gain compared to controls, Isl1CKO males exhibited significant growth impairment. Integrative analysis of Isl1CKO and control endocrine cells at postnatal day 9 (P9) and one month highlighted sex-specific upregulation of chromatin remodelers in the older mice. Notably, female Isl1CKO cells exhibited increased expression of transcriptional repressors, including Foxp1, Sin3b, and Gatad2b, which may mitigate transcriptional dysregulation. Furthermore, female cells showed enrichment in genes associated with the FOXO pathway, its target AP-1 complex, and Klf transcription factors, suggesting a molecular basis for their greater metabolic adaptability. Our findings suggest that sex-specific compensatory mechanisms, particularly those involving chromatin remodeling and transcriptional repression, may underlie the increased resilience of female Isl1CKO mice. These molecular differences provide insights into pancreatic endocrine development and identify potential therapeutic targets for improving β-cell maturation and diabetes treatment.

Friday, 13 of June, 2025

KS The human islet in diabetes – lessons learned from the Human Pancreas Analysis Program (HPAP)

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The Human Pancreas Analysis Program is designed to define the molecular pathogenesis of islet dysfunction by studying human pancreatic tissue samples from organ donors with T2D, T1D, or prediabetes. HPAP generates detailed datasets of physiological, histological, transcriptomic, epigenomic, and genomic information. Importantly, all data collected, generated, and analyzed by HPAP are made immediately and freely available through a centralized database, PANC-DB, thus providing a comprehensive data resource for the diabetes research community. By combining functional and transcriptomic profiling, we identified the first defect in pancreatic alpha cells in non-diabetic individuals which are GAD autoantibody positive, suggesting an early role for non-beta cells in the etiology of T1D. Furthermore, we discovered that the activation of an interferon response gene signature in beta cells of pre-diabetic and diabetic individuals correlates with high expression and poor editing of endogenous double-stranded mRNAs, suggesting an alternate pathway to the initiation of islet inflammation.

With regard to T2D, our extensive epigenomic profiling, including single nucleus chromatin accessibility, three-dimensional chromatin architecture in alpha, beta and acinar cells, and cell-type specific analysis of DNA methylation and hydroxymethylation suggest accelerated epigenomic aging in beta cells of T2D patients. In addition, our 3D chromatin maps reveal lineage-specific regulatory architecture of the genetic risk for T2D.

IS Dynamic crosstalk within the islet shapes insulin release

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Pancreatic beta cells are the only cell type in our body capable of producing and secreting the insulin necessary to instruct insulin-sensitive cells and tissues of our bodies to absorb nutrients after a meal. Accurate control of insulin release is of critical importance; too little insulin leads to diabetes, while an excess of insulin causes potentially fatal hypoglycaemia. Because we only become aware of the important contributions of the pancreas when it fails to maintain nutrient homeostasis, it is easy to forget just how well insulin release from a healthy pancreas is matched to insulin need. Beta cells achieve this feat in part by extensive crosstalk with the rest of the endocrine cell types in the islet, notably the glucagon-producing alpha cells and somatostatin-producing delta cells. The complex and dynamic intra-islet crosstalk that shapes insulin release is therefore best understood by quantifying the dynamic changes in calcium and cAMP across all three major islet cell types simultaneously. Pro-glucagon-derived peptides released by pancreatic alpha cells promote insulin secretion in the prandial phase. By restraining beta cell activity under resting conditions, somatostatin from pancreatic delta cells sets the fasting glucose setpoint of the islet. During fed conditions, somatostatin inhibits beta activity directly. However, a significant proportion of the inhibitory potential of intra-islet somatostatin on insulin release is mediated indirectly via the inhibition by somatostatin of alpha cells. Our observations generally affirm the important contributions by non-beta cells to the control of the timing and magnitude of insulin secretion.

IS The mitochondrial tRNA-derived fragment, *mt-tRF-Leu*^{TAA}, modulates both pancreatic insulin secretion and muscle insulin sensitivity

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University of Lausanne, Switzerland

The intricate interplay between glucose sensing, insulin secretion and glucose disposal is essential for metabolic homeostasis and its disruption contributes to diabetes development. Our study unveils a mechanism regulating mitochondrial metabolism via *mt-tRF-Leu*^{TAA}, a small RNA fragment derived from the cleavage of a mitochondrially-encoded tRNA. We see a coordinated modulation of *mt-tRF-Leu*^{TAA} levels in pancreatic islets and muscle in models of diabetes susceptibility and in response to changes in nutritional status. Mechanistically, *mt-tRF-Leu*^{TAA} interacts with core subunits and assembly factors of respiratory complexes in the mitochondrial electron transfer system, modulating ATP production. While *mt-tRF-Leu*^{TAA} depletion impairs glucose-stimulated insulin secretion in pancreatic β-cells, its inhibition enhances insulin sensitivity in skeletal muscle. Overall, these findings highlight *mt-tRF-Leu*^{TAA} as a pivotal regulator of systemic metabolic control, influencing both insulin secretion and action.

IS Calcium Messages Within and Between Beta Cells: Recent Contributions to their Understanding from Functional Multicellular Calcium Imaging

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Within the beta cell stimulus-secretion coupling cascade, changes in intracellular concentration of the secondary messenger Ca^{2+} are well synchronized with proximal membrane potential oscillations and serve as the triggering signal for the distal exocytosis of insulin. Under certain conditions, they are also synchronized between different beta cells by means of intercellular waves. These important aspects of Ca^{2+} messages were described more than 30 years ago in pioneering Ca^{2+} imaging studies, but some important details about their exact content remain to be described, especially with regard to their dependence on secretagogue concentration, the heterogeneity of Ca^{2+} responses in different beta cells during activation, activity, and deactivation, the possible role of specialized groups of cells in generating and conveying the Ca^{2+} signals, as well as with regard to translationally relevant interspecies differences and changes during development of diabetes, to name only a few. In the last decade, we have employed high-frequency confocal Ca^{2+} imaging of beta cells to investigate these aspects of Ca^{2+} messages and demonstrate some of the most important findings in the present contribution. The shape and active time of so-called fast Ca^{2+} oscillations or bursts of Ca^{2+} spikes encode the concentration and possibly also the nature of the stimulus. These fast oscillations usually originate in groups of cells at the periphery of islets and are transmitted to some or many other cells by means of intercellular waves that typically traverse more centrally located hub cells. Finally, their main characteristics seem to be conserved in human beta cells, and they seem to become enhanced in early and attenuated in later stages of diabetes.

CT Appropriate insulin secretion and glucose homeostasis in absence of α-cells

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Introduction: Glucose homeostasis relies on glucose sensing and regulated insulin secretion from pancreatic β-cells. Insulin secretion is tightly regulated by systemic cues and, locally, by adjacent islet α-, δ- and γ-cells. For instance, glucagon, produced by α-cells, is a major glucose counter-regulatory hormone that modulates β-cell activity. While it is well established that β-cells are essential for glucose homeostasis, whether α-cells are needed for euglycemia and accurate β-cell secretory function is still debatable.
Methods: We generated a diphtheria toxin-mediated islet cell ablation mouse model to efficiently remove in adult mice either the α-cells or the α-, δ-, and γ-cells simultaneously ("β-only" mice).
Results: α-cell-ablated mice remained normoglycemic under random-fed conditions and after short and prolonged fasting. The residual glucagonemia and other circulating counter-regulatory hormones (cortisol, growth hormone and epinephrine) probably prevented hypoglycemia. Hepatic glucose mobilization and glycogen storage were equivalent in β-only and control mice. Under glucagon signalling blockade, β-only mice maintained normoglycemia as non-ablated mice. *Ex vivo*, insulin secretion dynamics in islets composed of only β-cells was like in intact islets. Likewise, human islets composed exclusively with β-cells (monotypic pseudoislets) also exhibited proper regulated insulin secretion *ex vivo* upon glucose stimulation.
Conclusions: Glucagon and α-cells are not essential for blood glucose homeostasis, and the alternative counter-regulatory signalling may compensate the lack of glucagon signalling *in vivo*. Glucagon and α-cells are also dispensable for the β-cell secretory function, particularly *ex vivo*. This is relevant in diabetes, where α-cells become dysfunctional and worsen the disease's pathophysiology.

CT Optogenetic interrogation of metabolic and paracrine heterogeneity of pancreatic α-cells

Haiqiang Dou, ***Caroline Miranda***, ***Johan Tolö***, ***Cristiano Santos***, ***Rui Gao***, ***Thomas G. Hill***, ***Andrei I. Tarasov***, ***Quan Zhang***, ***Patrik Rorsman***

Department of Physiology, Metabolic Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden

Glucagon, produced by α-cells of pancreatic islets, is the body's primary hormone countering hypoglycemia. We used optogenetics in conjunction with electrophysiology, functional imaging of $[Ca^{2+}]_i$ and ATP/ADP and hormone release measurements to explore the intrinsic and paracrine regulation of glucagon secretion. We found that up to 50% of the α-cells were electrically silent at 1mM glucose. These silent α-cells can be activated by K_{ATP} channel blockade, a cocktail of amino acids and somatostatin receptor (SSTR) antagonism. Optoactivation of α-cells leads to $[Ca^{2+}]_i$ increase and stimulates glucagon secretion by 283% $(n = 4)$. Membrane depolarization and action potential firing in silent α-cells was associated with an increased cytoplasmic $ATP : ADP$-ratio.
Optoactivation of somatostatin-releasing δ-cells inhibits electrical activity and exocytosis (by 34%, $n = 10$) in adjacent (but not distal) α-cells and reduces glucagon secretion by 45%, at 1mM glucose. Interestingly, optoinhibition of δ-cells, or SSTR antagonism, activates only 70% spontaneously silent α-cells, while K_{ATP} blockade activates 100%, at 1mM glucose, suggesting both paracrine and intrinsic metabolic mechanisms determine α-cell activity. Amino acids stimulate

two-phases glucagon secretion, reflecting the activation and inactivation of silent and active α-cells, respectively. There is an inverse relationship between basal glucagon secretion (determined by fraction active α-cells) and the relative stimulatory effects of amino acids.
We conclude that islet α-cells are functionally heterogenous and that their electrical excitability and glucagon release are determined by K^+ channel activity due to variable mosaic of K_{ATP} and somatostatin-sensitive K^+ channels reflecting metabolic state and proximity to δ-cells, respectively.

IS Time zones of α- and β-cells: circadian orchestration of the islet cell function

Charna Dibner, Ph.D.

University of Geneva, Switzerland

The circadian clock system has evolved by most organisms as an anticipatory mechanism driving rhythmic oscillations of physiology with approximately 24-hour cycles (*circa diem*, from Latin "about a day"). A key function of the circadian timing system is to orchestrate metabolism in different organs according to the needs imposed by rest-activity cycles. Light exposure during the night, late meals, and reduced sleep hours associated with modern lifestyle desynchronize intrinsic clocks from environmental cycles. Type 2 diabetes mellitus (T2D), a major public health challenge today, is one of the consequences of circadian misalignment. Our works demonstrated that circadian oscillators operative in mouse and human α- and β-cells ensure temporal orchestration of transcriptional and lipid landscape impacting on the islet function, which is perturbed in context of type 2 diabetes (T2D). We are eager to dissect the molecular pathways linking the islet cellular clocks and insulin and glucagon secretion, and their disruption in T2D context, and to explore therapeutic potential of these newly discovered targe

IS Shedding light on pancreas regeneration and PDAC cell of origin with single cell sequencing?

Meritxell Rovira, Ph.D.

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Pancreatic ducts form an intricate network of tubules that secrete bicarbonate and drive acinar secretions into the duodenum. This network is formed by centroacinar cells, terminal, intercalated, intracalated ducts, and the main pancreatic duct. Ductal heterogeneity at the single-cell level has been poorly characterized; therefore, our understanding of the role of ductal cells in pancreas regeneration and exocrine pathogenesis has been hampered by the limited knowledge and unexplained diversity within the ductal network.
We used single cell RNA sequencing to comprehensively characterize mouse ductal heterogeneity at single-cell resolution of the entire ductal epithelium from centroacinar cells to the main duct. Moreover, we used organoid cultures, injury models, and pancreatic tumor samples to interrogate the role of novel ductal populations in pancreas regeneration and exocrine pathogenesis.

We have identified the coexistence of 15 ductal populations within the healthy pancreas and characterized their organoid formation capacity and endocrine differentiation potential. Cluster isolation and subsequent culturing let us identify ductal cell populations with high organoid formation capacity and endocrine and exocrine differentiation potential *in vitro*, including a Wnt-responsive population, a ciliated population, and Flrt3+ cells. Moreover, we have characterized the location of these novel ductal populations in healthy pancreas, chronic pancreatitis, and tumor samples. The expression of Wnt-responsive, interferon-responsive, and epithelial-to-mesenchymal transition population markers increases in chronic pancreatitis and tumor samples. In light of our discovery of previously unidentified ductal populations, we unmask potential roles of specific ductal populations in pancreas regeneration

and exocrine pathogenesis. Thus, novel lineage-tracing models are needed to investigate ductal-specific populations *in vivo*.

CT eGLP1/GSK3i Enhances β-Cell Function and Proliferation in Diabetes Models

Jin Feng[1,2,3], ***Sreya Bhattacharya***[1,2,3], ***Moya Wu***[4], ***Tobias Wiedemann***[4], ***Haydn Ball***[2,5,6,7], ***Elisabeth Kemter***[8], ***Patrick E. MacDonald***[9,10], ***Oliver Plettenburg***[2,5,6,7], ***Heiko Lickert***[1,2,3†]

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Background and Aim: Diabetes mellitus is characterized by progressive β-cell loss and dysfunction. While β-cell regeneration through self-replication holds therapeutic potential, current treatments fail to stimulate human β-cell proliferation effectively. Glycogen synthase kinase-3β (GSK3β) inhibition promotes β-cell expansion, but systemic inhibition poses safety concerns. To overcome this, we developed eGLP1/GSK3i, a β-cell-targeted conjugate combining an oestrogen GLP-1 analogue (eGLP1) with a GSK3β inhibitor (GSKi) via a pH-sensitive linker, ensuring selective β-cell delivery while minimizing off-target effects. This study evaluates the efficacy of eGLP1/GSK3i in improving β-cell function and proliferation in rodent, porcine, and human islet models.
Methods: eGLP1/GSK3i was validated using BRET-based GLP1 signalling assays. β-cell proliferation was assessed using EdU incorporation and Ki67 staining in INS1 cells, mouse islets, and porcine islets. Glucose-stimulated insulin secretion was evaluated in INS1 cells, mouse and human islets, and *in vivo* using hyperglycaemic clamp studies. Diabetic db/db and STZ-induced mice were treated with eGLP1/GSK3i for 7 weeks, followed by metabolic profiling, immunostaining, and single cell sequencing analysis.
Results: eGLP1/GSK3i enhanced β-cell proliferation via WNT/β-catenin activation, as indicated by increased β-catenin and PCNA expression. It potentiated insulin secretion in INS1 cells, mouse, and human islets, mimicking physiological biphasic insulin release. *In vivo*, eGLP1/GSK3i significantly reduced fasting glucose and HbA1c levels, expanded β-cell mass, and improved β-cell function.
Conclusion: eGLP1/GSK3i effectively restores β-cell function and promotes β-cell proliferation in diabetic models by combining targeted GSK3β inhibition with GLP-1R-mediated insulinotropic effects. These findings highlight the potential of eGLP1/GSK3i as a novel regenerative therapy for diabetes.

CT Single-cell quantification of β-cell secretory capacity in T2D

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A major determinant of disease progression in type 2 diabetes (T2D) is deteriorating insulin secretion from the pancreatic β-cells. However, the molecular changes that underlie β-cell dysfunction in T2D are still elusive. Recent single-cell studies have revealed transcriptional heterogeneity among β-cells, highlighting the need to investigate the relationship between β-cell molecular heterogeneity and functional decline in T2D. A limitation to current approaches is the difficulty in establishing functional profiles for different subtypes of β-cells. Here we perform fluorescent labelling of individual β-cells according to their secretory capacity and combine it with single-cell RNA-sequencing in the same cell. Our approach is based on the use of fluorescent tracers of endocytic membrane retrieval, which we show can be used as a proxy for insulin granule fusion and secretion. We use this methodology to characterize β-cell states associated with higher secretory function in nondiabetic donors and to identify potential mediators of β-cell failure during progression to T2D. By investigating islets from mouse models with increasing levels of β-cell dysfunction, as well as human islets from donors with and without T2D, we identified genes and pathways associated to decreased β-cell secretory capacity in both mouse and human β-cells. Some of these genes are known regulators of insulin exocytosis and mitochondrial function and are now being validated through mechanistic studies. Overall, our approach sheds light on the relationship between molecular heterogeneity and secretory capacity in β-cells and offers potential avenues for the development of new treatments targeting β-cell secretory failure.

CT Heterozygous Hnf1a Mutation in Mice Reveals Multiple Pathogenic Mechanisms of HNF1A-MODY

***Isaline Louvet*[1], *Ana Acosta-Montalvo*[1], *Chiara Saponaro*[1], *Maria Moreno-Lopez*[1], *Nathalie Delalleau*[1], *Julien Thevenet*[1], *Valery Gmyr*[1], *Julie Kerr-Conte*[1], *François Pattou*[1], *Adrian Liston*[2], *Caroline Bonner*[1]**

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Introduction: Heterozygous pathogenic variants in *HNF1A* cause HNF1A-MODY, the most common form of monogenic diabetes, characterized by age-dependent onset and progression. Despite HNF1A's established role in beta cell function, the mechanisms underlying disease progression remain understood, with conventional mouse models failing to recapitulate the gradual metabolic deterioration observed in patients.
Aims: To determine the tissue-specific role of *HNF1A* deficiency and identify the sequence of metabolic disruptions that drive disease progression.
Methods: We developed mice with a heterozygous deletion of the *Hnf1a* transactivation domain (Hnf1a$^{+/\Delta e4-10}$) and complemented these studies with *HNF1A* knockdown in human islets.
Results: Like their human counterparts, these mutant mice were normoglycemic at birth, with metabolic abnormalities emerging progressively. Male mutants exhibited a sequential dysfunction: testosterone deficiency during puberty (8-12 weeks), preceding fasting hyperglycemia in young adulthood (12-16 weeks), followed by glycosuria and glucose intolerance in mature adults (25-35 weeks) that worsened with advanced age ($>$ 45 weeks). Aging mutant mice displayed elevated fasting glucagon, increased endogenous glucose production, and hepatic fat accumulation, while maintaining normal plasma insulin levels compared to wild-type siblings. Islets exhibited decreased Androgen Receptor expression, hormonal dysregulation, impaired glucose-stimulated insulin secretion, and abnormal glucagon suppression, while

maintaining sulfonylurea sensitivity. Human islet studies demonstrated *HNF1A*'s critical role in alpha cells, where haploinsufficiency reduced SGLT2 expression while increasing glucagon content and secretion.

Conclusions: These findings establish *HNF1A* as a master regulator of multiple hormonal pathways and identify testosterone deficiency as a potential trigger for disease onset in males, suggesting therapeutic strategies for HNF1A-MODY beyond insulin-centric approaches.

List of Posters

Poster number: Session number + Poster number (*e.g. P-101= Session 1, Poster 01*)
Presenting authors are underlined

Session 1: Wednesday, 11 of June, 2025 16:55

P-101 EPDR1, an Obesity-Induced Protein, Support Autophagy and Functional Maturation of β-Cells

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Introduction and Aims: Ependymin-related protein 1 (EPDR1) was initially identified as an adipokine but is also expressed in pancreatic β-cells. Using knockdown (KD) models, we showed EPDR1 is essential for glucose-stimulated insulin secretion (GSIS) and mitochondrial metabolism—findings validated in human islets. Recombinant human EPDR1 (hEPDR1) enhances insulin secretion in both rodent and human β-cell models. EPDR1 expression rises during in vitro β-cell differentiation and is elevated in islets from obese and type 2 diabetic (T2DM) individuals, correlating with BMI. As autophagy and mitophagy are vital for cellular homeostasis and β-cell differentiation, we aim to demonstrate that EPDR1 is upregulated under metabolic stress to support these processes and enhance differentiation.
Materials and Methods: Ins1 832/13 and EndoC-βH1 cells were used. RNA-seq, Western blot, qPCR, and ELISA were performed under autophagy inducing/blocking conditions. hESC and iPSC differentiation was done over 35 days with hEPDR1 (2.5 μg/mL) added from day 17.
Results: RNA-seq identified autophagy as a key altered pathway in EPDR-KD cells. EPDR1 was upregulated by autophagy-inducing conditions and downregulated at the protein level by autophagy inhibition, suggesting post-transcriptional regulation. Despite KD, EPDR1 remained responsive to autophagy, with findings replicated in EndoC-βH1 cells. KD impaired autophagic flux, reduced basal p62, and increased PINK1 and mitochondrial complexes subunits, indicating defective autophagy/mitophagy. Elevated levels of early autophagy mediators suggested partial compensation for EPDR1 loss. Autophagy induction restored insulin production and secretion in KD cells. EPDR1 expression increased towards the end of β-cell differentiation, and hEPDR1 treatment enhanced differentiation outcomes in hESCs and iPSCs.
Conclusion: EPDR1 is required for efficient autophagy/mitophagy and promotes β-cell differentiation, representing a promising target in diabetes research.

P-102 Inflammatory stress drives isomiR production through $3'$ end trimming of microRNAs in human pancreatic islets and beta cells

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Introduction: Although miRNAs in pancreatic islets have been widely studied, their post-transcriptional modifications ($3'/5'$ nucleotides trimming/extensions and nucleotide variations), known as isomiRs, are often overlooked. Inflammation may influence the enzymes involved in isomiR biogenesis, potentially linking these molecules to inflammatory processes in beta cells during Type 1 Diabetes (T1D).
Aims: This study investigates the relationship between islet inflammation and changes in isomiR expression profiles.
Methods: IsomiR expression was assessed in three small RNA-seq datasets: Human islets treated or not with IL-1β+TNF-α+IFN-γ for 24h ($n = 3$) and 48h ($n = 10$). EndoC-βH3 ($n = 5$) untreated or treated with IL-1β+TNF-α+IFN-γ for 8h, 24h, or 48h. miRNAs and isomiRs were quantified and differential proportion analysis was performed to evaluate alterations of isomiRs. Gene Ontology Enrichment Analysis (GOEA) was performed on TargetScan-predicted targets of $3'$ trimmed isomiRs. RT-PCR was performed to quantify the expression of exoribonucleases.
Results: Significant $3'$ trimming of miRNAs was observed in response to inflammation ($FDR < 0.05$). In human islets, 31 miRNAs at 24h and 61 miRNAs at 48h showed increased $3'$ trimming, while 45 miRNAs were trimmed in EndoC-βH3 cells at 48h. GOEA revealed the regulation of immune-related processes by $3'$ trimmed isomiRs. The exoribonuclease *ISG20* was significantly upregulated after cytokines treatment and associated with $3'$ trimmed isomiRs, suggesting its role in this process.
Conclusions: $3'$ trimming of miRNAs represents a response to inflammation in human islets and beta-cell, likely mediated by *ISG20*. This mechanism may provide insights into pathological processes in islet inflammation associated with T1D, especially considering the potential differential affinity of 3' trimmed isomiRs for their targets.

P-103 A Dual Approach to Studying Acute Pancreatitis: Live Cell Calcium Imaging and Tissue Damage Evaluation

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Acute pancreatitis (AP) is characterized by inflammation of the exocrine pancreas, leading to autodigestion and tissue damage. The interplay between exocrine and endocrine functions is critical, as acinar cell damage can affect endocrine activity. However, the nature of these interactions remains unclear. Traditional histological techniques are incompatible with live-cell imaging, necessitating methods that can assess both tissue damage and live cell function. In this study, we combine the LiveDead fluorescence assay for tissue damage evaluation with live cell calcium imaging to assess endocrine function.
AP was induced in adult male NMRI mice through repeated cerulein injections. Two separate cohorts were used: one for validating classical histological methods, which is hematoxylin eosin staining and the other for calcium imaging and LiveDead staining. Pancreatic tissue slices from the second cohort were stained with a calcium indicator dye to monitor beta cell calcium dynamics via confocal microscopy, and the LiveDead assay assessed tissue damage.
Our results showed that AP increased beta cell oscillatory activity in response to glucose. Histological analysis revealed classic signs of AP, including edema, necrosis, and inflammatory infiltration. No significant regional differences in tissue damage were observed, and the islets of Langerhans remained intact. The LiveDead assay strongly correlated with histological methods in assessing pancreatic damage.
This study highlights that AP enhances beta cell activity and demonstrates the utility of the LiveDead assay for evaluating pancreatic damage while simultaneously assessing endocrine function. This dual approach provides insights into the relationship between exocrine damage and islet function in AP.

P-104 G-protein coupled and purinergic receptors collectively regulate lineage specification in the developing pancreas

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The G-protein coupled receptor (GPCR) family regulates several cellular processes during development and in the adult organism. Several GPCRs and their downstream effectors are expressed during pancreas development. Previous work showed that sphingosine-1-phosphate receptor 2 (S1pr2) signaling is necessary for the specification of the acinar and endocrine lineages in the mouse, with endocrine specification relying on the function of the Gai subunit. However, the broader role of GPCRs in pancreas progenitor differentiation and lineage allocation has not been fully explored.
This study aims to systematically address the role of Ga subunits and the GPCR-related purinergic receptor P2rx1 in pancreas progenitor differentiation and lineage specification.
We utilized air-to-liquid interface (ALI) organotypic cultures of 14.5 dpc mouse embryonic pancreata and employed selective inhibitors or activators to manipulate the signaling pathways. The survival, proliferation, and differentiation patterns of pancreatic progenitors were analyzed using immunofluorescence and quantitative image analysis. Our findings show that different signaling pathways regulate specific lineages through selective proliferation, cell death, and differentiation. Interestingly, RNA sequencing analysis did not show significant regulation of lineage markers at the transcriptional level. However, there was differential regulation of numerous genes encoding ribosomal protein subunits. Ribosome profiling of embryonic pancreata revealed a discordance between expressed and translated transcripts, uncovering a novel aspect of pancreas differentiation. These results suggest that GPCR and purinergic receptor signaling pathways contribute to pancreas differentiation through ribosomal translational regulation, offering new insights into lineage specification mechanisms.

P-105 A 94 bp deletion in the *SLC16A1* gene causes aberrant expression of MCT1 in pancreatic β-cells leading to hyperinsulinaemic hypoglycaemia

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Introduction: Hyperinsulinaemic hypoglycaemia is characterised by low blood glucose levels due to inappropriate insulin secretion. We identified a heterozygous 94 bp deletion in the promoter of *SLC16A1* in 31 individuals from 11 families with hyperinsulinaemic hypoglycaemia. *SLC16A1* encodes the monocarboxylate transporter 1 (MCT1) protein, which is expressed in most tissues except pancreatic β-cells, where its expression is silenced. It is hypothesised that the 94 bp deletion disrupts MCT1 repression in β-cells, allowing pyruvate entry and inappropriate insulin secretion following anaerobic exercise, causing exercise-induced hyperinsulinism (EIHI).
Aims: To investigate whether the 94 bp deletion in the *SLC16A1* promoter leads to inappropriate expression of MCT1 in the pancreatic β-cells of affected individuals.
Methods: Immunohistochemistry studies were conducted on FFPE pancreatic tissue from four individuals with the 94 bp deletion, all of whom had undergone surgery for the clinical management of persistent hypoglycaemia. Using OPAL technology, sections from these patients and age-matched controls were stained for insulin, glucagon, somatostatin, and MCT1. Slides were scanned with the VectraPolaris PhenoImager and quantitatively analysed using QuPath (v0.5.1). For qualitative analysis of MCT1 intracellular localisation, islets were imaged using confocal microscopy.

Results: Aberrant MCT1 expression was observed in the pancreatic tissue of the four affected individuals but not in age-matched controls. MCT1 was localised to the cell membrane and was expressed in both the islets and exocrine pancreas.
Conclusions: Our findings confirm that the *SLC16A1* 94 bp deletion causes EIHI by inducing aberrant MCT1 expression in pancreatic β-cells, likely triggering insulin secretion due to the inappropriate entry of pyruvate into β-cells following exercise.

P-106 Plant-Derived FT50 Prevents Obesity and Insulin Resistance in a Diet-Induced Mouse Model of Metabolic Syndrome

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Introduction Metabolic syndrome, a widespread metabolic disorder driven by rising obesity rates, markedly increases the risk of cardiovascular disease and type 2 diabetes. High-fat, high-carbohydrate diets are key contributors to obesity in both humans and rodents, making mice an essential model for studying its pathophysiology.
Methods and aims This study examines the impact of FT50, a plant extract, on obesity, glucose tolerance, insulin resistance, and metabolic syndrome progression. FT50 is thought to lower blood glucose levels by inhibiting carbohydrate digestion and absorption in the gastrointestinal tract. To evaluate its effectiveness, male C57BL/6J mice were fed a carbohydrate-rich Western diet for 12 weeks, leading to metabolic syndrome marked by significant weight gain, glucose intolerance, and insulin resistance.
Results Incorporating FT50 into the diet effectively prevented weight gain and metabolic dysfunction. FT50 supplementation not only halted the onset of glucose intolerance and insulin resistance but also improved glucose tolerance and insulin sensitivity. These effects suggest that FT50 mitigates metabolic dysregulation by modulating glucose absorption and improving insulin signaling.
Conclusions Our findings highlight FT50's potential as a plant-based therapeutic for metabolic syndrome and type 2 diabetes, supporting further investigation into its clinical applications.

P-107 Implication of spermine oxidase (SMOX) in pancreatic β-cell function

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Introduction and aims Type 2 diabetes (T2D), the most prevalent form of diabetes, arises from dysfunction and/or loss of insulin-producing β-cells. Our study aimed to better understand the metabolic pathways regulating pancreatic islet function.
Methods We identified a potential metabolic actor in islets of T2D patients using the RNA-seq biobank at Lund University Diabetes Center. To explore its role in β-cell function, its expression was silenced using siRNAs in rat INS-1 832/13 cells and human islets. Insulin secretion was measured using ELISA assay kits, and metabolic function by respirometry.
Results We found increased spermine oxidase (SMOX) mRNA in islets of T2D patients, correlating positively with insulin and negatively with glucagon expression. SMOX catalyzes spermine oxidation to spermidine, crucial for β-cell function. Nevertheless, the specific role of SMOX in β-cells remains unclear. Silencing of *SMOX* reduced glucose-stimulated insulin secretion in both of our models. In INS-1 832/13 cells, *Smox* silencing also impaired insulin secretion stimulated by pyruvate and KCl (a depolarizing agent inducing insulin exocytosis), and decreased insulin mRNA level, insulin

content and glucose-stimulated oxygen consumption. Finally, INS-1 832/13 cells exposure to high glucose and palmitate (mimicking the glucolipotoxic conditions encountered by β-cells in T2D) increased *Smox* mRNA expression.
Conclusion Our study suggests that *SMOX* upregulation in T2D may be a compensatory mechanism to maintain insulin output under metabolic stress, potentially via modulations of both insulin production and mitochondrial metabolism. A better characterization of *SMOX*'s impact on β-cell function could contribute to novel therapeutic strategies for T2D.

P-108 Generation of stem-cell derived isletswith enhanced purity and functionality for treatment of Type 1 Diabetes

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Primary islet transplantation is a promising treatment for type-1 diabetes, but limited availability of donors, poor yield and immune rejection are the major obstacles with primary islet transplantation. Yet stem cell derived-islets (SC-islets) provide an alternative to overcome challenges associated with primary islet transplantation. Although, several protocols have been developed by different labs, the composition and purity of these SC-islets remains a challenge. SC-islets, from most of the protocols, contains approximately 20% to 40% unwanted cells (ductal, exocrine and enterochromaffin) apart from alpha (SC-alpha) and beta cells (SC-beta). These unwanted cells can cause potential risk and threat to teratoma formation after transplantation; hence to get rid of these unwanted cells is very important for future transplantation studies and clinical trials. So, our aim was to find novel cell surface markers which can be utilized for enrichment of SC-alpha and SC-beta cells.
By using multiomic CITE-Seq and flow cytometry-based approaches, we identified a specific cell surface marker which is detected on SC-beta and SC-alpha, but not in the unwanted cells. We have utilized this antibody to enrich the alpha and beta cells while efficiently eliminating unwanted cells. This resulted in SC-islets with 98% purity (beta and alpha cells), devoid of exocrine, ductal or enterochromaffin cells. Moreover, the *in-vitro* functionality shows that elimination of the unwanted cells improved the functionality of the islets achieving more than 10-fold increased insulin release following glucose stimulation. To summarize, we have developed a method to generate pure and highly functional SC-islets for treatment of type-1 diabetes.

P-109 Increased somatostatin receptor 2 expression in alpha cells could contribute to decreased glucagon secretion and hypoglycaemia in Type 1 diabetes.

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Introduction: Glucagon secretion is reduced in Type 1 diabetes (T1D) and in autoimmune non-obese diabetic (NOD) mice compared to non-diabetic (ND) NODs. This is associated with increased somatostatin (SST) secretion within diabetic NOD and human T1D islets, contributing to the glucagon deficit and hypoglycaemic episodes in man. Islet paracrine suppression of glucagon by somatostatin is largely mediated via α-cell SST receptor 2 (SSTR2).
Aims: To identify α-cell SSTR2 and quantify T1D-related changes by morphometry in non-diabetic and diabetic NOD mouse islets and human donors.
Methods: SSTR2 was identified by immunofluorescence (IF) in wax-embedded pancreas from normoglycaemic and diabetic NOD mice and human non-diabetic (ND) and T1D donors ($n = 4$ for each group). Changes in IF labelling for SSTR2 in SST^{+ve} and glucagon^{+ve} cells were calculated using Image J and Pearson's co-localisation coefficient. To examine the effects of inflammation or hyperglycaemia, ND mouse islets were cultured o/n in a cytokine cocktail or at

20mM glucose and embedded in wax for IF and morphometry.
Results: α-cell SSTR2 expression in diabetic NOD islets was 2.3-fold ($p < 0.05$) higher vs ND islets; Pearson's coefficient: 0.7 vs 0.3. Similarly, SSTR2 was 2.2-fold higher ($p < 0.05$) in α-cells of T1D donors compared to ND; Pearson's coefficient: 0.58 vs 0.26. SSTR2 expression was similar in both human and mouse delta cells. SSTR2 was increased by cytokine treatment by 1.72-fold but unchanged by hyperglycaemia.
Conclusions: Alpha cell sensitivity for paracrine SST inhibition via SSTR2 is increased in T1D via cytokine-mediated receptor upregulation. This could contribute to hypoglycaemia-induced glucagon deficit in T1D.

P-110 Discovery of a Genetic Rescuer: A Novel Modifier Preventing Insulitis and Diabetes in Gimap5 Mutant BB Rats

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Introduction: The mechanisms underlying T-cell-mediated destruction of pancreatic islet β-cells in spontaneously diabetic BioBreeding (BB) rats remain unclear. Identifying genetic factors influencing this process is essential for understanding insulitis and diabetes pathogenesis.
Aims: This study aims to elucidate genetic interactions that modulate β-cell loss by using cross-intercross breeding to refine diabetes- and lymphopenia-associated loci on chromosome 4.
Methods: Since 1980, systematic breeding has introduced increasing proportions of Diabetes Resistant (DR) DNA into BB rats' Diabetes Prone (DP) background. Two congenic lines were generated: sBBM (1.02–1.26Mbp DP DNA) and xshBBM ($\sim$0.09Mbp DP DNA). Whole-genome sequencing (WGS) was performed to identify genetic variants.
Results: Both lines retain a frameshift mutation in *Gimap5*, linked to insulitis, diabetes, and lymphopenia. However, xshBBM rats remain diabetes-free, suggesting the presence of a protective genetic factor. Our WGS analysis identifies *Gimap4* as a candidate genetic rescuer. Wild-type (WT) *Gimap4* prevents insulitis and subsequent diabetes and eliminates serum IgE elevation, an early insulitis biomarker. Sequencing reveals that the mutated *Gimap4* carries a frameshift mutation that alters its protein sequence.
Conclusions: Our findings suggest a genetic interaction between *Gimap5* and *Gimap4*, where WT *Gimap4* mitigates the deleterious effects of the *Gimap5* mutation, preventing insulitis and diabetes development. This discovery offers new insights into the genetic regulation of T-cell-mediated β-cell killing and should prove helpful for novel therapeutic strategies.
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P-111 Discovery of a Genetic Rescuer: Expression of Gimap4 in the diabetes protected BB rat

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Introduction: Failure to express the *Gimap5* protein is associated with lymphopenia, insulitis and spontaneous diabetes in the diabetes-prone (DP) BB rat. Recently we discovered that another member of the *Gimap* family, *Gimap4*, may rescue the deadly effects of lacking the *Gimap5* protein.

Aim: To identify the thymic T cell expression of *Gimap4* in two congenic lines carrying the mutated *Gimap5* gene, however, one line is no longer lymphopenic and does not develop diabetes.
Methods: FACS sorting were conducted of thymus cells for gene expression to be determined by RNA sequencing. The protein sequences of *Gimap4* differs at the C-terminal end, between DP and DR (diabetes resistant). Peptides were synthesized and antibodies were raised in goats (Capra Science Antibodies AB, Sweden). Multiple color immunohistochemistry was used on paraffin sections.
Results: Sorted thymus cells from sBBM (representing $Gimap4_{DP}$ and $Gimap5_{DP}$) and from xshBBM (representing $Gimap4_{DR}$ and $Gimap5_{DP}$) revealing 4% double-negative cells, 60% double-positive, 20% CD4-positive and 15% CD8-positive cells. The $Gimap4_{DR}$ peptide generated high titer antiserum, different from low titer $Gimap4_{DP}$ antiserum. Immunostaining with CD4, CD8 and the $Gimap4_{DR}$ antibody revealed that $Gimap4_{DR}$ was predominantly detected in the medulla of the thymus along with CD4 positive cells. CD8 positive cells were mostly observed in the cortex and appeared negative for $Gimap4_{DR}$.
Conclusions: $Gimap4_{DR}$ may be predominantly expressed in CD4-positive and CD8-negative cells in the thymus. It is speculated that beta-cell reactive CD4-$Gimap4_{DR}$ positive cells may be eliminated in the thymus medulla, thereby preventing lymphopenia, insulitis and diabetes.
Funding: Supported by Barndiabetesfonden, Sven Mattssons Stiftelse, Diabetesfonden (DIA2023-844), Sydvästra Skånes Diabetesförening, the National Genomics Infrastructure, the Strategic Research Area Exodiab (2009-1039), and the Swedish Foundation for Strategic Research (IRC15-0067).

P-112 Volume reduction rate in human and rat pancreatic islets in culture after isolation

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Introduction: Accurate pancreatic islet volume estimation is essential for graft dose calculation and *in vitro* studies. Isolated islets undergo changes in morphology, which can impact data normalization in metabolic or secretory assays. Failure to account for early post-isolation shrinkage may lead to misinterpretation of results. However, individual islets volume dynamics remain uncharacterized.
Aims: To quantify the volumetric changes of individual rat and human pancreatic islets during the first hours in culture.
Methods: Human or rat islets were isolated and seeded in a μ-Slide with supplemented medium, within an hour after the end of isolation. Time-lapse imaging was performed using a Leica DMi8 microscope (37°C, 5% CO_2), acquiring images every 20 minutes for the first 3 hours, then hourly up to 17 hours. Images were segmented using the GraphCut plug-in in Fiji, and volumes were estimated from islet sizes and projection shapes using our Spinacle model.
Results: A total of 83 Wistar rat islets from four donors exhibited an average volume reduction of 14% (CV 0.07), while 42 human islets from a single donor showed a 14% reduction (CV 0.06). In both cases, 50% of the total volume loss occurred within the first 3 hours.
Conclusions: Freshly isolated pancreatic islets exhibit a rapid volume reduction of 14% on average, reaching half of that change within the first 3 hours. Great variability was observed among the individual islets, irrespective of the species or size. Supported by grant NU22-0100141.

P-113 δ-cells control subset of β-cells in mouse pancreatic islets

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Introduction: Somatostatin is a powerful inhibitor of insulin secretion and β-cell electrical activity, but the effects are weak in intact islets, possibly because of high intra islet somatostatin levels.
Aim: To interrogate the paracrine and electrical control of β-cells by δ-cells.
Methods: We used optogenetics combined to hormone secretion measurements, electrophysiology and $[Ca^{2+}]_i$ imaging.
Results: Opto-activation and -inhibition of δ-cells stimulated and inhibited their electrical activity and somatostatin secretion, respectively. Unexpectedly, neither optoactivation nor -inhibition of δ-cells had any effect on insulin secretion at 1 or 20 mM glucose. However, optoactivation of δ-cells at 6 mM glucose paradoxically increased insulin secretion by 113%, an effect that correlated with β-cell action potential firing. In $[Ca^{2+}]_i$ imaging experiments, optoactivation of δ-cells induced islet-wide β-cell $[Ca^{2+}]_i$ transients and synchronized the oscillatory pattern induced by 7 mM glucose. Conversely, optoinhibition of δ-cells and somatostatin secretion reduced rather than increased β-cell electrical activity and $[Ca^{2+}]_i$ in $<$10% of the β-cells and only in those situated $<$20 μm from δ-cells.
Conclusion: Somatostatin inhibits δ-cells. We therefore predicted that optical activation and inhibition of δ-cells would respectively inhibit and stimulate insulin secretion. Paradoxically, photoactivation of δ-cells at 6 mM glucose stimulated insulin secretion, causing inward depolarizing currents and action potential firing in β-cells. Conversely, optogenetic hyperpolarization of δ-cells interrupted $[Ca^{2+}]_i$ oscillations in $\sim$10% of β-cells, suggesting a bidirectional signal transmission via gap junctions. Optoactivation of δ-cells initiates a regenerative wave of β-cell activation, possibly linked to hub cells, facilitating rapid β-δ-β-cell electrical coupling. δ-cells, with their extended morphology, act as islet "interneurons."

P-114 Serum levels of the inflammatory protease cathepsin S are elevated at disease onset and positively associated with TNF-α and β-cells stress in new-onset type 1 diabetes.

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Introduction: We recently demonstrated that cathepsin S (CTSS) is induced and secreted from human β cells during inflammation and that CTSS serum levels are elevated in children with new-onset type 1 diabetes and autoantibody-positive siblings; suggesting a promising early biomarker potential.
Aims: To investigate the associations between circulating CTSS and β-cell function and inflammation in individuals with new-onset type 1 diabetes who underwent a randomized clinical trial.
Methods: Serum levels of CTSS and cytokines (IFN-γ, IL-8, IL-10, TNF-α) were quantified by ELISA and MSD, respectively, in 55 individuals with new-onset type 1 diabetes before and after 52 weeks of treatment with oral fenofibrate or placebo. Mixed-effect and linear regression models were used to assess the effect of fenofibrate on CTSS and cytokines and to evaluate the associations between CTSS and stimulated C-peptide, cytokines, and proinsulin/C-peptide ratio.
Results: Serum CTSS was decreased at 52 weeks compared to baseline in both the placebo ($p < 0.001$) and fenofibrate group ($p < 0.05$), whereas TNF-α was decreased in the placebo group only ($p < 0.01$). The decrease in both CTSS

and TNF-α was less in the fenofibrate group compared to placebo ($p < 0.05$). CTSS was inversely associated with stimulated C-peptide at baseline ($p < 0.05$) and positively correlated with IL-8 ($p < 0.05$), IFN-γ ($p < 0.05$), and TNF-α ($p < 0.01$) at baseline and/or 52 weeks. In the fenofibrate group, the change in CTSS was positively associated with the change in proinsulin/C-peptide ratio ($p < 0.001$).
Conclusions: Our findings support the potential of CTSS as an early biomarker of islet inflammation and β-cell dysfunction in new-onset type 1 diabetes.

P-115 Glucose regulates mitochondrial motility, localisation, and sub-plasma membrane ATP production in pancreatic α-cells

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Background and aim: Type 2 diabetes is associated with dysregulated glucagon secretion. Despite this, we do not understand the basic mechanisms governing glucagon secretion, nor the cause of impairment in diabetes. In α-cells, glucagon secretion is controlled by ATP generated in the mitochondria. Increased fatty acid oxidation elevates ATP, which closes K_{ATP} channels, leading to electrical activity and glucagon secretion. In neurons, mitochondrial motility enables local energy supply and facilitates neurotransmitter release. Here, we aimed to investigate the role of mitochondrial motility and localisation in the regulation of glucagon secretion from α-cells.
Methods: We developed and validated complementary imaging analysis tools to quantify the spatiotemporal dynamics of mitochondria in α-cells from isolated mouse islets using live-cell confocal time-lapse imaging. This method was used in combination with electron microscopy, hormone secretion assays, immunofluorescence staining, and shRNA gene silencing.
Results: We show that mitochondrial motility is reduced ($26.5 \pm 5.1\%$) with increased secretion in 1 mM glucose (1G) ($0.4 \pm 0.05\%$ of content) compared to 5 mM (5G) ($0.2 \pm 0.04\%$ of content). Moreover, mitochondria localised closer to the cell periphery in 1G compared to 5G (distance from nucleus 2.09 ± 0.13 μm vs. 1.64 ± 0.08 μm, respectively), causing a higher ATP/ADP ratio in the sub-plasma membrane compartment (1.22 ± 0.02 vs. 1.18 ± 0.003 PercevalHR$_{488/405\,nm}$ fluorescence). While mitochondrial motility in α-cells was regulated by glucose, this was not mediated by the mitochondrial Rho GTPase, Miro1.
Conclusion: These findings suggest that mitochondrial movement and sub-plasma membrane ATP production play a critical role in the regulation of glucagon secretion independently of Miro1.

P-116 Mesenchymal stromal cells mitigate the detrimental effects of endoplasmic reticulum stress on insulin secretion from mouse islets.

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Introduction: Islet β-cell dysfunction has been linked to endoplasmic reticulum (ER) stress. Our previous studies demonstrated that preculturing islets with bone marrow-derived mesenchymal stromal cells (BM-MSCs) enhanced insulin secretion. However, it remains unclear whether BM-MSCs can also improve insulin secretion from islets experiencing ER stress.
Aims: To investigate the impact of BM-MSC co-culture on insulin secretion from β-cells under ER stress.
Methods: Islets isolated from KINGS mice, a model characterised by severe β-cell ER stress, and WT mice were cultured for 72 h on a BM-MSC monolayer. Islets were retrieved and incubated for 1 h in the presence of 2 mM and 20 mM glucose, then insulin secretion was measured by radioimmunoassay. Statistical analysis was performed by two-way ANOVA followed by the Holm-Sidak test.
Results: Basal and glucose-stimulated insulin secretion from male and female KINGS islets was lower than from WT

islets, with a pronounced impairment in male KINGS islets. While 20 mM glucose did not significantly stimulate insulin secretion from male KINGS islets (2 mM glucose: 0.033±0.0097 ng insulin/islet/h, 20 mM glucose: 0.057±0.0094 ng insulin/islet/h; $p > 0.5$), BM-MSC co-culture restored their ability to secrete insulin in response to glucose (2 mM glucose: 0.028±0.0042 ng insulin/islet/h, 20 mM glucose: 0.170±0.036 ng insulin/islet/h; $p < 0.001$). Female KINGS islets showed a significant insulin secretory response to 20 mM glucose (2 mM glucose: 0.069±0.055 ng insulin/islet/h, 20 mM glucose: 0.850±0.0097 ng insulin/islet/h, $p < 0.05$), which was not further improved by BM-MSCs.
Conclusions: BM-MSC co-culture enhances glucose-stimulated insulin secretion from male KINGS islets characterised by severe ER stress.

P-117 Minute levels of beta-cell stress elicits compensatory proliferation in the murine pancreatic islets of Langerhans

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Introduction: Regeneration involves damaged tissue or organ replacement in adulthood. The murine pancreas shows remarkable plasticity, adapting its response based on the extent and cause of beta-cell loss. When destruction is total, new beta-cells arise through transdifferentiation, while partial loss triggers proliferation of surviving cells. However, how islets respond to local beta-cell stress without significant cell loss remains underexplored.
Aims: This study investigates the murine islet response to minimal beta-cell injury in vivo, focusing on adaptive responses that occur without a significant beta-cell population reduction.
Methods: We induced beta-cell stress using a single low-dose of streptozotocin (STZ, 50 mg/kg) and collected physiological, transcriptomics and morphometric data at different periods after injury. These were compared to our previous results (Mathisen, Larsen et al., *Comms. Biol*, 2024) on a partial (50%) ablation model.
Results: Two-weeks post STZ injection, we observed a five-fold, transient, increase of Ki67^{+} in alpha- and beta-cells compared to citrate buffered-saline injected controls, which continued through the first month post-injury. Interestingly, both alpha- and delta-cell populations increased over time. Transcriptomics analyses indicated an increase in the STZ samples' proliferation at 30 days post ablation (30DPA) compared to their DT-50% counterpart. Furthermore, the proliferative signature was similar to the one characterizing the early DT effect (5DPA). This compensatory proliferation event was triggered in the two conditions by different molecular mechanisms, being defined by distinct activity profiles of Notch-, Fgf- or Hippo-signaling.
Conclusion: A single low dose of STZ can induce compensatory proliferation by a specific molecular mechanism, revealing a local intra-islet interplay between cell populations under stress.

P-118 Comprehensive multi-omics profiling of endocrine progenitors across organs and species

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The intestine and endocrine pancreas are central to metabolic regulation through their specialized hormone-producing cells. Despite Neurogenin-3 (Ngn3) being a definitive marker for both pancreatic and intestinal endocrine progenitors,

the transcriptional landscapes and regulatory mechanisms underlying their lineage commitment remain largely unresolved. Deciphering these regulatory networks is essential for advancing regenerative and cell-replacement therapies for metabolic disorders such as obesity and diabetes. Here, we utilized the Ngn3-Venus fusion (NVF) reporter mouse line, which faithfully reflects endogenous Ngn3 expression, to conduct paired single-nucleus RNA-seq and ATAC-seq analyses in the pancreas (E14.5–E16.5) and in enteroendocrine lineage-enriched intestinal epithelial cells. Our multi-omics approach uncovered complex gene regulatory networks governing pancreatic and intestinal endocrinogenesis, including transcription factor (TF) binding landscapes and their associated downstream target genes. Comparative analysis of human and mouse embryonic pancreatic datasets identified both conserved and species-specific TFs that orchestrate endocrine progenitor fate decisions and lineage commitment. To further validate lineage-specific transcriptional signatures, we performed proteomics analysis and integrated it with transcriptomics data, providing protein expression dynamics in endocrine progenitors and differentiated cell populations, thereby reinforcing the validity of the identified regulatory networks. Finally, cross-organ comparison revealed both shared regulatory programs and organ-specific factors guiding progenitor differentiation. Collectively, this study provides a comprehensive profiling of endocrine progenitors, offering new insights into cellular heterogeneity and lineage dynamics during endocrinogenesis across species and organs with implications for therapies targeting obesity and diabetes.

P-119 Inhibition of G protein-coupled receptor kinases 2 and 3 disrupts islet GLP-1 receptor-mediated signalling in mouse islets.

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Introduction and Aims: G protein-coupled receptor kinases (GRKs) regulate GPCR signalling through receptor desensitisation mechanisms. As alterations in GRK expression are associated with disease progression, we assessed the role of GRKs in islet function and GLP-1 receptor (GLP-1R) regulation using the GRK2/3 inhibitor Cmpd101.
Methods: Insulin secretion and cAMP accumulation were measured in mouse islets incubated with 1μM Cmpd101 $\pm$ 20nM Liraglutide (GLP-1R agonist) using radioimmunoassay and HTRF cAMP assay. 20-hour exposure to apoptotic stressors (TNFα, IL-1β, IFNγ cytokine cocktail or 500μM palmitate) were quantified by Caspase 3/7-GLO assay in islets pre-treated with compounds of interest for 48-hours. Data are expressed as mean$\pm$SEM% control and analysed by one- or two-way ANOVA (n=4-5 experiments).
Results: Cmpd101 alone did not affect glucose-induced insulin secretion (2mM vs 20mM glucose: $p = 0.006$; Cmpd101: $p = 0.153$ vs 20mM glucose), but it reduced GLP-1R-mediated potentiation of insulin secretion (+Liraglutide: $p = 0.007$ vs 20mM glucose; +Liraglutide/Cmpd101: $p = 0.18$ vs 20mM glucose). Cmpd101 also suppressed increases in cAMP stimulated by GLP-1R activation (+Liraglutide: 465$\pm$91%, $p = 0.032$ vs control; +Liraglutide/Cmpd101: 206$\pm$43%, $p = 0.21$ vs control). 48-hour treatment with 10nM/100nM/1μM Cmpd101 did not affect islet apoptosis. Cytokines induced caspase-mediated cell death (Cytokines: 288$\pm$58%, $p < 0.001$ vs control) and Cmpd101 treatment did not alter islet responses to cocktail exposure. Liraglutide mildly protected islets from lipotoxicity but this was diminished by co-incubation with Cmpd101 (Palmitate: 288$\pm$58%, $p = 0.0013$ vs control; +Liraglutide: 161$\pm$23%, $p = 0.038$ vs palmitate; Liraglutide+Cmpd101: 241$\pm$36%, $p = 0.85$ vs palmitate).
Conclusions: Inhibition of Grk2/3 alone had no detrimental effects on key β cell functions, but it impaired GLP-1R-mediated signalling.

P-120 Glucose-Dependent Regulation of m^{6}A Epitranscriptomic Machinery in Pancreatic β-Cells

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Introduction: Hyperglycemia, a key feature of type 2 diabetes mellitus (T2DM), impairs pancreatic β-cell function via glucose toxicity. Emerging studies link RNA modification N6-methyladenosine (m^{6}A) with cellular responses to metabolic stress. However, the glucose-induced regulation of m^{6}A machinery in β-cells remains poorly understood. Aim: To investigate how short-, medium-, and long-term glucose stimulation affects m^{6}A methylation levels and the expression of m^{6}A regulators in primary murine pancreatic islets and INS-1 β-cells. **Methods**: RNA sequencing, RT-qPCR, immunochemical analysis, and global m^{6}A/m methylation quantification. **Results**: In murine islets, total m^{6}A/m methylation levels were unchanged under glucose stimulation. However, key m^{6}A "reader" proteins showed altered expression: Igf2bp2, Ythdf2, Hnrnpd (mRNA stability/degradation), and Eif3, Ythdc2 (translation regulation). In INS-1 β-cells, a transient rise in global m^{6}A/m methylation was observed specifically after 24 hours of high glucose exposure, but not after 2 or 72 hours. This effect correlated with downregulation of the m^{6}A "eraser" ALKBH5 at both mRNA and protein levels. **Conclusion**: Our findings reveal distinct epitranscriptomic regulation in murine islets and INS-1 cells exposed to stimulating glucose. In murine islets, glucose affects m^{6}A machinery without altering global methylation. In INS-1 β-cells, transient m^{6}A/m increase is driven by ALKBH5 downregulation, highlighting a potential epitranscriptomic mechanism linking glucose levels to β-cell function and T2DM pathogenesis. **Funding**: This research was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) - Funded by the European Union - Next Generation EU, and Charles University Grant Agency project n. 243423

P-121 IFIH1-Mediated Immune Response and Differential Viral Susceptibility in Pancreatic α- and β-Cells

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Introduction Enteroviral infection of pancreatic islets has been linked to T1D. MDA5, encoded by the *IFIH1* gene, detects cytosolic enteroviral infection and triggers an interferon (IFN)-mediated antiviral response. β-cells are potentially more susceptible to enteroviruses than α-cells, due to differential expression of viral entry receptors and antiviral immune responses.
Aim This study examines β-cell susceptibility to enteroviral infection and the role of MDA5 in antiviral defense using stem-cell-derived islets (SC-islets).
Methods Wild-type (WT) and *IFIH1*-knock-out (*IFIH1*^{KO}) isogenic iPSC lines were differentiated into SC-islets and infected with Coxsackievirus-B (CVB) with or without IFNα pre-stimulation. Virus receptors (CAR and DAF), viral tropism, and immune responses were analyzed using flow cytometry (FC), IHC, RT-qPCR and live-imaging.
Results scRNA-seq data from SC-islets indicated lower *IFIH1* and higher *CAR* expression in β-cells compared to α-cells. FC analysis showed that the CAR receptor was predominantly expressed on β-cells while α-cells were largely CAR negative and DAF positive. A higher fraction of β-cell than α-cells were infected by CVB, independent of *IFIH1* expression. Extended live-cell imaging indicated a higher rate of infection in *IFIH1*^{KO} cells compared to WT during prolonged infections, partially mitigated by IFNα pre-stimulation. RT-qPCR analysis suggested that IFNα pre-stimulation induced *MDA5* expression, thereby enabling virus-infected cells to increase the expression of IFN-I (*IFNb*), IFN-III (*IFNλ1*), and the innate-immune response marker *CXCL10*.

Conclusion β-cells have higher levels of cell surface expression of the CVB receptor CAR and are more permissive to infection than α-cells. MDA5 is needed for a robust antiviral IFN response in SC-islets.

P-122 Synaptotagmin-13 modulates insulin secretion in pancreatic β-cells

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Introduction Restoring insulin secretion in dysfunctional β-cells is a key therapeutic target for type-2 diabetes (T2D). Synaptotagmins (Syts) are pivotal in coordinating insulin secretion. Misexpression of the atypical calcium-independent *Syt13* in islets from patients with T2D has been reported, but its impact on β-cells remains understudied.
Aims This study aims to decipher the role of *Syt13* in pancreatic β-cell function and how depletion of *Syt13* impacts insulin secretion in rodent and human models.
Methods We developed three β-cell-specific conditional knockout (CKO) mouse lines and generated a human stem cell-derived β-cell line deficient in *SYT13* using an *in vitro* differentiation protocol. We employed various *in vivo* and *in vitro* techniques, including glucose-stimulated insulin secretion assays, electron and high-resolution microscopy, proteomics, transcriptomics, and biochemical assays.
Results Mice lacking *Syt13* exhibit improved glucose tolerance and enhanced insulin secretion *in vivo*. Depletion of *SYT13* increased insulin granule exocytosis *in vitro* in both mouse and human β-cells. *Syt13* localized on insulin secretory granules and translocated to the plasma membrane upon glucose stimulation. Deletion of *Syt13* increased levels of soluble SNAP-receptor (SNARE) proteins mediating insulin granule exocytosis. Mechanistically, *Syt13* modulates the abundance of plasma membrane proteins at the secretory domain and subsequent endosomal sorting and degradation.
Conclusions This study demonstrates an endocytic function of *Syt13* modulating the abundance of a subset of plasma membrane proteins, thereby affecting insulin secretion. We provide novel mechanistic insights into the coupling of insulin secretion and endocytic sorting, thereby offering new potential for therapeutic development of T2D.

P-123 Role of Stac2 adaptor protein on membrane excitability and hormone secretion of endocrine cells

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Introduction: Stac adaptor proteins (Stac1–Stac3) have been identified as novel regulators of L-type voltage-gated Ca^{2+} channel (CaV) expression and biophysical properties. Functionally, it has only been shown in heterologous cell systems or cultured hippocampal neurons that Stac2 overexpression abolishes L-type CaV channel voltage-dependent inactivation.
Aims: Here we set to investigate if genetic ablation of the endogenous Stac2 protein alters mouse chromaffin cells (MCCs) or pancreatic β-cells CaV channels' biophysical properties, membrane excitability, and hormone release.
Methods: For this we used a combination of voltage-clamp, current-clamp, capacitance measurements, and glucose-induced dynamic insulin release.
Results: In MCCs, Stac2 deletion did not alter whole-cell calcium currents amplitude or inactivation kinetics but

significantly shifted the voltage-dependence of activation to more hyperpolarized potentials. Stac2$^{-/-}$ MCCs did not show altered resting membrane potential or spontaneous action potential (AP) firing frequency. However, the AP depolarization threshold was significantly reduced and step current injection elicited APs with higher initial firing frequency in Stac2$^{-/-}$ compared to WT. Interestingly, Stac2 deletion impaired the time course of MCC vesicle exocytosis. In pancreatic β-cells, Stac2 deletion caused a significant increase in glucose-induced membrane depolarization. While the AP-train duration and frequency were not affected, the AP threshold was significantly higher in Stac2$^{-/-}$ compared to WT. Contrary to increased excitability, 15 mM glucose stimulation showed an almost complete loss of first-phase insulin release albeit only a trend towards reduced total insulin release.
Conclusion: Cumulatively, we show that Stac2 is an important regulator of endocrine MCC and β-cell excitability and vesicle exocytosis.

P-124 Preferential transfer of mitochondria from mesenchymal stromal cells to stressed beta cells via extracellular vesicles improve islet oxygen consumption and functional survival

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Introduction: Human islet transplantation is compromised by the loss of functional beta cells. We have demonstrated that co-culture with MSCs enhanced islet beta cell insulin secretion and survival, which is associated with the transfer of mitochondria. Here we assess MSC-EVs as a route for mitochondrial transfer (mitotransfer) and investigate their effects on islet function.
Methods: Mitochondria in mouse/human bone marrow-derived MSCs were labelled with Bacman-GFP then MSC-EVs were purified from the conditioned medium using the Total Exosome isolation kit and characterised by Nanosight, immunoblotting (IB) and transmission electron microscopy (TEM). Human islets were treated with MSC-EVs (24–72h), after which mitotransfer was assessed by flow cytometry and confocal microscopy. Islet oxygen consumption rate (OCR) was measured using a Seahorse analyser. Islet function was assessed by measuring cytokine-induced apoptosis (caspase3/7) and glucose-stimulated insulin secretion (GSIS).
Results: GFP+ mitochondria from MSCs were detected in human islets after their treatment with purified EVs ($0.15 \pm 0.08\%$). GFP-mito signals co-localised with Mitotracker fluorescence in recipient beta cells, consistent with functional mitochondria. Mitochondrial proteins (TOM20/ATPB) were detected in $CD63+$ EVs by IB and immunoTEM. Exposing islets to stressors (cytokines or hypoxia) significantly increased mitotransfer ($23.2 \pm 0.3\%$; $p < 0.01$). GFP-mito from MSCs were observed predominantly in cells that expressed the stress markers Bip-1/HIF1-α. Treating islets with MSC-EVs increased OCR ($153 \pm 26.1\%$, $P < 0.05$), GSIS ($138 \pm 13.2\%$, $P < 0.001$) and protected against cytokine-induced apoptosis ($37 \pm 8.2\%$, $p < 0.01$).
Conclusion: MSC-derived EVs are sufficient to recapitulate some of the beneficial effects of MSC co-culture on islet function. These effects may be mediated by EV-dependent transfer of mitochondria.

P-125 Single-nucleus transcriptomics reveals cell type-specific regulatory profiles in human pancreas in obesity and type 2 diabetes

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The cell type-specific regulatory programs underlying obesity and type 2 diabetes (T2D) in the human pancreas remain poorly understood. Here, we performed single-nucleus RNA sequencing on pancreatic tissue from 19 non-diabetic lean (ND-Lean), 7 non-diabetic obese (ND-Obese) and 8 T2D pancreas donors, profiling a total of 58,018 nuclei. We captured a broad range of pancreatic cell types, including classical endocrine islet cells (α, β, δ, and γ), as well as diverse acinar and ductal subtypes. Notably, our dataset also includes immune, endothelial, and stellate cells, providing a comprehensive view of the pancreatic cellular landscape. We identified differentially expressed genes associated with obesity and T2D, uncovering key regulatory pathways and potential causal mechanisms. In addition, we investigated intercellular communication between islet endocrine cells and between endocrine and exocrine cell types, revealing remodeling of cell–cell signaling networks in obesity and T2D. Overall, our findings provide a comprehensive atlas of pancreatic remodeling across metabolic states and provide insights into potential targets for therapeutic intervention.

P-126 Characterising repressed epigenetic states at combinations of transcription factor binding sites in pancreatic islets

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Introduction: The loss of repression of beta-cell disallowed genes can lead to disease. Non-coding variants near *HK1* and *SLC16A1* genes lead to a failure of repression and subsequent loss of beta-cell function and hyperinsulinism. Work characterising *HK1*, identified that variants disrupted NFAT, NKX2-2 and FOXA2 binding sites, as well as *HK1* repression being mediated by a H3K27me3 polycomb domain. This raises the question of whether polycomb domains are found near this or any other combination of islet transcription factors (TFs).
Methods: ChIP-seq peaks for islet TFs (CTCF, FOXA2, MAFB, NKX2-2, NKX6-1, PDX1) were intersected to identify locations where TF pairs bind. We then calculated enrichment of polycomb repressed domains identified from islet ChromHMM epigenetic states, in the proximity ($+/-$ 1Kb) of either islet TFs alone or pairs of islet TFs.
Results: We found that polycomb domains are depleted near all islet TF bound loci with the exception of CTCF for which we find an excess (Odds ratio 2.13, $p < 10^{-300}$). For combinations of TFs we found that the depletion effect strengthens when CTCF is not involved, but we found that polycomb domains are enriched at FOXA2, NKX2-2, and PDX1 sites that co-bind with CTCF.
Conclusions: The enrichment of polycomb domains near CTCF is consistent with its structural role. These results suggest that islet TF binding sites that are co-bound with CTCF have a distinct role from those that do not. This work helps characterise a transcription factor signature for gene repression in pancreatic islets.

P-127 Activation of the glucose sensor enzyme glucokinase — a rational concept for future diabetes therapy?

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The glucose sensor enzyme glucokinase (GCK) is still a target structure of interest for medication of diabetes. The first GCK activator (GKA) was published 2003. However, glucose-independent continuous stimulation of GCK by GKAs led to severe hypoglycaemia. Because GKAs also showed liver toxicity, clinical trials were discontinued 2013. Recently, the GKA dorzagliatin (sinogliatin, HMS-5552, Hua Medicine) was published and approved in China for the treatment of T2DM. The aim was to compare dorzagliatin with the well-studied first-generation GKA RO-28-1675.
Human β-cell GCK was prepared recombinantly. Enzyme activity was measured photometrically at 2, 5 and 10 mmol/l

glucose in the absence or presence of 10 μM dorzagliatin or RO-28-1675.
Without GKA treatment, GCK enzyme activity was significantly ($p = 0.016$) threefold higher at 10 compared to 2 mmol/l glucose. Dorzagliatin, like RO-28-1675, led to a fivefold increase in GCK enzyme activity at 2 mmol/l glucose ($p < 0.0001$). At 5 and 10 mmol/l glucose, both compounds resulted in a significant doubling of GCK enzyme activity ($p < 0.01$). The increase in GCK enzyme activity was not significant in the presence of either RO-28-1675 or dorzagliatin comparing 2 versus 10 mmol/l glucose.
Both GKAs do not differ in their effect on GCK enzyme activity. In contrast to recent publications, our results cannot confirm that dorzagliatin activates GCK in a glucose-dependent manner. While dorzagliatin is already used in several clinical trials for the treatment of T2DM and MODY, experimental studies are lacking. These appear to be necessary to clarify the actual benefit of dorzagliatin.

P-128 Modulation of pancreatic islet function and viability by chemogenetically generated H_2O_2 via mitochondria-targeted HyPer7-DAAO fusion construct

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Excessive hydrogen peroxide (H_2O_2) levels contribute to oxidative stress and diabetes pathogenesis. Conversely, low H_2O_2 concentrations have been proposed as essential signaling molecules for insulin secretion. However, its causal role in pancreatic islet function remains unclear due to conflicting evidence and methodological limitations.
Here, we applied a chemogenetic D-amino acid oxidase (DAAO)-based approach to selectively induce mitochondrial H_2O_2 production in pancreatic islets upon D-alanine exposure. We evaluated islet function through hormone secretion, calcium dynamics, viability, and transcriptomic profiling.
We confirmed glucose-dependent mitochondrial H_2O_2 generation with increasing D-alanine concentrations. Acute D-alanine exposure (10 mM) elevated H_2O_2 without affecting static insulin secretion across glucose levels (2.8–20 mM). Under dynamic perifusion, acute H_2O_2 increased insulin secretion and calcium oscillations at low glucose (5.6 mM). In contrast, chronic exposure to higher D-alanine (20 mM) reduced insulin secretion. Remarkably, both acute and chronic H_2O_2 elevation abolished glucagon secretion at low glucose (0.5 mM) without impairing islet viability.
Transcriptomic analysis revealed that chronic mitochondrial H_2O_2 exposure upregulated pathways related to endoplasmic reticulum (ER) stress, secretion, and metabolic processes, while downregulating genes involved in ATP production, glycolysis, and mitochondrial complexes.
Together, our findings suggest that mitochondrial H_2O_2 may support insulin secretion at low-glucose conditions but impairs secretion chronically by disrupting islet metabolism and activating ER stress pathways. Additionally, mitochondrial H_2O_2 consistently exerts detrimental effects on glucagon secretion.

P-129 Dysregulation of innate antiviral immune pathways as an explanation of enterovirus-induced type 1 diabetes

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Introduction: Since the rising incidence of type 1 diabetes (T1D) cannot be linked to genetic drift, it has raised the question as to how non-genetic risk factors, e.g. viral infections, induce autoimmunity and β-cell destruction in T1D. Preliminary data indicate that β-cells exhibit lower expression of key innate antiviral immune pathway (InAIP) genes (*TLR3*, *TLR7*, *OAS1*) compared to α-cells, and a genome-wide association study identified 661 T1D-associated SNPs in 19 InAIP genes, suggesting a heightened vulnerability to viral infections in cases with T1D compared to control.
Aims: To investigate the role of enterovirus, namely Coxsackievirus B3 (CVB), in triggering β-cell autoimmunity (either directly or indirectly) and to characterize dysregulation of InAIP genes associated with T1D.
Methods: We infected EndoC-βH1 (direct) and THP-1 cells (indirect) with CVB3 Nancy (300 MOI) or Mock control (1h infection + 5h rest). From THP-1 cells, supernatant was collected, heat-inactivated and added to EndoC-βH1 cells (indirect). Infection was investigated by FACs using a CVB3 specific VP1 antibody. RNA was collected for bulk RNA-seq.
Results: The results are currently incomplete; however, preliminary FACs data revealed viral replication of CVB3 in EndoC-βH1 cells only. Furthermore, RNAseq indicate no major transcriptional changes in THP-1 cells infected with CVB3 compared to Mock. EndoC-βH1 RNAseq data analysis is currently ongoing and will be ready for the conference.
Conclusion: No conclusion can be made at this point in time; however, we anticipate being able to identify transcriptional changes and clarify the interaction between immune cells and CVB3 infection of EndoC-βH1 cells.

P-130 Impact of islet derived stellate cells on EndoC-βH1 cell viability, function and gene expression

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Introduction: Through AI-informed histopathological analysis, we have demonstrated presence of (peri)islet activated stellate cells in cystic fibrosis (CF), temporo-spatially associated with likely progression to CF-related diabetes (CFRD).
Aims: To establish human islet-derived stellate cells (ISCs) in primary culture and evaluate the impact of the secretome on gene expression in a human β-cell line.
Methods: ISCs were grown out of isolated human islets in 2D adherent culture with medium samples collected at culture Day 1/3/5/7 and cytokine secretion analysed (MSD). EndoC-βH1 cells were incubated with these medium samples or a cytomix (1 ng/ml IL-1β, 20 ng/ml TNF-α, 20 ng/ml INF-γ) for 72 hours. Impact on viability (MTS), glucose-stimulated insulin secretion (GSIS) and insulin content was assessed. Beta-cell bulk RNA-sequencing was performed using TruSeq Standard mRNA library prep kit.
Results: ISCs secreted cytokines including IL-1β, TNF-α, IL6 and IL8. No significant impact of ISC treatment / cytomix on EndoC-βH1 viability was observed. Incubation with ISC medium was associated with reduced insulin content. GSIS and insulin content were reduced following cytomix incubation.
Key β-cell transcription factors including MafA and NKX6.1 were down-regulated (1.6–2.3 fold) on incubation with ISC medium. Ingenuity Pathway Analysis revealed upregulation of interferon $\alpha/\beta/\gamma$-induced genes with ISC medium / cytomix; and IL6-activated genes with ISC medium.
Conclusion: Cultured ISCs secreted proinflammatory cytokines. Incubation of EndoC-βH1 with ISC secretome was associated with reduced insulin content. RNAseq revealed upregulation of cytokine-induced pathways and down-regulation of key β-cell transcription factors supporting a role of activated stellate cells in CFRD pathogenesis.

P-131 Assessing inter-islet synchronization with multicellular calcium imaging in pancreatic tissue slices

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Introduction: Pancreatic beta cells display complex oscillatory behavior, crucial for controlling insulin release. Achieving the pulsatile secretion patterns seen at a larger scale necessitates synchronized activity both within individual islets and across the entire islet population. Although the mechanisms driving synchronization within islets—primarily through electrical coupling via gap junctions, vascular interactions, and paracrine signaling—are well-documented, the processes governing coordination between islets remain largely unexplored and not fully comprehended.
Aims: To assess the degree of synchrony of fast, electrically driven oscillations and slow, metabolically driven oscillations in pancreatic tissue slices, both within individual islets and between them, and to investigate whether this synchrony can be influenced by external stimuli.
Methods: Confocal calcium imaging was performed in pancreatic tissue slices containing two closely positioned islets. The tissue was exposed either to constant stimulatory glucose or periodic glucose pulses of varying patterns. Fast and slow oscillatory components were analyzed separately, with a focus on their individual properties and the degree of synchrony.
Results: Our findings show that under constant glucose stimulation, both components of the calcium oscillations are well synchronized within individual islets, while no synchrony is observed between islets. In contrast, during pulsatile stimulation, inter-islet synchrony is virtually never observed in the fast component. However, the slow component can become synchronized across islets—but only when glucose variations are sufficiently large. This inter-islet synchrony in the slow component is linked to the effectiveness of entrainment.
Conclusions: While varying glucose levels can induce synchronized activity across islets, this requires relatively large amplitude variations. It is likely that additional mechanisms contribute to islet synchronization *in vivo*, particularly neural communication, which is disrupted during tissue slicing or islet isolation procedures.

P-132 Transcriptomic analysis of pancreatic islets suggests cell cycle activation in mice lacking Sodium-glucose cotransporter 1

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Sodium-glucose cotransporter 1 (SGLT1) is essential for intestinal glucose absorption and has been increasingly recognized as a therapeutic target for diabetes. In addition to its established role, emerging evidence links SGLT1 to pancreatic islet function and cellular composition. Previous published work shows that SGLT1 knockout (KO) in mice alters islet cytoarchitecture and function, though the underlying molecular mechanisms remain unclear. Here, we investigated the pancreatic islet transcriptome under SGLT1 ablation to better understand its role in islet cell integrity and maintenance.
Pancreatic islets were isolated from SGLT1 wildtype (WT) and KO mice and bulk RNA sequencing was performed to assess global gene expression profiles. Differentially expressed genes (DEGs) were categorized and functional annotation was performed to assess changes in biological pathways and processes involved. Transcriptomic analysis indicates that loss of SGLT1 results in upregulation of cell cycle associated genes in pancreatic islets, such as genes belonging to the cell division cycle (CDC) family, cyclins (*CCNA*, *CCNB*), and genes encoding the minichromosome maintenance (MCM) complex. Additionally, few genes for chemokine

receptors and ligands connected to immune response are upregulated in KO islets.
Although further research needs to be conducted to confirm transcriptomic changes at the protein level and to assess their impact on proliferation, these findings provide valuable insights into the role of SGLT1 in pancreatic islet physiology. By mapping the molecular pathways affected by SGLT1 ablation, we better our understanding of the role sodium-glucose transporters play in islet architecture and function, thereby providing new insights into endocrine pancreas biology.

P-133 Deficiency of stearoyl-CoA desaturase 1 activity propagates mitochondrial failure through compositional recalibrations of cardiolipin in lipid-laden pancreatic β-cells

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Introduction: Obesity-associated type 2 diabetes is a multifaceted malady in which insulin signaling is impaired from reaching peripheral effectors, combined with pancreatic β-cell demise. The accumulation of excess lipids in the pancreas accelerates mitochondrial dysfunction and unbalanced dynamics, which are considered early indicators of T2D. The availability of the FA pool determines the composition of the mitochondrial signature-cardiolipins (CL). Stearoyl-CoA desaturase 1 (SCD1) is the rate-limiting enzyme that catalyzes the biosynthesis of monounsaturated fatty acyl moieties and affects the overall rate of β-cell survival.
Aims: This study investigated the molecular effect of SCD1 depletion on the regulation of mitochondrial bioenergetics and architecture in pancreatic β-cells undergoing lipotoxic insult.
Methods: Mitochondrial morphology and CL composition were verified under pharmacological/genetic SCD1 deprivation in INS-1E cells and in pancreatic islets of SCD1$^{-/-}$ mice using imaging techniques and GC-MS analysis.
Results: Ablation of SCD1 activity led to more severe impairment of mitochondrial bioenergetics. This effect occurred in parallel with higher amounts of compromised mitochondria, and alterations in CL-linked OXPHOS complexes. The β-cells and pancreatic islets fraction of CL was enriched in 16:1, 18:1, 18:2, 20:4 and 20:3n-6 FAs. Such lipid rearrangements coincided with abnormal CL synthesis, altered abundance of the CL remodeling/fatty acyl moieties distribution and cristae shaping effectors. Increased hallmarks of lipid peroxidation complemented observations of palmitate-mediated collapse of cristae microarchitecture upon SCD1 deficiency.
Conclusions: Our findings uncover a bridging role of $\Delta 9$ desaturation integrating the CL acyl side chain composition and mitochondrial homeostasis to counteract lipotoxicity-derived mitochondrial damage in pancreatic β-cells.
Acknowledgements: This work was supported by a grant from the National Science Centre, Poland (UMO-2015/19/D/NZ4/03705).

P-134 Application of porcine pancreatic tissue slices for functional and physiological analyses

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Pancreatic tissue slices provide a valuable *ex vivo* model for studying pancreatic physiology and pathology while preserving the native microenvironment. Due to their anatomical and physiological similarities to humans, pigs serve as an excellent model for pancreatic research. This method allows simultaneous investigation of different pancreatic cell types, including β-cells and acinar cells, under near-physiological conditions. However, its application in the porcine

pancreas remains largely unexplored.
The aim of this study was to establish a reliable method for preparing viable porcine pancreatic tissue slices and to assess their suitability for calcium imaging and immunohistochemical analysis.
Fresh porcine pancreas was obtained and sectioned into thin tissue slices using a vibratome. Tissue viability was evaluated through live/dead staining. Functional imaging of β-cells and acinar cells was performed using calcium-sensitive dyes and fluorescence microscopy, while immunohistochemical staining was employed to identify specific cellular markers.
We successfully prepared porcine pancreatic tissue slices while preserving structural integrity and cellular functionality. Calcium imaging revealed dynamic responses in β-cells and acinar cells, confirming their functional viability. β-cells exhibited glucose-dependent calcium activity, while in acinar cells, calcium responses were triggered by acetylcholine. Immunohistochemical analysis successfully identified distinct pancreatic cell populations, and live/dead staining confirmed high tissue viability.
Porcine pancreatic tissue slices represent a promising *ex vivo* model for investigating pancreatic physiology and pathology. This approach enables real-time functional imaging and immunohistochemical analysis while preserving native tissue architecture. Our findings highlight the application of this method for future studies in diabetes research and pancreatic disease modeling.

P-135 Endogenous Glucocorticoid Receptor Activation Modulates Early-Stage Cell Differentiation in Pancreatic Progenitors of Mice and Humans

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Introduction: Glucocorticoids mediate ancestral signaling pathways involved in stress responses. The glucocorticoid receptor (GR) and its associated hormones were among the first signaling pathways ever studied. Their role during development, however, has been largely eclipsed by their critical function in late-gestation organ maturation, particularly in the lungs. Earlier developmental roles have predominantly been linked to pathological conditions, where atypical signaling (*e.g.*, due to stress or malnutrition) triggers premature GR activation. Previous reports suggest that glucocorticoids do not play a significant role in mouse pancreas development before the second transition.
Aims: Investigate GR signaling during early pancreas development in mice and humans.
Methods: We analyzed responses to GR activation in pancreatic progenitors using scRNA-seq, immunofluorescence, and RT-qPCR.
Results: In this study, we demonstrate that, under physiological conditions, the GR is selectively active in mouse pro-acinar and early endocrine cells from E11.5, silenced in bipotent progenitors, and reactivated during endocrine commitment. In mouse pancreatic explants, ectopic GR activation globally promotes acinar fate. Surprisingly, GR activation in human *in vitro*-derived multipotent pancreatic progenitors steers lineage commitment toward a bipo-

tent/endocrine trajectory and upregulates novel genes whose expression profiles resemble those of *SOX9* and *HES1* during human embryonic pancreatic bipotential and endocrine progenitor fate choice. Our combined epigenomic and single-cell transcriptomic analyses suggest that these novel marker genes may play important roles in human pancreas development.
Conclusions: Taken together, our findings position the GR pathway as an endogenous developmental modulator of early-stage pancreatic progenitor cell differentiation and provide insights into the underlying transcriptional mechanisms involved.

P-136 Revisiting beta-cell disallowed genes using human transcriptomic data

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Introduction Pancreatic β-cells require the repression of certain genes for proper glucose-stimulated insulin secretion. For example, *SLC16A1* and *HK1* are repressed to ensure insulin is not secreted in response to circulating pyruvate or low glucose, respectively. Variants interrupting regulatory elements of these genes, causing loss of their repression, cause congenital hyperinsulinism, a rare monogenic condition. Previous studies have implicated more than 100 putative beta-cell disallowed genes, however these used heterogeneous microarray datasets obtained exclusively from rodent models, producing discordant gene lists.
Aim We aimed to curate a list of pancreas-repressed genes in humans, leveraging multiple publicly available transcriptomic datasets.
Methods We examined gene-level bulk RNA-seq data from the Genotype-Tissue Expression (GTEx) project and tissue-based "pseudo-bulked" single-cell RNA-seq data from the newest release of the Tabula Sapiens consortium. We applied bespoke conditions to identify pancreas-repressed genes in both datasets.
Results We identified 251 pancreas-repressed genes from GTEx and 2,327 in Tabula Sapiens, 98 of which were shared (Fisher's Exact Test Odds Ratio = 7.84, right-tailed P-value < 0.001). Of these 98, only two, *SLC16A1* and *HIGD1A*, had been reported in earlier microarray studies. Additionally, we found 14 genes repressed in either GTEx or Tabula Sapiens that were previously categorised as β-cell disallowed (Fisher's Exact Test Odds Ratio = 3.52, right-tailed P-value < 0.001).
Conclusions Modern transcriptomic datasets provide new insights into pancreas-specific gene repression and highlight genes relevant to β-cell function and disease. We are now investigating the epigenomic regulation of these genes further, querying β-cell repressive marks at prioritised genes.

P-137 Enhancing NAD^+ biosynthesis alleviates blocked glycolytic metabolism in pancreatic islets, following chronic hyperglycaemia.

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Introduction/Aim: Chronic hyperglycaemia restricts glucose metabolism in pancreatic islets and β-cells via inactivation of glycolytic enzyme, glyceraldehyde 3-phophate dehydrogenase(GAPDH). A reason for this may be due to limited availability of the GAPDH coenzyme, nicotinamide adenine dinucleotide (NAD^+). Hence, in the current study we have investigated whether enhancing NAD^+ biosynthesis can restore GAPDH activity, glucose metabolism and insulin secretion in islets following chronic hyperglycaemia.
Methods: We utilised the βV59M mouse model where hyperglycaemia is initiated via a tamoxifen-inducible KATP channel activating mutation in pancreatic β-cells. Islets were studied after 2 weeks of hyperglycaemia (>20mmol/l). To increase NAD^+ biosynthesis, islets were cultured with the NAD^+ precursor, nicotinamide mononucleotide (NMN), for

24 hours. Mitochondrial metabolism was assessed by oxygen consumption rate (OCR, Seahorse), GAPDH activity was measured biochemically and insulin secretion from islets was determined by ELISA (Mercodia).

Results: Results: GAPDH activity was reduced in βV59M islets (Control = 0.58 ± 0.07 vs βV59M = 0.06 ± 0.01, pmol NADH/min/islet, $p < 0.01$, $n = 3 - 4$), but was comparable to control after 24 hour culture with 100 μmol/l NMN. NMN also increased glucose-stimulated OCR in βV59M islets (βV59M = 12.54 ± 2.07 vs βV59M+NMN = 90.59 ± 11.93 pmol O_2/min/50 islets, $p < 0.001$, $n = 5$). Finally, NMN partially restored glucose-stimulated insulin secretion in βV59M islets (βV59M = 0.06 ± 0.02 vs βV59M+NMN = 0.20 ± 0.02 ng/ml/islet/hour, $p < 0.001$, $n = 5 - 7$).

Conclusion: These data show that supplementing NAD^+ biosynthesis restores glucose metabolism and glucose-stimulated insulin secretion in islets, following chronic hyperglycaemia. Therefore, enhancing NAD^+ biosynthesis may be of therapeutic relevance for preventing/reversing β-cell failure during type 2 diabetes.

Session 2: Wednesday, 11 of June, 2025 17:50

P-201 Modelling Alpha-Cell Dysfunction in T1D with Reaggregated Primary Human Islet and Proinsulin-Specific Cytotoxic T Lymphocyte Co-Cultures

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Pancreatic α-cell glucagon secretory response at low glucose is impaired in type 1 diabetes (T1D), increasing the risk of hypoglycaemia. Underlying mechanisms remain incompletely understood, partly due to poor translatability of rodent models and limited availability of islets from humans with T1D. Here we describe an *in vitro* T1D model of α-cell dysfunction, where the β-cell remains the primary target of autoimmune attack, while α-cells are exposed to the inflammatory environment and disruptions in α-β-cell interactions.
We co-cultured uniform reaggregated human islets with cytotoxic CD8$^+$ T lymphocytes (CTL) that recognize a preproinsulin peptide presented on HLA-A2 molecules for 3 days. Next, we assessed α- and β-cell functions by quantifying insulin and glucagon secreted at 2.8 and 16.7 mM glucose or measuring intracellular insulin and glucagon contents. We employed 3D microscopy to assess α- and β-cell death and T-cell infiltration using cell-specific markers *ARX*, *NKX6.1*, and *CD3*.
CTL attack resulted in increased basal and decreased glucose-stimulated insulin release and reduced intracellular insulin content. Interestingly, glucagon content and secretion, particularly at 2.8 mM glucose, were also significantly reduced. 3D microscopy revealed significant decline in *NKX6.1*-positive cell count in correlation with T-cell count, along with a small loss of *ARX*-positive cells following co-culture with higher T-cell counts. Despite the specific targeting of β-cells in our model, the collateral damage to α-cells resulted in functional disruptions similar to those observed in T1D. The described biomimetic human islet-CTL co-culture assay thus represents a valuable tool for studying α-cell dysfunction and exploring therapeutic approaches at scale.

P-202 In-vivo human β-cells dysfunction is associated with in-situ upregulation of miR-148a-3p

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Introduction: MiRNAs regulate β-cell function and mediate pancreatic islet impairment in type 2 diabetes. Human studies directly linking miRNAs to *in vivo* β-cell function are still lacking. This study explores miRNA expression in human islets from metabolically characterized living donors to identify key pathways underlying progression of β-cell dysfunction.
Methods: 34 patients underwent partial pancreatectomy and OGTT/ hyperglycemic clamp before pancreatic biopsy. Metabolic parameters were obtained including β-cell Glucose Sensitivity (GS) and Rate Sensitivity (RS). Small RNA Sequencing was performed on Laser Capture Microdissected islets. Differential Expression Analysis ($p_{adj} < 0.05$) and

linear regression between miRNAs and clinical data were carried out. Validation of differentially expressed miRNAs was performed through ddPCR. miRNAs target gene prediction was performed with TargetScan7.2. EndoC-βH1, MIN6 cell lines and primary human islets were transfected with miR-148a-3p mimic. mRNA and protein levels of selected target gene were evaluated by Real-time PCR and Western Blot.
Results: Subjects were stratified by β-cell dysfunction severity using *in vivo* GS/RS: functional (high GS/RS; $n = 16$), partially dysfunctional (high GS, low RS; $n = 10$), and severely dysfunctional (low GS/RS; $n = 8$). Six miRNAs resulted differentially expressed; *miR-148a-3p* (validated by ddPCR) increased progressively in parallel with β-cell dysfunction severity. Regression analysis associated *miR-148a-3p* to lower GS and higher glycemia. TargetScan analysis identified *ATP6AP2* (involved in GLP1R signaling) as a *miR-148a-3p* target. In MIN6, EndoC-βH1, and human islets cells, *miR-148a-3p* overexpression reduced *ATP6AP2* mRNA and protein levels ($p < 0.05$).
Conclusions: Islet *miR-148a-3p* is associated to β-cell dysfunction *in vivo* and suppresses *ATP6AP2*, a putative regulator of incretin-mediated insulin secretion.

P-203 Mannose-6-phosphate receptor (M6PR) controls insulin secretion and incretin responses in β-cells.

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Introduction & Aims: Defective insulin secretion by β-cells contributes to hyperglycaemia and type 2 diabetes (T2D) progression. We have demonstrated that miR-125b negatively regulates β-cell glucose-stimulated insulin secretion (GSIS) and glucose homeostasis. MiR-125b overexpression results in hypertrophic lysosomes with reduced levels of the major hydrolase cathepsin D, likely by targeting the hydrolase's transporter cation-dependent mannose-6-phosphate receptor (*M6PR*). Here, we investigate the unexplored role of *M6PR* in β-cells.
Methods: *M6PR* expression was measured by RT-qPCR and Western blot in mouse and human islets, and β-cell lines (INS-1(832/3)). GSIS, cAMP and receptor internalization were assessed via time-resolved fluorescence, molecular probes and microscopy following siRNA/shRNA-mediated *M6PR* knockdown.
Results: Islets from mice fed a high-sugar, high-fat diet contained elevated miR-125b levels (1.42-fold, $p < 0.05$) and reduced *M6PR* protein (0.72-fold, $p < 0.05$) with a strong negative correlation ($r = -0.655$, $p < 0.001$). Unexpectedly, *M6PR* levels increased (2-fold, $p < 0.05$) rather than decrease in mouse and human islets cultured at high glucose for 48h. ShRNA-induced *M6PR* knockdown in INS-1(832/3) cells impaired insulin secretion in response to glucose (0.75-fold, $p = 0.0508$) and GLP-1 (0.53-fold, $p < 0.05$), and reduced *GLP-1R* (1.45-fold, $p < 0.001$) and *GIPR* internalization (1.8-fold, $p < 0.001$). ShRNA-induced *M6PR* knockdown in mouse and human islets decreased cAMP production in response to both GLP-1 (0.7-fold, $p < 0.01$) and GIP (0.8-fold, $p < 0.01$).
Conclusions: *M6PR* expression is reduced by diet-induced hyperglycemia and obesity, likely through miR-125b repression. *M6PR* is required for adequate secretory and signalling responses to glucose and incretins. We are currently studying its role in lysosomal function. Targeting the miR-125b-*M6PR* axis may improve β-cell function in the onset of hyperglycaemia.

P-204 A Specific Missense Variant in *CNOT1* Reveals a Putative Role in Nuclear Receptor-Mediated Transcriptional Regulation of Pancreatic Development.

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Introduction:
CNOT1 is the scaffold of the CCR4-NOT complex, a regulator of mRNA stability and transcriptional repression. While most pathogenic *CNOT1* variants cause a neurodevelopmental disorder, the missense variant p.R535C causes holoprosencephaly and pancreatic agenesis, highlighting this gene's role in pancreas development. Mouse studies of the p.R535C variant revealed a defect in pancreatic development but the underlying molecular mechanism remains unclear.
Aims: To investigate how the p.R535C variant disrupts pancreatic development through understanding how the impact of different variants on CNOT1 function.
Methods: Missense *CNOT1* variants from 27 individuals with a range of neurodevelopmental disorders, including 5 individuals with the p.R535C variant (4 with pancreatic agenesis) were mapped onto an AlphaFold3-generated model of CNOT1 to assess spatial proximity to functional motifs.
Public ChIP-seq of other CCR4-NOT members (used as proxy for CNOT1) was analysed against public ChIP-atlas database of all human transcription factor binding. RNA-seq of *CNOT1* KD was used to identify CNOT1 transcriptional targets.
Results: Structural modelling revealed that R535 lies in a distinct region of CNOT1 protein, located far away from the majority of variants that do not cause a pancreatic phenotype. R535 lies spatially near an LXXLL motif that has been shown to be a nuclear receptor interaction site that represses RXR mediated transcription. Analysis of chromatin localisation of the CCR4-NOT member CNOT3 (as proxy for CNOT1) reveals significant overlap with RXR and RAR binding. Transcriptomic analysis of *CNOT1* KD revealed genes transcriptionally dysregulated in proximity to CNOT3/RXR binding sites.
Conclusions: These findings support the hypothesis that this missense CNOT1 variant disrupts pancreatic development by altering the transduction of retinoic acid signalling. We are now investigating downstream target genes revealed by transcriptomic analysis.

P-205 Ghrelin and LEAP2 affect insulin and glucagon secretion in mouse but not in human islets, and their effects in mouse islets are mediated by somatostatin

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Introduction and aims: Ghrelin, known as the "hunger hormone", impairs glucose tolerance, whereas LEAP2 enhances it. We investigated the effects of ghrelin (a GHS-R1a receptor agonist) and three presumed GHS-R1a inverse agonists (LEAP2, LEAP2$_{(38-47)}$ (a LEAP2 fragment upregulated after Roux-en-Y gastric bypass), and PF-5190457 (PF)) on pancreatic hormone secretions in mice and humans, and determined the role of somatostatin (SST) in their effects.
Methods: Human and mouse islets from control (Sst$^{+/+}$) or SST knockout (Sst$^{-/-}$) mice were incubated for 1h with different glucose concentrations (1-7-15mM, G1-G7-G15) and GHS-R1a modulators (0.1-100nM/1μM).
Results: In Sst$^{+/+}$ islets, ghrelin dose-dependently inhibited glucagon secretion at G1 (35-50% with 0.1-100nM), while only 100nM ghrelin inhibited insulin secretion at G15 (20%). LEAP2, at 100nM only, increased glucagon secretion at G1 (45%) and insulin secretion at G15 (19%). Ghrelin ($\geq$1nM) stimulated SST secretion in Sst$^{+/+}$ islets, whereas LEAP2 ($\geq$10nM) inhibited it. Ghrelin and LEAP2 did not affect insulin and glucagon secretion in Sst$^{-/-}$ islets, demonstrating that their effects in Sst$^{+/+}$ islets are mediated by SST. LEAP2$_{(38-47)}$ and PF (0.1nM-1μM) had no effect on glucagon or insulin secretion in Sst$^{+/+}$ mouse islets. In human islets, neither ghrelin nor LEAP2 affected pancreatic hormone secretion, except for a 60% increase of SST secretion induced by 100nM ghrelin at G15.

Conclusion: In mouse islets, ghrelin inhibits, whereas LEAP2 stimulates, glucagon and insulin secretion via SST. $LEAP2_{(38-47)}$ and PF do not reproduce the effects of LEAP2. In human islets, neither ghrelin nor LEAP2 affect insulin or glucagon secretion, although ghrelin stimulates SST secretion.

P-206 Metformin counteracts lipotoxic stress in pancreatic β-cells by regulating lipid droplet-mitochondria dynamics

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Introduction: Lipotoxicity that is caused by excess lipid accumulation is a major factor that contributes to gradual impairments of pancreatic β-cell function and the development of type 2 diabetes. The antidiabetic drug metformin has shown protective effects against lipid-induced damage in β-cells, but its specific mechanisms of action within pancreatic islets remain unclear.
Aims: The present study sought to delineate the direct modulatory effects of metformin on lipid droplet-mitochondria dynamics in pancreatic β-cells.
Methods: Experiments were conducted using the INS-1E β-cell line, treated with metformin alone or in combination with palmitate. Mitochondrial morphology and lipid metabolism enzymes were analyzed using kinetic tests, electron microscopy, and functional assays. Lipid droplets, fatty acid content, and β-cell functionality were assessed using BODIPY staining, gas chromatography-mass spectrometry and ELISA, respectively.
Results: Our results showed that metformin reduced both the number and size of lipid droplets in palmitate-treated INS-1E cells. This was followed by an increase in the association between mitochondria and lipid droplets and an increase in fatty acid utilization. Under conditions of palmitate overexposure, metformin limited the activity of adipose triglyceride lipase and lipogenic regulators, such as stearoyl-CoA desaturase 1, and suppressed fatty acid uptake into cells. Additionally, metformin alleviated triglyceride and free fatty acid accumulation and partially reversed palmitate-induced impairments in insulin secretion in INS-1E cells that were subjected to lipotoxicity.
Conclusions: These findings provide additional mechanistic insights toward better understanding the pleiotropic effects of metformin and its role in regulating β-cell function.
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P-207 Characterization of a novel monoclonal antibody panel for pancreas biology

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Introduction: Monoclonal antibodies (mAbs) are a great tool for studying and treating pancreatic disease by e.g cell sorting of relevant populations or exerting functional effects to specific diseased pancreatic subpopulations. Still not many mAbs are currently available for extensive use in the pancreas field.
Methods: To address this gap, we have generated and characterized a novel panel of mAbs targeting pancreatic antigens. We used human islets to immunize a rat and characterized a panel of mAbs using FACS, histological and sequencing-based applications.
Results: Our approach generated 59 affinity-purified mAbs targeting both exocrine and endocrine pancreatic populations. We validate the expression pattern of the endocrine cell- enriched antibodies in non-diabetic and diabetic pancreata and across different species. Moreover, we identified a promising mAb candidate specific to the endocrine cell compartment that can be used to enrich for islet cells from low purity human islet preparations and from stem-cell derived islets. Using this mAb we enriched for islet cells and in vitro assays revealed improved safety profile compared to current enrichment methods.
Conclusions: In this work, we present a large mAb panel against human antigens for many applications and we validate a new pan-endocrine mAb as a cell sorting reagent to generate safe islet cell preparations for transplantation in type 1 diabetes patients.

P-208 Proteomic analysis of beta cell loss and dysfunction in human islets

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Introduction: Decreased beta cell insulin secretion is a hallmark of type 2 diabetes (T2D), which may be attributed to beta cell loss and dysfunction. beta cell loss and alpha cell gain in human diabetic islets have been observed in many, but not all, studies.
Aim: We aim to estimate islet cell proportions and elucidate the mechanisms underlying the variations in insulin

secretion caused by beta cell loss or dysfunction.
Method: Leveraging our large human islet atlas (humanislets.com), we conducted cell-type deconvolutions from proteomics and benchmarked results, including the cell proportions already available in the atlas.
Results: The results suggested slightly decreased beta cells and increased alpha cells in T2D, but not prediabetes. The endocrine proportions of beta and alpha cells had a strong negative correlation among all donors. beta cell proportion explained $\sim$10% of the variance in the glucose-stimulated insulin secretion in linear regression. Among the hundreds of proteins altered in T2D, a small number of proteins remained significantly different after accounting for the β cell proportion, including *HADH*, *HHATL*, *ARG2*, *PFKFB2*, *SOD3*, *CYP2U1*, *MDK* and *ATP2A3*. They might be key players associated with beta-cell or islet dysfunction. We also identified proteins and pathways associated with insulin secretion after adjusting for beta cell proportion.
Conclusions: Our data suggest that altered beta cell proportion has a significant impact on islet function, and taking it into account may help identify key mechanisms behind islet dysfunction. Future work will establish a more accurate beta cell type deconvolution for our expanding human islets atlas.

P-209 Evaluating cell-specific gene expression using single-cell and single-nuclei RNA-sequencing data from human pancreatic islets

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Background: Single-cell RNA sequencing (scRNA-seq) and single-nuclei sequencing (snRNA-seq), the latter of which can also be used on frozen samples, allow for cell-specific transcriptome analysis. However, available reference-based cell type annotation methods were developed for scRNA-seq data and may not be ideal for snRNA-seq.
Aims: To compare scRNA-seq and snRNA-seq data generated from pancreatic islets of the same four human donors, and assess the differences in gene detection, cell type composition, reference-based annotation accuracy, and marker gene identification.
Methods: Cells/nuclei for scRNA-seq/snRNA-seq were obtained from human pancreatic islets of the same four non-diabetic male donors. scRNA-seq and snRNA-seq were performed using the 10X Chromium platform, and sequencing was performed using NovaSeq6000. Cell types were assigned using three different annotation approaches: 1) manual annotation based on known marker genes using Seurat's FindAllMarkers, 2) reference-based annotation with Azimuth's pancreasref dataset, and 3) reference-based annotation using the HPAP non-diabetic dataset and Seurat CCA.
Results: There were differences in predicted cell type proportions between scRNA-seq and snRNA-seq, particularly for acinar, stellate, immune, and endothelial cell types, but to a minor extent also for beta cells. Reference-based annotation methods, based on scRNA-seq reference datasets, were suboptimal for snRNA-seq. Also, the overlap of scRNA-seq and snRNA-seq marker genes was low, ranging from 4% (acinar cells) to 26% (ductal cells). We confirmed previous snRNA-seq markers and identified novel snRNA-seq marker genes, which may improve snRNA-seq-based cell type identification.
Conclusions: Our findings highlight differences between scRNA-seq and snRNA-seq, emphasizing the need for tailored snRNA-seq annotation strategies.

P-210 Activity of Pancreatic Cells in Health and Type 1 Diabetes

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The pancreas is an essential organ responsible for metabolism and blood glucose homeostasis. A great deal of effort has been devoted to understanding the physiology of the pancreatic islets of Langerhans, particularly β cells.
Pancreatic tissue slice preparation allows for the investigation of all cell types present within pancreatic tissue while preserving tissue architecture, including both intra- and intercellular interactions. The major advantage of this approach is that it enables the study of small and disrupted islets, which are commonly found in type 1 diabetes.
Employing the pancreatic tissue slice method in combination with high-resolution Ca^{2+} imaging using laser-scanning microscopes allows for the simultaneous tracking of activity responses in multiple cell types at single-cell resolution. Human pancreatic tissue slices from healthy lean donors and type 1 diabetic donors were challenged with low glucose to reveal α cell activity, high glucose to activate β cells, and an amino acid cocktail mimicking post-meal plasma conditions to stimulate acinar cells and trigger a cascading response in ductal cells. To confirm the location of endocrine cells, I performed post-staining using antibodies against endocrine cell hormones.
Through this study, I confirmed the lack of β-cell function in type 1 diabetic donors and successfully characterized multiple response phenotypes of pancreatic cells, including both endocrine and exocrine cells.
The characterization of phenotypes in human pancreatic tissue slices at high spatial and temporal resolution has provided valuable insights. Single-cell resolution calcium activity imaging in pancreatic tissue has revealed distinct cellular phenotypes.

P-211 A lncRNA associated to T1D risk regulates PD-L1 expression in pancreatic β cells

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Type 1 diabetes (T1D) is a complex autoimmune disease that develops in genetically susceptible individuals upon exposure to environmental factors. Several T1D susceptibility variants are located in non-coding regions of the human genome, including long non-coding RNAs (lncRNAs), suggesting their potential implication in the pathogenesis of the disease.
In the present work, we scrutinized the Human Pancreas Analysis Program (HPAP) database to identify lncRNAs differentially expressed in pancreatic β cells of individuals with T1D. We identified a lncRNA that harbors a SNP associated to T1D risk that was highly expressed in pancreatic β cells in comparison with other islets cells. Moreover, β cells of T1D individuals showed a 10-fold increase in the expression of the lncRNA when compared to β cells of control individuals. Interestingly, HPAP data analysis revealed that the expression of this lncRNA was highly correlated with the expression of PD-L1 in pancreatic β cells ($r = 0.79$; $p < 0.0001$).
Pro-inflammatory stimuli, such as IL1β+IFNγ or IFNβ, upregulated the expression of the lncRNA and PD-L1 in the EndoC-βH1 cells. Concordantly, lncRNA knockdown reduced cytokine-induced PD-L1 protein expression. Co-culture experiments of β cells with T cells showed reduced T cell activation when co-cultured with β cells in which the expression of the lncRNA was inhibited, and consequently, PD-L1 expression reduced. These

preliminary results point to a regulatory role of this lncRNA in PD-L1 expression in β cells. Modulation of this lncRNA in β cells may serve as a therapeutic approach to induce pancreatic β cell immune tolerance through activation of the PD-L1/PD-1 immune checkpoint.

P-212 Glucose-Dependent Calcium Dynamics in Human Pancreatic Islets

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Introduction Animal models have provided essential insights into pancreatic islet physiology; however, they insufficiently capture critical species-specific differences for understanding human type 2 diabetes (T2D). Studying human pancreatic tissue directly could bridge this gap.
Aims This study aimed to characterize glucose-stimulated calcium signaling dynamics and insulin secretion from pancreas tissue slices and isolated islets obtained from non-diabetic and diabetic human donors.
Methods Pancreatic tissue was obtained from three deceased donors (two non-diabetic, one diabetic). Tissue slices (130-140 μm) produced from the pancreatic tail underwent confocal microscopy to assess calcium dynamics during perifusion with glucose concentrations (3-, 6-, 9-, and 12-mM). Pancreatic tissue was also enzymatically processed to isolate islets for insulin secretion assays. Additionally, calcium dynamics were performed in tissue slices from surgical donors.
Results In tissue slices of deceased donors, beta cells displayed heterogeneous calcium responses, including baseline activity at 3 mM glucose and rapid oscillations at higher glucose concentrations. Non-diabetic islets showed clear glucose-dependent increases in calcium oscillation frequency and insulin secretion. In contrast, diabetic islets exhibited impaired glucose responsiveness, characterized by minimal calcium oscillation changes and markedly reduced insulin secretion, despite a modest rise in activity between 3- and 12-mM glucose.
Conclusions This preliminary investigation highlights distinct glucose-dependent calcium signaling patterns and insulin secretion profiles between non-diabetic and diabetic human pancreatic islets. Our findings indicate impaired calcium dynamics as a possible mechanism underlying beta-cell dysfunction in human T2D, underscoring the value of human-based approaches for elucidating diabetes pathophysiology.

P-213 Pericytic CCL2 regulates islet macrophages to support glucose homeostasis

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β-cells are regulated by signals from the islet microenvironment, particularly immune and vascular cells. Pericytes, mural cells of the islet vasculature, have emerged as key regulators of islet homeostasis. Macrophages are the predominant immune population in the islets and can play either a supportive or distractive role in β-cell function. CCL2 is a critical regulator of immune recruitment and activation, and its dysregulation was implicated in β-cell dysfunction. We identified pericytes as the primary source of the chemokine CCL2 in health and found that a subset of islet macrophages expresses its receptor, CCR2. Here, we test the hypothesis that pericyte-derived CCL2 regulates islet macrophage populations to support β-cell function. To this end, we generated mice with a selective deletion of the

Ccl2 gene in pancreatic pericytes. Pericytic CCL2 deletion resulted in fewer islet macrophages in neonatal and adult mice; however, the proportion of CCR2^{+} macrophages remained unchanged. Mice lacking pericytic CCL2 were glucose intolerant, while their insulin sensitivity was unaffected. Although β-cell mass was preserved in transgenic mice, we observed impaired expression of key β-cell transcription factors in the absence of pericytic CCL2. Our findings indicate that pericyte-derived CCL2 is required for maintaining islet macrophage numbers and β-cell phenotype in health, highlighting a novel vascular-immune axis critical for islet homeostasis and glucose regulation.

P-214 LRR kinase modulates glucose homeostasis by controlling beta-cell insulin secretion

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Background: Leucine-Rich Repeat Kinase 2 (LRRK2) is a kinase widely expressed in the central nervous system (CNS), and mutations in its coding gene, PARK8, have been associated with autosomal dominant Parkinson's Disease. In the CNS, LRRK2 controls neurotransmitter vesicles trafficking and release. Based on the functional analogy between neurons and pancreatic β-cells, here we explored the expression and function of LRRK2 in β-cells and its possible contribution to glucose homeostasis.
Methods: LRRK2 expression was confirmed in human and rodent islets and βTC3 cells by Western blotting and PCR. ELISA and Total Internal Reflection Fluorescence Microscopy (TIRFM) were used to study the effect of LRRK2 kinase activity on insulin secretion and granule dynamics. The in vivo impact of LRRK2 kinase on glucose homeostasis was assessed in BAC mice expressing the PD-related LRRK2 G2019S mutant, characterized by increased kinase activity.
Results: Pharmacological inhibition of LRRK2 reduced stimulated insulin secretion by modulating secretory granule trafficking. Conversely, expression of the hyperactive LRRK2 mutant G2019S increased basal insulin release without affecting stimulated secretion. Compared to the control group, G2019S mice exhibited several metabolic defects, including a significant body weight increment, hypoglycaemia, and hyperinsulinemia under fasting conditions, highlighting the importance of LRRK2 for β-cell function and systemic glucose homeostasis.
Conclusion: Our results identify LRRK2 as a novel regulator of glucose homeostasis and vesicular trafficking in pancreatic β-cells. We are currently exploiting pull-down assays combined with proteomics to identify LRRK2 interacting partners and substrates and to elucidate the molecular mechanisms involved.

P-215 Metabolic, Transcriptional and Structural Regulation of Pancreatic β-Cell Maturation: lessons to develop strategies to improve iPSC-islet function.

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Background: Pancreatic β-cell maturation is a complex process involving transcriptional, metabolic, and structural adaptations necessary for functional insulin secretion. Understanding these processes is critical for improving the functionality of human pluripotent stem cell-derived β-cells (iPSC-islet) for diabetes treatment. This study investigates the relationship between gene expression, mitochondrial function, and translation regulation during β-cell maturation in mice.
Methods: Pancreatic islets were isolated from C57 mice at different developmental stages: neonatal (P3, P9, P20), juvenile (P28), and adult. We assessed gene expression, glucose-stimulated insulin secretion, translation by puromycin

incorporation, mitochondrial function and β-cell ultrastructure was by electron microscopy.
Results: The expression of key β-cell identity and maturation genes (*INS*, *MAFA*, *PAX6*, *GCK*, *GLUT2*, *ERO1*) increased from P9 onward, but efficient GSIS was only achieved after P20. Translation analysis revealed that immature β-cells (P9) exhibited higher basal translation but were unresponsive to glucose stimulation, unlike mature β-cells. Mitochondrial potential was lower in neonatal islets compared to adults, suggesting reduced mitochondrial function. Ultrastructural analysis revealed that mature β-cells had elongated mitochondria with dynamic fission-fusion processes, while immature β-cells contained rounded, less functional mitochondria.
Conclusion: These findings suggest that β-cell maturation involves a temporal dissociation between transcriptional and functional development, largely influenced by mitochondrial adaptation and translation regulation. This study provides insights into strategies to enhance iPSC-islet maturation for diabetes therapy.

P-216 Clocking Inflammation: How Circadian Rhythms and Proinflammatory Cytokines Drive Type 2 Diabetes

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Introduction: Disruption of circadian clocks is associated with the development of type 2 diabetes (T2D). We previously reported that in normoglycemic participants, molecular oscillators operative in skeletal muscle regulate glucose uptake, lipid metabolism, and rhythmic secretion of the proinflammatory myokines interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein 1 (MCP-1). Furthermore, in human pancreatic islet cells, circadian clocks orchestrate insulin and glucagon secretion but are disrupted in people with T2D.
Aims: As proinflammatory cytokines may contribute to T2D pathogenesis, we investigated the interaction between molecular clocks and cytokine secretion in a systemic and tissue-specific manner.
Methods: We applied parallel assessment of molecular clockwork by reporter-based circadian bioluminescence recording in human primary pancreatic islet cells, skeletal myotubes, and skin fibroblasts established from non-diabetic (ND) and T2D donors, along with temporal cytokine secretion by continuous perifusion followed by ELISA/multiplex arrays. In sera, IL-6 levels were measured over 24h using ProQuantum kit.
Results: Serum IL-6, IL-8, and MCP-1 showed diurnal variations and increased levels in T2D participants. Skeletal myotubes from T2D donors displayed altered BMAL1-luciferase circadian oscillations compared to ND. IL-6 secretion was rhythmic and elevated in T2D skeletal myotubes and pancreatic islets. Measurements in FACS-separated islet cells revealed non-β-cells as the primary source of cytokines. Cytokine secretion by skin fibroblasts was rhythmic in ND but remained unchanged in T2D.
Conclusions: Proinflammatory cytokine secretion exhibits 24-hour variations in sera, islets, skeletal myotubes, and skin fibroblasts. Temporal IL-6 levels are up-regulated in T2D patients, with rhythmic profiles being altered in a tissue-specific manner.

P-217 Optimizing Single-Cell Long-Read Sequencing for Enhanced Isoform Detection in Pancreatic Islets

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Alternative splicing joins exons in different combinations or retains introns, producing diverse protein isoforms. In pancreatic islets, cytokine treatment induces aberrant splicing, and about 25% of splicing events are dysregulated in donors with type 2 diabetes (T2D). Since alternative splicing can alter protein-binding capacities, it raises the question of whether it contributes to neoantigen generation. Additionally, splicing may affect beta cell function and stress responses, making cells more prone to immune attack. As beta cells rely on the endoplasmic reticulum (ER) for protein folding and secretion, splicing changes in ER stress-related genes may impair stress handling and enhance immunogenicity. In this study, we apply single-cell long-read sequencing to achieve isoform-level resolution in mouse pancreatic islets. This is the first use of long-read sequencing in islet cells and offers new insight into mechanisms underlying type 1 diabetes (T1D). By generating reads that span exon-exon junctions, our method reveals isoform variants missed by short-read technologies. We hypothesize that specific isoforms may increase beta cell vulnerability to immune attack. Our analysis identifies distinct beta cell subpopulations defined by unique splice variants and reveals differential splicing across endocrine cell types. Future work will compare transcriptomes from non-obese diabetic (NOD) mice before and after islet infiltration to identify inflammation-driven splicing changes. We will also compare islets from NOD and wildtype mice prior to infiltration to detect disease-specific alterations. This study aims to uncover the role of isoform variation in beta cell biology and its contribution to T1D pathogenesis.

P-218 Functional and Molecular Characterization of the H55Y-Inceptor Mutant Protein: Implications for Beta-Cell Dysfunction and Diabetes Risk

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Introduction: Pancreatic beta cells with diminished (pro)insulin levels have increased susceptibility to immune insults and dietary factors, thereby diabetes risk. Identifying genetic variants influencing (pro)insulin availability is crucial for advancing personalized diabetes medicine. A GWAS meta-analysis identified the SNP rs74920406 (H55Y) in *ELAPOR1* as associated with lower proinsulin levels in non-diabetic individuals. *ELAPOR1* encodes to the insulin inhibitory receptor (inceptor), a transmembrane protein in beta cells that functions as a lysosomal sorting receptor for insulin and proinsulin degradation.
Aims: To investigate the molecular and cellular mechanisms by which the H55Y-*ELAPOR1* variant reduces proinsulin levels and its physiological implications in *Elapor1*$^{H55Y/H55Y}$ mice.
Methods: Alpha3Fold predicted H55Y-inceptor folding and ligand interaction. Beta cell function (GSIS), inceptor subcellular localization (IFI) was assessed in INS-1 cells expressing H55Y-inceptor-Venus. The metabolic phenotype of *Elapor1*$^{H55Y/H55Y}$ mice was determined, while dynamic GSIS evaluated islet function *ex vivo*.
Results: H55Y mutation does not affect inceptor folding, however it affects inceptor dimerization domain potentially increasing protein stability. In INS-1 cells, H55Y-inceptor-Venus predominantly localizes to the trans-Golgi network, unlike WT-inceptor-Venus, which also appears at the plasma membrane. H55Y-inceptor expression significantly reduces insulin secretion, consistent with reduced proinsulin levels in humans. At 8 weeks, *Elapor1*$^{H55Y/H55Y}$ male mice exhibit normal weight and glucose tolerance but show reduced insulin secretion upon glucose challenge. Similarly, H55Y-islets show diminished insulin secretion after glucose stimulation.
Conclusions: H55Y-inceptor may enhance proinsulin degradation, representing a gain-of-function mutation that reduces

(pro)insulin availability. H55Y-*ELAPOR1* mutation could be classified as a risk mutation associated with diabetes.

P-219 Expanded Multi-Omics Capabilities and an Integrated Metabolic Atlas at Human-Islets.com

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The study of human pancreatic islets is essential for elucidating diabetes pathophysiology and identifying new therapeutic targets. However, accessibility, integration, and exploration of high-quality human islet datasets remain limited. To address this challenge, HumanIslets.com was developed as a centralized resource for accessing and exploring human islet data generated by the Alberta Diabetes Institute IsletCore program. This study introduces significant advancements in HumanIslets.com 2.0 to enhance its utility for diabetes research. The updated resource includes expanded transcriptomic, proteomic, and single-cell datasets, as well as new data on genetic risk, ancestry, metabolomics, and environmental contaminants. Polygenic scores and process-specific partition scores for Type 1 and Type 2 diabetes enable the comparison of genetic risk factors to phenotypic and molecular signatures. Insulin processing (proinsulin, C-peptide) measurements provide insight into β-cell function and insulin production efficiency. A dedicated multi-omics analysis module enables integrated exploration across molecular layers. Correlated gene–protein pairs were found to be enriched in pathways such as EGFR tyrosine kinase inhibitor resistance. Additionally, HumanIslets.com 2.0 features a human islet metabolic atlas built from genome-scale models tailored to donor-specific transcriptomes, enabling the investigation of donor-specific metabolic signatures and comparisons across diabetes phenotypes. The advancements in HumanIslets.com 2.0 make it a more robust and comprehensive resource for the scientific community, providing improved tools and datasets to advance research on islet function, diabetes mechanisms, and potential therapeutic targets.

P-220 Hide and seek: Protecting human β-cells in T1D by modulating their PDL1 and HLA Class I expression

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In type 1 diabetes (T1D), β-cells express two molecules with opposing actions: HLA Class I (HLA-I), which increases antigen presentation and immune targeting, and PDL1, which provides partial immune protection. Strategies to uncouple their expression could aid new T1D therapy development. We developed an assay platform to assess whether previously suggested (e.g., *TYK2*, *STAT2*) or novel targets can decouple PDL1 from HLA-I and/or protect β-cells from T1D-relevant stress.
Reaggregated uniform islets were cultured with proinflammatory cytokines, activated peripheral blood mononuclear

cells, or HLA-A2-restricted preproinsulin-specific cytotoxic T lymphocytes. Biochemical and 3D microscopy-based methods were used to assess β-cell function, β-cell death, T-cell infiltration, cytokine secretion, HLA-I and PDL1 expression at single-islet resolution. Gene expression was modified by shRNAs delivered via adeno-associated viruses during islet aggregation.
β-cell stress manifested as increased basal but decreased glucose-stimulated insulin release, reduced insulin content, loss of first-phase insulin response, and elevated proinsulin-to-insulin ratios. 3D microscopy showed elevated HLA-I and PDL1 expression, extensive T-cell infiltration, and β-cell killing. Liraglutide and HLA-I blocking antibodies demonstrated anti-inflammatory and immune-protective effects, serving as effective controls for future studies. *TYK2* inhibition preserved intracellular insulin, improved fold increase in insulin secretion, and reduced both HLA-I and PDL1 expression. *STAT2* knockdown had milder, non-significant effects in cytokine treated islets.
The described biomimetic assays offer scalable *in vitro* models for early-stage T1D immunopathology and β-cell dysfunction. Our findings confirm the anti-inflammatory effects of GLP-1 receptor agonists, immune evasion via HLA-I interference, and *TYK2* inhibition as a potential β-cell protective strategy during T1D progression.

P-221 Targeting *ARX* to turn human α-cells into insulin producers

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Introduction: The reprogramming of α-cells into insulin production is a promising approach to treat diabetes. We have shown that human α-cells can secrete insulin in response to glucose by forcing the expression of $Pdx1$ and $Mafa$ (PM). Although converted α-cells retained the α- cell transcriptomic signature, they restored euglycemia when transplanted into diabetic mice.
Aim: We hypothesize that silencing the transcription factor ARX, key for α-cell identity maintenance, will foster the reprogramming of human α-cells by reducing their α-cell profile.
Methods: We have generated five Cas9-mediated *Arx* homozygous knock-out subclones using mouse αTC1.9 cells. We have also optimized a protocol for the Cas9-mediated deletion of *ARX* in isolated purified primary human α-cells. The impact of *Arx/ARX* loss was assessed at functional and transcriptomic levels.
Results: In murine α-cells (αTC1.9 cell line), *Arx* loss led to reduced glucagon secretion at low glucose. Bulk RNA-sequencing revealed that the functional phenotype was associated with decreased α-cell identity and changes in secretion-associated processes. In human α-cells, *ARX* loss decreased their α-cell identity and promoted the acquisition of β-cell identity traits, such as the expression of the β-cell genes *PDX1* and *PCSK1*.
Conclusion: These preliminary observations suggest that decreasing α-cell identity fosters α-cell plasticity and facilitates the PM-driven conversion of human α-cells.

P-222 Spatial RNA profile of pancreatic beta cell populations to investigate beta cell identity gene signatures in isolated islets and biopsies from living or dead-brain donors

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Introduction: Human islets are extensively used to investigate endocrine cell physiology, study diabetes, and most importantly, transplant diabetic patients. Islets are isolated from brain-dead donors through a process in which they are detached from their vasculature and exposed to both cold- and warm ischemia. The impact of this process on islet biology has, however, been largely ignored. In this study, we aim to uncover the impact of cold ischemia on gene expression and to compare endocrine cell transcriptomes from peri-operative biopsies from living patients to both biopsies of brain-dead donors and isolated islets.
Methods: GeoMx DSP is used to perform the RNA profiling of endocrine cells in fresh frozen sections of biopsies or isolated human islets. The spatial profile of the whole transcriptome is performed in populations of cells identified by antibody staining (e.g. anti-insulin antibody).
Results: The impact on beta cell gene expression of exposure to either short (4-6h) or long (17-22h) periods of cold ischemia has been analyzed in brain-dead donor biopsies. Differential expression analysis showed that in short-cold ischemia there is an enrichment of beta cell identity genes while pathways related to inflammation, apoptosis, and hypoxia are enriched in long-cold ischemia. Preliminary data of biopsies from living versus dead-brain donors reveal that pathways associated with olfactory transduction and resting membrane potential are enriched in beta cells of living donor biopsies. Finally, when comparing living donor biopsies versus isolated human islets we identified insulin and IAPP as genes enriched in the biopsies while in isolated islets the enrichment of several beta cell-specific transcription factors is observed.

P-223 TMEM55A-mediated PI5P signaling regulates alpha cell actin depolymerization and glucagon secretion

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Introduction and Aims: Diabetes is associated with the dysfunction of glucagon-producing pancreatic islet alpha cells, although the underlying mechanisms regulating glucagon secretion and alpha cell dysfunction remain unclear. While insulin secretion from pancreatic beta cells has long been known to be controlled partly by intracellular phospholipid signaling, very little is known about the role of phospholipids in glucagon secretion. Using patch-clamp electrophysiology and single-cell RNA sequencing, we previously found that expression of PIP4P2 (encoding TMEM55A, a lipid phosphatase that dephosphorylates phosphatidylinositol-4,5-bisphosphate [PIP2] to phosphatidylinositol-5-phosphate [PI5P]) correlates with alpha cell function. We hypothesize that TMEM55A is involved in glucagon secretion and aim to validate the role of TMEM55A and its potential signaling molecules in alpha cell function and glucagon secretion.
Methods: Patch-clamp measurement of ion channel activity and exocytosis, hormone secretion measurements, siRNA knockdown, immunofluorescence, qPCR, and western blot.
Results: TMEM55A knockdown in both human and mouse alpha cells reduces glucagon exocytosis at low glucose. This is rescued by direct intracellular dialysis of PI5P. Accordingly, PI5P (but not PIP2) increases glucagon secretion

from intact mouse islets. This results from a remodeling of cortical F-actin dependent on TMEM55A lipid phosphatase activity, which occurs in response to oxidative stress. Finally, TMEM55A- and PI5P-induced F-actin remodeling is dependent upon the inactivation of GTPase, RhoA, rather than RAC1 or CDC42.
Conclusion: We reveal a novel pathway by which TMEM55A regulates alpha cell exocytosis and glucagon secretion in mice and humans by controlling intracellular PI5P and the F-actin network.

P-224 Pro-inflammatory cytokines prime human islets for gasdermin D-mediated pyroptosis

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Introduction and Aim: Type 1 diabetes (T1D) is characterized by immune-mediated destruction of the insulin-producing β cells. While apoptosis has been considered the primary mode of β-cell death in T1D, its inherent anti-inflammatory nature contrasts with the progressive islet inflammation observed during disease development. This suggests that additional pro-inflammatory cell death pathways may contribute to T1D pathogenesis. Pyroptosis is a highly inflammatory form of programmed cell death driven by inflammasome activation and gasdermin-mediated pore formation, leading to the release of cytokines like interleukin-1β (IL-1β) and IL-18, which exacerbate immune responses. Here, we aimed to investigate β-cell pyroptosis focusing on the role of the key pyroptosis executioner gasdermin D (GSDMD) for β-cell killing in isolated human islets exposed to pyroptosis-relevant stimuli.
Methods: Human pancreatic islets were pretreated with IL-1β, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α), and subsequently exposed to lipopolysaccharide (LPS) and nigericin. Pyroptotic activation was determined by evaluating GSDMD cleavage, cytotoxicity, and release of IL-18.
Results: Pro-inflammatory cytokine treatment upregulated full-length GSDMD in human islets and EndoC-βH5 cells but did not trigger its cleavage. However, cytokine-primed islets responded to LPS and nigericin with GSDMD cleavage and activation, increased cytotoxicity, and IL-18 release.
Conclusion: These findings suggest that GSDMD-mediated pyroptosis may contribute to β-cell demise and sustained intra-islet inflammation in T1D. Further investigations of islet pyroptosis in T1D-like settings are warranted and could open new avenues for therapeutic interventions aimed at preserving β-cell function and mitigating islet inflammation.

P-225 Unraveling Glucose-Induced Oxidative Modifications in INS-1E β-Cells

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Insulin-secreting β-cells are essential for the regulation of blood glucose levels and carbohydrate metabolism. The process of glucose-stimulated insulin secretion (GSIS) is tightly regulated and involves, among other relevant post-translational modifications (PTMs), physiological changes in cellular redox status. However, oxidative distress, e.g. due to nutrient overload, can lead to β-cell dysfunction, impaired insulin secretion, and contributing to type 2 diabetes development. It is therefore essential to gain understanding of the dual role of ROS in β-cell function – both as

signalling molecules and as drivers of dysfunction.
Therefore, this study aims to characterise the dynamic redox modifications of GSIS in insulin-secreting INS-1E cells by redox proteomics. Sequential alkylation was used to distinguish reversible oxidative modifications from unmodified cysteine residues. In combination with tandem mass tag labelling, this allowed the quantification of time-resolved changes in oxidative PTMs following glucose exposure. In addition, oxidative status was assessed by measuring H_2O_2-production using Amplex Red®.
GSIS of INS-1E cells results in a biphasic insulin release response accompanied by increased H_2O_2-formation during the initial phase (5-10 min) of the insulin secretion process. Redox-proteomic analysis identified dynamic protein oxidations that were differentially regulated between the early and late phases of insulin secretion. Early-phase oxidative modifications predominantly affected proteins involved in intracellular trafficking and cytoskeletal organisation, whereas late-phase modifications affected metabolic enzymes and insulin processing machinery.
Through this comprehensive investigation, we aim to improve our understanding of the intricate signalling pathways that control insulin secretion and gain insight into the role of oxidative stress in β-cell function and dysfunction.

P-226 miR-125a-5p overexpression in insulin secreting cells affects cellular structure and function

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Background and aim: MicroRNAs regulate post-transcriptional gene expression, including pathways involved in glucose homeostasis. This study investigates the effects of miR-125a-5p overexpression on beta-cell function and structure. While miR-125a-5p influences various cellular functions, including those in pancreatic cells, its specific role in pancreatic beta cells requires further investigation to fully elucidate its effects.
Material and methods: Lentiviral transduced INS-1E cells were used to compare miR-125a-5p overexpression (OE) and control (NTC) groups. Techniques included proteomics, glucose-stimulated insulin secretion (GSIS) assays, cell viability tests, RT-qPCR, and immunofluorescent confocal imaging.
Results: MiR-125-5p was over-expressed $\sim$2–3 fold compared with NTC-cells. Proteomic analysis identified 90 differentially expressed proteins upon miR-125a-5p overexpression, linked to cell adhesion, localization, and metabolic processes. In cells cultured at low (5 mM) glucose, there was no effect of miR-125aOE on beta-cell function, while miR-125aOE increased GSIS in cells precultured at high (20 mM) glucose for 48 hrs. MiR-125a-5p overexpression also significantly upregulated *Gcg* transcript levels but did not change *Pdx1* or total *Ins1* & *Ins2* transcript levels. Immunofluorescent imaging indicated increased levels of p120ctn, actin, and RhoA, consistent with proteomics data. In human islet preparations, levels of miR-125-5p correlated positively with *Gcg* mRNA levels ($r = 0.297, p < 0.01, n = 62$ donors) and insulin peptide levels ($r = 0.315, p = 0.007, n = 74$ donors).
Conclusion: MiR-125a-5p overexpression in INS-1E cells enhances insulin secretion at high glucose levels, suggesting a positive role in beta-cell function. It also affected cellular adhesion, cytoskeleton, shape and volume suggesting a connection between these parameters.

P-227 Metabolic wiring of pancreatic progenitors determines β-cell functionality by impacting on epigenetics and gene expression

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Diabetes modeling and cell therapies rely on human pluripotent stem (hPS) cell-derived β-cells. Growing evidence suggests that a precisely regulated differentiation of pancreatic progenitors (PPs) into the endocrine lineage is crucial for subsequent β-cell functionality. We have shown that the mitochondrial aldehyde dehydrogenase 1b1 (Aldh1b1) is expressed in mouse PPs, but not in differentiated cells, that it regulates the timing of PP differentiation, and that it is necessary for β-cell functionality. *Aldh1b1* null PPs differentiate earlier, and *Aldh1b1* null adult mice develop multi-level β-cell dysfunction and age-related hyperglycemia.
We hypothesized that Aldh1b1 acts as a metabolic regulator linking the self-renewal and differentiation of the PPs with the functionality of their progeny.Metabolic profiling of *Aldh1b1* null PPs suggested that Aldh1b1 moderates reactive oxygen species (ROS) levels and helps channel metabolic fluxes towards key substrates for epigenetics-regulating enzymes, including Acetyl-CoA.Changes in the availability of these metabolites may alter chromatin accessibility, leading to transcriptomic changes that would affect endocrine development and β-cell functionality. EpiCyTOF experiments confirmed chromatin restructuring, whereas ATAC-seq and RNA-seq analyses revealed reduced chromatin accessibility in *Aldh1b1* null PPs and changes in the expression of oxidative stress response and β-cell development genes, possibly via disruption of the GPCR–ERK signaling cascade. To link these early PP deficiencies with β-cell function, we are performing the same multilevel analyses on postnatal β-cells.
Deciphering the metabolic programming and molecular mechanisms that govern pancreatic progenitor differentiation and β-cell maturation will contribute to achieving full maturation of hPS cell-derived β-cells for diabetes modeling and cell therapy.

P-228 Age-associated DNA methylation patterns in human pancreatic islets

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Introduction: Age is a risk factor for type 2 diabetes (T2D) and ageing is associated with changes in DNA methylation. We previously investigated DNA methylation of a limited number of sites in pancreatic islets of a small human cohort. We are now expanding on this analysis, investigating a larger cohort and much larger number of methylation sites.
Aims: Identify age-associated DNA methylation changes in islets that alter the risk for T2D.
Methods: DNA methylation of >850,000 sites was analysed with Illumina EPIC arrays in pancreatic islets from 117 donors without a diabetes diagnosis, aged 24–81.
Results: Methylation at 9680 sites associated with age ($q < 0.05$, corrected for sex, BMI, purity, and days in culture). Comparison with analyses in human adipose tissue and liver showed that $1/4 - 1/3$ of the identified sites associate with age in these tissues, with almost all overlapping sites concordant. The 9680 methylation sites are annotated to 4767 unique genes. These include 121 out of 395 genes that we previously identified as differentially expressed genes (DEG) in islets from donors with T2D. Interestingly, 6 age-associated methylation sites are annotated to *PAX5*, the top T2D-associated candidate islet DEG. Moreover, 227 T2D candidate genes identified by GWAS also exhibit age-associated methylation. Pathway analysis on the genes with age-associated methylation sites show an enrichment for a T2D pathway, as well as pathways involved in cellular signalling and development.
Conclusions: Ageing is associated with wide-spread DNA methylation changes in human pancreatic islets that may influence islet cell function and the risk for T2D.

P-229 Transcription factor footprintng provides insights into the mechanism of ZNF808-mediated pancreatic agenesis

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Introduction: Homozygous loss-of-function variants in the primate specific *ZNF808* gene, were recently identified as a cause of pancreatic agenesis. *ZNF808* is a KRAB Zinc Finger transcription factor, that primarily represses the domesticated *MER11* transposable elements and plays a vital role in regulating the development of the pancreas. The loss of *ZNF808* unmasks the regulatory potential of the *MER11* elements, leading to an aberrant activation of liver specific genes during pancreas differentiation. However, this mechanism is not completely understood. Therefore, we investigate the possible transcription factors that bind to the unmasked *MER11* elements and cause this dysregulation in gene expression.
Methods: We analysed scATAC-seq data generated from wild type and *ZNF808* knock out H1 human embryonic stem cells at the first three stages of pancreatic differentiation (SC: Stem Cell, DE: Definitive Endoderm, S2: Primitive Gut Tube). We performed transcription factor (TF) footprinting using TOBIAS to identify the enriched JASPAR motifs, followed by a GO enrichment analysis using enrichR.
Results: We identified 64 differentially accessible *MER11* regions between pseudo-bulk wild type and *ZNF808* knockout cells, that revealed footprints of 20, 25 and 18 TF families at the SC, DE, and S2 stages respectively. Notably we find footprints of pluripotency factors *POU/KLF* at SC; nuclear receptors *RXR/RAR/ESR* and *FOX* factors at SC and DE; and *GATA* factors at DE and S2 stages.
Conclusion: Identifying TFs that gain binding sites on the unmasked *MER11* elements, helps understand the regulatory mechanisms of *ZNF808* and its potential role in the endoderm gene regulatory network.

P-230 Dual Role of NOX4-Mediated Redox Signaling in Pancreatic β-Cell Health and Dysfunction

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Glucose-stimulated insulin secretion in pancreatic β-cells relies on tightly regulated redox signaling mediated by NADPH oxidase 4 (NOX4), which generates hydrogen peroxide to activate redox relay pathways. Under physiological conditions, hydrogen peroxide acts as a critical metabolic coupling factor that facilitates insulin exocytosis and maintains β-cell function. By iodoTMT analysis we revealed that glucose through NOX4-derived hydrogen peroxide orchestrates redox-dependent cascades, enhancing ATP-sensitive K^+channel closure and calcium oscillations necessary for efficient insulin secretion. However, chronic overactivation of NOX4—driven by sustained nutrient excess (8 weeks of high fat diet)—shifts cellular redox balance towards oxidative stress. This disrupts β-cell homeostasis, triggering NLRP3 inflammasome activation, IL-1β maturation, and proinflammatory signaling. Thus, β-cell-specific NOX4- knockout mice remained protected from systemic inflammation under these conditions. Interestingly, these mice exhibited accelerated senescence in aging islets characterized by increased β-galactosidase positive cells, p21, p16, and Nrf2 expression. These findings underscore NOX4's dual role: transient hydrogen peroxide production supports insulin secretion, while chronic overactivity promotes β-cell inflammation and dysfunction. However, basal NOX4 activity is crucial for protection against islet senescence. Our data identify redox imbalance as a central mechanism linking metabolic stress to type 2 diabetes pathogenesis. Properly timed therapeutic strategies targeting NOX4 activity or

redox buffering capacity may preserve β-cell health by maintaining the delicate equilibrium between redox signaling and oxidative damage.
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P-231 ASCT1 – A Critical Mediator of Amino Acid-induced Glucagon Secretion

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Background and aim Amino acids are potent stimulators of glucagon however, the mechanisms that drive amino acid induced glucagon secretion are still elusive. Most amino acid uptake is sodium-driven, and depolarization through increased sodium influx is therefore a favoured hypothesis. However, alanine and cysteine, two potent glucagonotropic amino acids, use the same alanine/serine/cysteine transporters (ASCTs). Here we aimed to explore the role of ASCTs in amino acid stimulated glucagon secretion.
Methods Glucagon secretion, Ca^{2+} oscillations and immunostaining was measured in islets/ alpha cells from C57B6N female mice.
Results Pancreatic islets express both ASCT1 and 2, however only *ASCT1* colocalised with glucagon expression. Supra-physiological levels of alanine and cysteine (3 mM) stimulated glucagon secretion at 1 mM glucose (1.6- and 2-fold, respectively), but at 5 mM glucose only cysteine elevated glucagon (2.8-fold). Physiological levels of alanine (0.5 mM) or cysteine (0.35 mM) only stimulated glucagon secretion at 1 mM (1.3-fold) or 5 mM glucose (1.4-fold), respectively. ASCT1 inhibition with 4-chlorophenylglycine removed the effect of 3 mM alanine and cysteine on glucagon secretion. *ASCT1* is a neutral amino acid transporter with a chloride conductance, we therefore speculated that changes in electrical activity may drive the effects of alanine and cysteine on glucagon secretion. Accordingly, stimulation of alpha cells with alanine increases Ca^{2+} oscillations at 1 and 5 mM glucose, which can be inhibited by 4-chlorophenylglycine. Cysteine similarly increased Ca^{2+} oscillations. However, this effect could not be inhibited by 4-chlorophenylglycine.
Conclusion The data suggests stimulation of glucagon secretion by alanine and cysteine is *ASCT1*-mediated.

P-232 Selective Modulation of Interferon Responses by JAK Inhibitors in Beta Cells and Gut Epithelium

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Introduction Interferons (IFNs) play a crucial role in antiviral defense. They are also implicated in the pathogenesis of type 1 diabetes (T1D) through the induction of HLA-I hyperexpression. IFNs signal via JAK1/JAK2 or JAK1/TYK2 pathways, and inhibiting these signals may be a promising strategy to delay or prevent T1D. Baricitinib, a JAK1/2 inhibitor, was recently shown to slow C-peptide decline in recent-onset T1D. Given that type III IFNs are vital for protecting against viruses linked to T1D and which primarily infect through the gut—treatment with JAK inhibitors (JAKis) may increase susceptibility to these infections.
Aim This study aimed to evaluate the efficacy of two JAKis, Baricitinib (JAK1/2 inhibitor) and Deucravacitinib (selective TYK2 inhibitor), in blocking IFN-induced HLA-I expression in β-cells and their impact on gut antiviral defense.
Methods EndoC-bh1 cells, stem cell-derived (SC) islets and intestinal epithelial cell lines (IECs) were stimulated with

JAKis and/or type I or III IFNs. HLA-I and gene expression was measured by FACS and qPCR, respectively. Coxsackie B virus infection was measured as cytopathic effect.
Results Baricitinib and Deucravacitinib attenuated type I and III IFN-induced HLA-I expression in EndoC-bh1 cells and SC-islets. Baricitinib impaired type I and III IFN-induced antiviral defense mechanisms in IECs. Deucravacitinib blocked type I IFN while having lesser impact on type III IFN-induced antiviral defense mechanisms in IECs.
Conclusions A TYK2-specific inhibitor limits HLA-I expression in β-cells while preserving critical antiviral defense in the gut. Further evaluation is recommended to determine if TYK2 inhibitors should be preferred for T1D intervention.

P-233 A genome-wide CRISPR-Cas9 knockout screen in MIN6 cells identifies novel genes implicated in insulin expression and beta cell function

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Introduction Insufficient insulin release is a culprit in Type 2 diabetes and the regulation of insulin production is not fully understood.
Aim Discover new genes regulating insulin expression using a genome-wide CRISPR screen.
Methods MIN6 cells expressing GFP linked to the proinsulin c-peptide sequence of *Ins2* was generated. Cells were transfected with Cas9 and lentiviral vectors containing CRISPR guide libraries targeting 20,000 genes (4 guides per gene) and exposed to low or high glucose for 24 h. Cells were FACS sorted and subjected to RNAseq to identify single-guide RNAs (sgRNAs) affecting insulin production. Novel genes were validated for expression in human islets (scRNAseq and IHC) and for insulin-regulation using siRNA in INS-1 cells.
Results In low glucose, 736 genes affected *Ins2* expression (310 depleted, 426 enriched), and in high glucose, 495 genes were identified (196 depleted, 299 enriched). *Ins2* sgRNA was consistently depleted in both conditions. 161 genes affected *Ins2* expression similarly under both glucose conditions (104 enriched, 56 depleted). GSEA revealed a positive enrichment of spliceosome-related genes among these latter genes. Notably, *Arglu1*, a splicing regulator not previously related to insulin expression, was top-ranked in both conditions. *Arglu1* co-expressed with insulin in human islets and its silencing reduced the expression of *Ins1* in INS-1 cells.
Conclusions The CRISPR/Cas9 screen combined with the *Ins2*-GFP readout enables cost-efficient parallel interrogation of tens of thousands of genes involved in insulin production. Our results highlight the potential role of splicing factors in insulin production, pinpointing genes with hitherto unknown functions in beta cells.

P-234 Mesenchymal stromal cell-derived extracellular vesicles improve islet graft function

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Introduction: Human islet transplantation offers potential for treating type 1 diabetes, but islet viability is compromised by inflammatory, oxidative, and hypoxic stresses. Mesenchymal stromal cell-derived extracellular vesicles (MSC-EVs) have regenerative properties, and MSCs enhance islet function and survival.
Aims: Assess MSC-EV effects on islet function in vitro and in vivo.
Methods: Mouse bone marrow MSC-EVs were purified using Total Exosome isolation and characterised by NanoSight, immunoblotting and transmission electron microscopy. C57BL/6J mouse islets were treated with MSC-EVs for 72-hours before their functional assessment via quantification of apoptosis (caspase3/7) and glucose-stimulated insulin secretion (GSIS). 200 EV-treated or control islets were transplanted under kidney capsules of streptozotocin-induced diabetic C57BL6/J mice. Glycaemic control was monitored through daily blood glucose (BG) measurements and glucose

tolerance test (GTT) at day 14.
Results: Pre-treating islets with MSC-EVs increased GSIS (20 mM glucose: 405±34.8% of 2 mM glucose control; +MSC-EVs: 603±48.6%, $p < 0.01$) and protected against apoptosis (caspase activity: cytokine-positive control 296±21.3%; +MSC-EVs 150±13.5, $p < 0.05$). Mice transplanted with MSC-EV-treated islets had lower BG concentrations 16 days after transplantation compared to non-transplanted (NT) mice and showed an overall trend towards lower BG compared to mice transplanted with control islets (NT: 28.4±3.20 mM, control: 18.73±2.15, MSC-EV: 14.15±3.18, $p < 0.05$). Mice transplanted with MSC-EV-treated islets showed improved glucose tolerance upon challenge compared to the NT and control groups (GTT AUC, NT: 150.0±1.53, control 106.1±4.38, MSC-EV: 81.78±3.50, $p < 0.001$).
Conclusion: EV treatment of islets improves their function in vitro and in vivo. This therefore represents a promising cell-free therapeutic avenue for improving islet function for transplantation.

P-235 Lost in Transition: Mapping the Maturation Roadblocks of Stem Cell-Derived β-Cells

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Background and Methods: To investigate the relationship between gene regulation and functional maturation in embryonic stem cell-derived β-cells (SCβs), we performed multi-omic single-cell profiling, combining patch-clamp electrophysiology, single-cell RNA sequencing, and regulatory network inference (SCENIC). We analyzed SCβs across maturation states (stage 7; immature D23–33, maturing D45–57) and compared them to primary human β-cells. Functional validation included insulin secretion assays, oxygen consumption rates (OCR), and transmission electron microscopy (TEM).
Results: SCβs exhibited larger Na^+ and Ca^{2+} currents and exocytosis responses compared to primary β-cells, correlated with ion channel transcript expression (*KCNJ11*, *SCN3A*, *SCN9A*), yet did not secrete insulin in response to glucose. Mitochondrial pathways essential for β-cell function (e.g., ATP synthesis, Oxphos, ETC components) were underrepresented in SCβs, aligning with higher basal OCRs, reduced glucose respiration, and higher proton leak than primary β-cells. Despite high expression of cytochrome c oxidase transcripts, SCβs showed reduced activity of oxidative stress and metabolic regulators (NFE2L2(+), ATF4(+), SREBF1(+), XBP1(+)), suggesting insufficient control of mitochondrial function. These regulons were found to be anticorrelated to exocytosis, suggesting dysregulated coupling of transcriptional and functional maturation. Notably, TEM revealed mitochondrial abnormalities and secretory granule immaturity. SCβs had reduced activity of key β-cell identity regulons (MAFA (+), PDX1(+), NEUROD1(+)), while progenitor-associated regulons (LMX1A/B(+), FOXA1/3(+), SOX4(+)) remained active, indicating incomplete lineage commitment.
Conclusions: Our multi-omic framework reveals that SCβs display transcriptional and functional immaturity, particularly in metabolic regulation and terminal β-cell identity. Targeting upstream regulatory bottlenecks may be key to advancing SCβ maturation for therapeutic applications in T1D.

P-236 Towards vascularized SC-islets

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Vascular cells – pericytes (PCs) and endothelial cells (ECs) – interact with pancreatic progenitors (PPs) to promote endocrine differentiation and maturation. Additionally, islet vascularization promotes β-cell functionality. Including vascular cells in hPS cell-derived islets (SC-islets) may promote endocrine differentiation and maturation as well as systemic integration after transplantation.
We have developed robust procedures for the generation of very highly enriched PPs and essentially pure PCs and ECs from the same hPS cell line. To maximize the yield in PDX1$^+$/SOX9$^+$/NKX6.1$^+$ PP cells, we introduced changes in both early and PP specification steps. Single-cell RNA Seq confirmed a highly synchronous and efficient differentiation process. To increase the robustness of the procedure, we are using bright field images of three stages (PS cell, monolayer and PP) from over 100 differentiations and machine learning to develop an algorithm that would predict the differentiation outcome. To generate vascular cells, we adapted a protocol that takes advantage of the common developmental origin of PCs and ECs. The resulting PC and EC progenitors are separated by magnetic-activated cell sorting (MACS) and further differentiated into pure PCs and ECs. These procedures are applicable to different hPS cell lines.
PPs, PCs and ECs are then co-clustered in microwells and differentiated together into vSC-islets that contain functional β-cells. Vascular cells are maintained and appear well-distributed in the SC-islets. Strikingly, SC-islets grew larger in the presence of vascular cells, suggesting a meaningful interaction. We are currently evaluating the impact of vascular cells on β cell maturation and function and following transplantation.

P-237 Evaluation of apoptosis-regulatory effects of mediator complex subunits in cytokine-exposed human EndoC-βH5 cells

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Introduction. Transcription factors are crucial mediators of cytokine-induced beta-cell dysfunction and apoptosis thereby playing important roles in the pathogenesis of type 1 diabetes. Several transcription factors including nuclear factor kappa B (NFκB) and signal transducer and activator of transcription (STAT) have been widely investigated and are known to regulate important downstream effector genes involved in e.g. apoptosis. The large multi-subunit mediator complex (MED) is a conserved regulator of gene expression that facilitates binding of transcription factors attached to enhancer regions with the preinitiation complex (PIC) assembly at promotor regions and RNA polymerase II recruitment to initiate gene transcription. Despite this vital role of the MED, its role and the function of MED subunits in cytokine-induced detrimental effects in beta-cells are unclear. We hypothesize that MED and specific subunits play essential roles in the regulation of beta-cell apoptosis.
Aim. This project aims to establish the roles of individual MED subunits in mediating cytokine-induced apoptosis in human EndoC-βH5 cells.
Methods. siRNA was used to knock down the expression of 10 individual MED subunits. Following transfection and cytokine treatment, apoptosis was quantified by caspase 3/7 activity in the cells and cytotoxicity was quantified by cytoplasmic protease activity released to the media.
Results. The results showed that knockdown of MED subunits MED13 or MED15 caused increased cytokine-induced apoptosis, compared to mock-transfected cells. Conclusion. Our findings indicate that the MED subunits MED13

and MED15 have anti-apoptotic effects in cytokine-exposed EndoC-βH5 cells. Further, investigations are ongoing to decipher the genes affected by these two MED subunits.

Session 3: Thursday, 12 of June, 2025 17:40

P-301 Disrupted α-cell function in human and murine islets devoid of other endocrine cells

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Introduction: Pancreatic α-cells release glucagon to counteract hypoglycemia and suppress its secretion during hyperglycemia. This regulation is influenced by glucose levels and paracrine signals from islet cells. In diabetes, glucagon secretion is dysregulated, with elevated plasma glucagon during hyperglycemia and blunted α-cell responses to hypoglycemia. While multiple diabetes-related factors have been linked to α-cell dysfunction, the exact mechanisms remain unclear.
Aim: To investigate whether disrupted intra-islet communication drives glucagon dysregulation.
Methods: Monotypic pseudoislets were generated by sorting and re-aggregating purified human α- and β-cells. Glucagon and insulin secretion were measured at physiological glucose levels from basal (5.6 mM) to either 8.6 mM or 3 mM and compared with pseudoislets containing all cell types. Plasmatic glucagon was assessed in α-only mice (Rip-DTR, Sst-DTR, and Ppy-DTR transgenes) following diphtheria toxin (DT)-induced ablation of β-, δ-, and γ-cells ("non-α-cells"). After islet remodeling, glucagon was measured during hypo- and hyperglycemia and compared with control mice.
Results: Human β-cells maintain proper secretory function without α-, δ-, and γ-cells, whereas α-cells require paracrine regulation. In absence of non-α-cells, α-cells exhibit an inverted secretory pattern with increased glucagon release at 8.6 mM and reduced secretion at 3 mM. Similarly, α-only mice show impaired glucagon suppression during hyperglycemia and decreased secretion in hypoglycemia.
Conclusion: Human and murine α-cells rely on intra-islet communication for proper function. Without non-α-cells, α-cells display a dysregulated secretory pattern, mirroring postprandial hyperglucagonemia and impaired α-cell hypoglycemic responses in diabetes. This suggests that glucagon dysregulation in diabetes stems from inadequate α-cell interactions within the islet.

P-302 Direct reprogramming of human fibroblasts into pancreatic β-cells using pancreatic-specific transcription factors in recombinant protein form

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Introduction: Transdifferentiation of one terminally differentiated somatic cell into another, bypassing the pluripotent stage, minimizes the risk of tumorigenesis and genetic mutations. While β -like cells have been successfully derived from related endodermal cell types through the forced expression of developmental transcription factors (TFs), generating them from distant lineages, such as fibroblasts, remains a challenge. Direct nuclear reprogramming via key transcription factors (Pdx1, Ngn3, MafA, and Pax4) initiates this process, yet its clinical application is hindered by viral genome integration and associated mutagenesis risks. This study presents an alternative approach, inducing human dermal fibroblasts (HDFs) into insulin-producing cells (IPCs) using the TFs in the form of recombinant proteins.
Research Gap: A major challenge in reprogramming is developing transgene-free methods while ensuring that factors like Ngn3 and Pax4, which are absent in mature β -cells, can be turned off or regulated as needed. Recombinant proteins offer precise control over dosage and timing, addressing this limitation.

Materials and Methods: Protein transduction domains (PTDs), such as the HIV1-TAT peptide, have been used to facilitate protein uptake in various cell types, including stem cells. Here, we engineered pancreatic transcription factors with PTD sequences and introduced them into HDFs.
Results: Each transcription factor was efficiently internalized and localized in the nuclei, retaining biological functionality. RT-qPCR and immunocytochemistry confirmed de novo insulin synthesis in HDFs using the transcription factors. Additionally, modifying the epigenetic landscape of HDF using HDAC and DNMT inhibitors significantly altered reprogramming kinetics.

P-303 Restoring balance: normalization of impaired beta cell function in type 2 diabetes with caloric restriction

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Diet-induced obesity (DIO) mouse models have proven pivotal in unveiling pathophysiological mechanisms of type 2 diabetes mellitus (T2D). However, existing models have some inherent methodological drawbacks, such as beta cell plasticity and composition of the diet used to induce T2D. Clinical studies hint at T2D remission with caloric restriction in humans, however limited mechanistical explanation is available at the level of beta cell function.
We therefore constructed a novel mouse model of DIO that more closely reflects T2D in humans. Male and female C57BL/6J mice were fed a western diet (WD) for 12 weeks starting from the age of 12 weeks, after which they exhibited a T2D phenotype in the form of fasting hyperglycemia, impaired glucose clearance and increased insulin resistance. 7 days of caloric restriction (35% of the caloric intake of the control group) completely reversed the diabetic phenotype, with normalization of body mass, glucose handling and insulin sensitivity.
To provide a mechanistical explanation for both the DIO and remission following caloric restriction at the level of beta cell function and glucose sensitivity, we performed functional multicellular confocal calcium imaging on acute pancreatic tissue slices. A left shift in glucose dependence was detected in the WD group, which together with hyperglycemia accounted for hyperinsulinemia observed *in vivo*. Short term caloric restriction completely reversed the compensatory left shift in beta cell function and decreased their oscillatory activity.
Our findings further illuminate the impact of caloric restriction on T2D and our model provides a novel platform for studying T2D.

P-304 Reforming islets with mesenchymal stromal cells (MSCs) promotes deposition of extracellular matrix (ECM) and improves secretory function.

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Background: ECM forms an important component of the islet microenvironment and its loss during enzymatic islet isolation can contribute to poor graft survival post-transplantation. We investigated whether MSCs can deliver ECM to reformed islets and improve islet function.
Methods: Dispersed mouse islets were reformed either with a $1:1$ proportion of murine bone marrow-derived MSCs (MSC-reformed) or alone (reformed). Glucose-stimulated insulin secretion (GSIS), insulin content and oxygen consumption rate (OCR: via Seahorse) were measured in MSC-reformed, reformed and whole islets. Fluorescence immunohistochemistry was used to visualise islets and assess ECM deposition.
Results: MSC-reformed islets initially formed mixed-cell aggregates, but after two days in culture, islet cell clusters

'budded' from a distinct MSC core. GSIS was unchanged in MSC-reformed islets ($20mM$ glucose: whole: $1.1 \pm 0.8ng$/islet/hour, reformed: 0.8 ± 0.6, MSC-reformed:0.8 ± 0.5, two-way ANOVA) whilst insulin content was reduced compared to whole and reformed islets (whole:$35.8 \pm 8.0ng$/islet, reformed:31.8 ± 7.5, MSC-reformed:19.4 ± 3.8, one-way ANOVA, $p < 0.05$). Consequently, insulin secretion as a proportion of content was improved in MSC-reformed islets. Both reformed and MSC-reformed islets showed a trend towards increased OCR compared to whole islets (AUC: whole:9023 ± 1657, reformed:13167 ± 4513, MSC-reformed:12590 ± 2566, one-way ANOVA, $p = 0.198$). Fluorescent imaging suggested that reformed islets have reduced laminin content (likely a result of islet dispersion), but this effect was attenuated when islets were reformed with MSCs.
Conclusion: Reforming islets with MSCs increased GSIS as a proportion of insulin content and preliminary data suggests it may enhance ECM deposition in islets. Future studies will investigate whether MSC-reformed islets show improved viability or function in response to stressors and in diabetic mice.

P-305 Voltage-dependent Ca^{2+} entry inhibits GLP-1-induced cAMP generation in β-cells

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Introduction: Ca^{2+} and cAMP play crucial roles in insulin secretion from β-cells. Insulin granule exocytosis is triggered by voltage-dependent Ca^{2+} entry and amplified by cAMP-increasing agents, such as glucagon-like peptide-1 (GLP-1). Ca^{2+} regulates both cAMP generation by adenylyl cyclases and degradation by phosphodiesterases, but the resulting effect on cAMP kinetics remains unclear.
Aim: To clarify how voltage-dependent Ca^{2+} influx influences GLP-1-receptor-stimulated cAMP signalling in β-cells.
Methods: Using TIRF microscopy, sub-plasma-membrane concentrations of cAMP and Ca^{2+} ($[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$) were recorded in MIN6, primary mouse- and human β-cells using fluorescent reporters.
Results: Depolarization of MIN6 β-cells with high K^+ resulted in $[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$ increases. GLP-1 and semaglutide (10 nM) induced stable $[cAMP]_{pm}$ elevation. Subsequent depolarization consistently reduced $[cAMP]_{pm}$, an effect prevented by omission of extracellular Ca^{2+}. Depolarization counteracted GLP-1-stimulated $[cAMP]_{pm}$ elevation also in primary mouse and human β-cells. In mouse β-cells, insulin (0.5 μM) lowered $[cAMP]_{pm}$ in the presence of GLP-1, but the insulin receptor antagonist S961 (1 μM) did not prevent depolarization-induced $[cAMP]_{pm}$ reductions, indicating that these were not caused by auto/paracrine actions of insulin. Depolarization prevented $[cAMP]_{pm}$ elevation by subsequent GLP-1 stimulation. This effect apparently reflected inhibition of cAMP generation rather than stimulated degradation, since it could not be prevented by phosphodiesterase inhibition with IBMX.
Conclusion: Voltage-dependent Ca^{2+} entry inhibits GLP-1-receptor-mediated cAMP formation in β-cells, potentially via Ca^{2+}-inhibited adenylyl cyclases. This effect may be important to prevent excessive stimulation of insulin secretion by GLP-1.

P-306 Increased beta cell–specific MANF protects against beta cell death and hyperglycemia in streptozotocin (STZ)-induced diabetic and MANF knockout mice

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Background: Endoplasmic reticulum (ER) stress and sustained unfolded protein response (UPR) contribute to increased beta cell immunogenicity and death in type 1 diabetes (T1D). Mesencephalic astrocyte–derived neurotrophic factor (MANF) is an ER stress–regulating factor with protective and immunomodulatory roles. MANF knockout mice develop diabetes due to sustained UPR activation resulting in beta cell loss. In humans, MANF deficiency is associated with syndromic diabetes. In vitro, MANF promotes beta cell proliferation and protects from ER stress–induced death.
Aims: To investigate whether transgenic beta cell–specific MANF overexpression protects against STZ-induced T1D and hyperglycemia in *Manf*$^{-/-}$ mice.
Methods: We generated doxycycline-inducible beta cell–specific MANF-overexpressing mice (INS-MANF) and induced diabetes with multiple low doses of STZ. Protective mechanisms were studied using RT-qPCR, immunohistochemistry, mRNA sequencing, and immunophenotyping. Additionally, INS-MANF mice were crossed with *Manf*$^{+/-}$ mice to generate *Manf*$^{-/-}$ mice with beta cell–specific MANF overexpression.
Results: Overexpressed MANF protected against STZ-induced beta cell death, insulitis and hyperglycemia. Transcriptomics analysis of MANF-overexpressing islets revealed decreased expression of genes involved in the UPR, immune cell activation, antigen-presenting molecules, and p53-mediated cell cycle arrest. Immunophenotyping showed reduced number of CD4^{+} lymphocytes in pancreatic lymph nodes. The protective effect of MANF was associated with reduced expression of thioredoxin interacting protein (TXNIP). MANF overexpression in beta cells prevented hyperglycemia in MANF-deficient mice.
Conclusions: Increased MANF protects beta cells by reducing cellular stress, thereby attenuating autoimmune cell responses and beta cell death in mice, constituting a promising therapeutic avenue for T1D.

P-307 Pharmacological inhibition of SOS1-RAS interaction promotes pancreatic β-cell regeneration

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Restoring a sufficient mass of functional β cells has emerged as a promising therapeutic strategy for diabetes, yet achieving this through pharmacological stimulation of endogenous β-cell regeneration remains a challenge.

We demonstrate here that ectopic expression of a constitutively active form of Kras (Kras^{G12D}) specifically in pancreatic endocrine cells impairs islet growth, causing a dramatic reduction in β-cell numbers and islet size. Conversely, we show that BI-3406, a potent and selective SOS1-RAS interaction inhibitor, promotes unprecedented levels of β-cell proliferation in primary human islets, both in culture and following transplantation in immunocompromised diabetic mice.

Using mouse models of chemically-induced diabetes, we show that BI-3406 treatment restores β-cell mass, leading to a gradual normalization of blood glucose and insulin levels, as well as to sustainable improvement in glucose tolerance. Similarly, in a genetic mouse model of diabetes (db/db;Lepr^{db}), BI-3406 induces β-cell proliferation, enhances β-cell maturation and sustains elevated serum insulin levels.

Our data provide the first pre-clinical evidence of an orally bioavailable KRAS inhibitor that can directly induce β-cell regeneration and mitigate the diabetic phenotype.

P-308 Humanized anti-inceptor antibody enhances β-cell function and preserves β-cell mass in healthy and Type 2 Diabetes

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Introduction: Type 2 diabetes (T2D) is characterized by insulin resistance and progressive β-cell loss and dysfunction. Insulin signalling is crucial for β-cell survival, proliferation, and insulin secretion. In this context, the insulin inhibitory receptor (inceptor) has been identified as a novel regulator of insulin receptor (INSR) signalling desensitization, making it a promising target for β-cell protection.
Aims: This study aimed to investigate the therapeutic potential of humanized anti-inceptor antibody (HAb) in enhancing β-cell function and preserving β-cell mass in physiological and T2D settings.
Methods: We developed humanized anti-inceptor HAb targeting the native inceptor ectodomain. The antibodies

were tested *in vitro* using INS1 cells and primary mouse islets, and *in vivo* in hyperglycaemic clamp studies and a severe T2D mouse model (db/db). Pharmacokinetics, biodistribution, and glucose-dependent target engagement were characterized. Findings were further validated in human islets and EndoC-βH5 cells to confirm translational relevance.
Results: Anti-inceptor HAb exhibited prolonged pharmacokinetic stability and pancreatic targeting. Treatment significantly enhanced glucose-stimulated insulin secretion in a dose-dependent manner in healthy mice. In db/db mice it improved β-cell proliferation, preserved β-cell mass and increased glucose-stimulated insulin secretion. In human islets and β-cell lines, HAb potentiated insulin secretion and enhanced INSR signalling, leading to increased AKT activation and improved β-cell responsiveness.
Conclusion: Humanized anti-inceptor HAb improves β-cell function in healthy and diabetic mice by sensitizing INSR signalling. These findings highlight the potential of anti-inceptor HAb as a potential therapy for T2D, offering a novel approach to maintain glycaemic control.

P-309 Does glycine correct alterations in glucose-stimulated calcium influx and insulin secretion in human pancreatic islets cultured under glucotoxic conditions?

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Introduction: Prolonged culture of human islets under glucotoxic conditions alters their Ca^{2+} and insulin secretion responses to subsequent glucose stimulus (GSCaR and GSIS). Reduced expression of glycine receptor alpha 1 subunit (*GLRA1*) may contribute to these alterations.
Aim: Test whether glycine corrects the glucotoxic alterations of GSCaR and GSIS in human islets.
Methods: Upon reception, islets were precultured 3-7days in RPMI containing 5.5mM glucose and 10% FBS, then cultured 1week in RPMI containing 5.5, 8.8 or 22mM glucose (G5-, G8-, and G20-islets). Gene mRNA levels were measured by RNA sequencing. Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and insulin secretion were measured during stepwise stimulation with glucose (0.5, 5, 10 and 30mM) and glycine 0.1-10mM.
Results: *GLRA1* mRNA levels were 50% and 75% lower in G8- and G20-islets than in G5-islets. Compared to G5-islets, G8-islets were more sensitive to glucose and secreted more insulin at 10-30mM glucose; G20-islets displayed higher $[Ca^{2+}]_c$ and insulin secretion at low glucose, variable glucose-induced change in $[Ca^{2+}]_c$, and little secretion response to glucose. At 0.5mM glucose, glycine transiently increased $[Ca^{2+}]_c$ more in G8- and G20-islets than in G5-islets. Glycine increased $[Ca^{2+}]_c$ and secretion at 10-30mM glucose in G5-islets and at 5-30mM glucose in G8-islets. It also increased $[Ca^{2+}]_c$ but not secretion at 5-10mM glucose in G20-islets and failed to prevent their acute repolarisation and drop in $[Ca^{2+}]_c$ by 30mM glucose.
Conclusion: Despite the strong reduction in *GLRA1* expression, glycine increased $[Ca^{2+}]_c$ in glucotoxic human islets, except at 30mM glucose. However, it failed to stimulate insulin secretion.

P-310 AdoShell®: A Permselective and Non-Fibrotic Scaffold for Human Islet Transplantation

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Introduction: AdoShell® is a fully retrievable, implantable scaffold for islet transplantation as a potential diabetes cure without immunosuppression. Its permselective hydrogel allows insulin diffusion while blocking immune cell invasion and antibody diffusion. Allogenic rat islet transplants in immunocompetent diabetic rats showed prolonged islet survival, insulin secretion, and glycemic control, demonstrating immuno-protection. We aim to demonstrate AdoShell® compatibility with human primary islets and Stem Cell-Derived Islets (SCDI), its maintenance of long-term islets functionality, viability, and immunoprotective properties in vitro and *in vivo*.
Results: Human islet viability, assessed via LIVE/DEAD and TUNEL assays, was maintained after encapsulation. Morphology and architecture were preserved, as confirmed by immuno- histochemistry of α- and β-cells. AdoShell® showed glucose-responsive insulin secretion with a slight 2-minute delay compared to naked islets (perifusion experiments). Functionality was maintained for up to 4 months *in vitro*. To confirm immunoisolation of encapsulated human primary islets, they were co-cultured with IL- 2-activated allogeneic PBMCs for 4 days. They showed sustained viability, IEQ number, and total insulin content compared to naked islets exposed to the same conditions. *In vivo*, AdoShell® human Islets in immunodeficient mice exhibited stable C-peptide secretion for more than 2 months (n=10 mice, 2 studies). *In vitro* secretion indexes of explants were similar to pre-implantation levels. Similarly, encapsulated SCDI matured *in vivo* and demonstrated functionality for at least 2 months.
Conclusions: AdoShell® supports primary human islet immunoprotection, long-term survival and functionality, and enables the in vivo maturation and functionality of SCDI.

P-311 Regulation of islet lipid metabolism and mitochondrial reprogramming by incretin receptors

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Glucolipotoxicity is a major metabolic trigger for β-cell dysfunction linked to induction of β-cell oxidative stress. Strategies to reduce lipid accumulation and promote mitochondrial adaptation are required to halt progression towards overt type 2 diabetes.
A previous investigation from our lab identified a multiprotein GLP-1R signalosome at the ER-mitochondrial interface involved in acute β-cell mitochondrial fragmentation. In parallel, we identified protein-protein interactions (PPIs) between active incretin receptors and a range of lipid metabolism and fatty acid oxidation (FAO) enzymes, as well as with the lipid droplet (LD) small GTPase Rab18. As changes in mitochondrial morphology are closely associated with modified lipid transfer and nutrient utilisation, with a strong correlation between mitochondrial fragmentation and FAO, we hypothesised that these PPIs might be involved in the control of β-cell lipid metabolism coupled with mitochondrial adaptation towards lipid utilisation under glucolipotoxic conditions.
Using a FluoSTEP-based split fluorescent biosensor system, we demonstrate GLP-1R/GIPR-dependent cAMP/PKA signalling specifically from LDs, with overnight incretin exposure resulting in reduced LD numbers in high-fat fed mouse islets ($74 \pm 4\%$ reduction, $p = 0.0155$). Western blot quantification of PKA-phosphorylated hormone sensitive lipase indicated increased (3.18 ± 0.22-fold) lipolysis following acute incretin exposure in lipid-loaded β-cells, accompanied by increased LD-mitochondrial contacts and lipid transfer between both organelles. Oxygen consumption rates $\pm$ etomoxir, assessed by Seahorse assay, indicated increased FAO levels following incretin exposure of lipid-loaded β-cells under high but not low glucose conditions.

Our results reveal a prominent role for LD-localised incretin receptor signalling in the control of β-cell lipid metabolism and mitochondrial adaptation to glucolipotoxicity.

P-312 Gabapentin enhances mouse pancreatic β-cell excitability but reduces KCl-induced insulin release without affecting Ca_V calcium influx

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Introduction: Pancreatic β-cell electrical activity and insulin secretion are regulated by high voltage-gated calcium channels (HVCCs). Previously, we demonstrated that genetic ablation of the $\alpha_2\delta$-1 HVCC subunit reduces β-cell calcium influx by $\sim$60%, severely impairing insulin release and causing glucose intolerance. The antiepileptic and analgesic gabapentinoids target $\alpha_2\delta$ subunits and reduce HVCC calcium currents. However, gabapentinoid use has paradoxically been linked to hyperinsulinism and hypoglycemia.
Aims: To explore this discrepancy, we investigated whether chronic gabapentin (GBP) treatment affects β-cell glucose-dependent excitability and insulin release in an HVCC-dependent manner.
Methods: Isolated mouse pancreatic islets were incubated with 1 mM GBP for 36–40 hours. The effects were characterized at single β-cell level using electrophysiology (voltage- and current-clamp), and at whole islet level using calcium imaging and dynamic insulin release.
Results: Half of the GBP-treated islets exhibited spontaneous calcium transients at subthreshold glucose levels (5 mM), unlike untreated islets, which remained silent. In 10 mM glucose, $\sim$66% of the GBP-treated islets showed continuous calcium transients instead of a rhythmic activity. Consistent with increased excitability, GBP-treated β-cells displayed a higher resting membrane potential and plateau potential. However, KCl-induced calcium transients and dynamic insulin release were significantly reduced. Importantly, these effects were not due to decreased β-cell HVCC calcium influx.
Conclusion: Our findings suggest that GBP treatment exerts a biphasic effect on β-cell function, enhancing excitability at low physiological glucose levels but impairing insulin secretion under maximal stimulation.

P-313 Spontaneous sub-plasma membrane Ca^{2+} microdomains in beta cells involve Ca^{2+} release from the endoplasmic reticulum

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Background and aims: Ca^{2+} signaling governs glucose homeostasis by precisely regulating insulin secretion from pancreatic β cells. While global cytoplasmic Ca^{2+} signals in β cells are well-studied, subcellular spatiotemporal Ca^{2+} kinetics remains largely unexplored. This study aimed to characterize local Ca^{2+} signaling in the sub-plasma membrane space in β cells.
Material and methods: Changes in sub-plasma membrane Ca^{2+} concentration ($[Ca^{2+}]_{pm}$) were recorded in MIN6 and primary mouse β cells using a membrane receptor-anchored fluorescent Ca^{2+} indicator and total internal reflection fluorescence microscopy.
Results: β cells maintained at sub-stimulatory conditions (3 mM glucose) showed transient and recurring $[Ca^{2+}]_{pm}$ increases, each lasting for 1–5 seconds, in spatially restricted microdomains (diameter: $2.1 \pm 0.1\mu$m). Increasing the extracellular $[Ca^{2+}]$ from 1.3 to 2.6 mM increased both the frequency of $[Ca^{2+}]_{pm}$ spikes and the number of microdomains, while removal of Ca^{2+} from the buffer completely abolished the $[Ca^{2+}]_{pm}$ elevations. In MIN6 cells, inhibition of L-type voltage-dependent Ca^{2+} channels with nifedipine was without effect, whereas depletion of endoplasmic reticulum (ER) Ca^{2+} using cyclopiazonic acid (CPA), and inhibition of IP3-receptors using 2-aminoethoxydiphenyl borate (2-APB), suppressed the $[Ca^{2+}]_{pm}$ spiking (CPA: $n = 32$, $p = 0.001$; 2-APB: $n = 66$, $p < 0.0001$).
Conclusion: Unstimulated β cells exhibit spontaneous sub-membrane Ca^{2+} microdomains, dependent on both extracel-

lular Ca^{2+} and release of the ion from the ER. Continued studies will clarify the potential involvement of ER-membrane contact sites and role in insulin granule trafficking and exocytosis.

P-314 Beyond Weak: The Resilient Antioxidant Defense of Pancreatic β-Cells

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Pancreatic β-cells were shown to express low amounts of antioxidant defense enzymes, but the reason for this remains enigmatic. Here we bring evidence supporting the currently accepted view that the low levels of antioxidant enzymes are associated with glucose-induced increased redox sensitivity as redox signaling mediated by hydrogen peroxide is required as an important metabolic coupling factor for insulin secretion. Using RNA-seq analysis of primary mouse islets and the INS1-E rat insulinoma cell line, we demonstrated that glucose stimulation triggers a transcriptional upregulation of thioredoxin/peroxiredoxin systems and superoxide dismutase—key components of the β-cell's antioxidant machinery. Glutathione peroxidases are also present and are activated primarily under oxidative stress. Strikingly, primary β-cells exhibited heightened prooxidant sensitivity compared to INS1-E cells, suggesting cell-line adaptation to *in vitro* conditions. To dissect glucose-dependent redox signaling, we employed iodoTMT-based proteomics, revealing enhanced reversible cysteine oxidation in antioxidant enzymes upon glucose stimulation. This highlights their dual role in scavenging excess reactive oxygen species while facilitating redox signal transduction. Our findings indicate that primary β-cells sustain a dynamic antioxidant system essential for balancing insulin secretion and cytoprotection. We conclude that β-cells strategically limit classical antioxidant defenses to preserve redox sensitivity for GSIS while deploying thioredoxin/peroxiredoxin pathways as primary safeguards. Disruption of this equilibrium compromises insulin secretion and contributes to diabetic pathophysiology. These insights underscore the therapeutic potential of targeting redox signaling nodes to restore β-cell function in diabetes.
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P-315 Sorting of human pancreatic islets by flow cytometry into alpha and beta cells reveals genes associated with sex in the different cell types.

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Background and aim: Human islets have several cell types that regulate glucose homeostasis. These include α- and β-cells, secreting glucagon and insulin, respectively. Dysfunction in these cell types contributes to type 2 diabetes (T2D). Additionally, we previously found lower insulin secretion and differential gene expression in islets from women and men. Here, we aimed to associate sex with gene expression in sorted α- and β-cells from human pancreatic islets.
Material and methods: Human islets from 18 donors were sorted by flow cytometry into α- and β-cells, followed by RNA isolation and RNA-sequencing. Downstream bioinformatic analyses were done using R. Associations between sex and gene expression in α- and β-cells, respectively, were analyzed using linear models.
Results: We successfully sorted ≈600,000 cells from each islet donor and found 13,001 and 12,875 genes to be expressed in sorted human α- and β-cells, respectively. Additionally, sex was associated with differential expression of 34 and 31 genes in human α- and β-cells, respectively ($q < 0.05$). Based on gene ontology, the sex-associated genes in α-cells are involved in metabolic pathways and steroid biosynthesis, while those found in β-cells are involved in H3K27me2/H3K27me3 demethylase activity. Interestingly, *LDHA*, and *SLC2A1(GLUT1)*, as well as *OPRD1* and *PLCD4* are among the sex-associated genes in α- and β-cells, respectively.

Conclusion: We identified genes, such as *OPRD1*, that we previously found to be down-regulated in islets from human donors with T2D, to also be associated with sex in β-cells. Interestingly, down-regulation of *OPRD1* in clonal β-cells was associated with reduced glucose-stimulated insulin secretion.

P-316 Non-genotypic animal model of chronic type 2 diabetes: evaluation of pancreatic tissue

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Background and Aims: A complete understanding of the pathogenesis of type 2 diabetes (T2D) remains unclear. This could be related with a lack of appropriate pre-clinical models. Existing animal models, mostly genetic and monogenic, have limitations in replicating diabetes. In previous studies we observed that a short-term treatment of 15 days with Tacrolimus (TAC) induced beta-cell toxicity only in obese animals. This damage is comparable to that observed in diabetes. Basing on this, we propose a non-genotypic animal model of chronic T2D. **Materials and methods:** 26 male Sprague Dawley rats were fed with high-fat diet to induce obesity. Then, animals were treated with TAC or placebo for 9 months. The first 15 days animals were treated daily with a dose of 0.5 mg/kg. Then, treatment frequency was gradually reduced. Blood TAC levels were measured monthly. Intraperitoneal glucose tolerance test and insulin tolerance test were regularly performed. At endpoint, histological analysis of the pancreas was performed. **Results**: Animals treated with TAC developed diabetes, exhibiting higher glucose levels from day 15 to 9 months. Blood TAC levels were similar to those observed in clinical practice. In the pancreas, diabetic animals showed a reduced number and area of Langerhans Islets, increase in fibrosis, imbalance in alpha/beta cell ratio, decrease in insulin content and reduced expression of beta-cell nuclear factors MafA, FOXO1A, PDX-1 and NeuroD1. **Conclusion**: The observed phenotypes closely resemble human type 2 diabetes. This could be a more appropriate model for studying the pathophysiology of diabetes and, eventually, the related organ damage.

P-317 Roles of complement protein CD59 isoforms in metabolism and exocytosis in pancreatic β-cells

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Pancreatic β-cell dysfunction is a major cause of type 2 diabetes, owing to defects in its metabolism and insulin secretion machinery. CD59 is an inhibitor of the complement system whose canonical function is to inhibit the lytic membrane attack complex. Our previous studies identified that the intracellular CD59 isoforms, IRIS-1 and IRIS-2, play a crucial role in insulin exocytosis in pancreatic β-cells. Lacking glycosylphosphatidylinositol (GPI) anchor of the membrane-bound CD59, these intracellular variants interact with insulin granules and SNARE proteins, facilitating insulin release at different phases. However, the precise molecular mechanisms underlying their function remain unexplored. This study employs CRISPR-Cas9 gene editing to generate CD59 knockout and introduce an overexpression of human IRIS-1 or IRIS-2 in rat β-cell line INS-1 to investigate molecular functions of these CD59 isoforms. RNA sequencing analysis revealed that CD59 isoforms significantly influence pyruvate metabolism, a key determinant of β-cell function, as pyruvate fuels mitochondrial oxidative phosphorylation, triggering insulin secretion. In addition, glycogen accumulation, related to pyruvate metabolism in β-cells, is reported to cause metabolic dysfunction and reduced insulin secretion. Further, our protoarray data indicated that CD59 binds to Noc2, an effector of exocytosis. Our in vitro assays confirmed that IRIS-1 and IRIS-2 also have the binding capacity to Noc2, which could consequently regulate intracellular insulin transport. Our study highlights the novel metabolic and intracellular trafficking functions of IRIS-1 and IRIS-2. Understanding the mechanisms by which these CD59 isoforms regulate insulin secretion could

provide insight into the non-canonical roles of CD59 in insulin-secreting β-cells.

P-318 ISL1 modulates epigenetic landscape to repress genes associated with endocrine progenitor states during pancreatic endocrine development

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Pancreatic endocrine cells are essential for glucose homeostasis and energy metabolism, with their dysregulation contributing to metabolic diseases such as diabetes. All pancreatic endocrine cells originate from a common progenitor, and the transcription factor ISL1 plays a crucial role in regulating cell-fate decisions and differentiation. Using conditional ISL1 knockout mice (Isl1CKO) as a model, we assayed transcriptional changes induced by loss of ISL1. scRNA-seq revealed a significant shift in transcriptome of Isl1CKO mice compared to their littermate controls. These changes led to altered α-cell identity, loss of γ- and δ- cells, and an immature β-cell phenotype. These defects resulted in adaptive metabolic responses, severe diabetes, and premature death. ISL1 was previously shown to participate in chromatin remodelling. Using Cut&Tag targeting H3K27me3 and H3K27ac we therefore assessed the chromatin landscape at the gene regulatory regions of Isl1CKO endocrine cells. Interestingly, the analysis showed that in absence of ISL1 the chromatin was enriched with activating H3K27ac mark, in stark contrast to control condition where majority of regulatory regions were marked by repressive H3K27me3. Additionally, several genes with differential gene expression and histone modifications in Isl1CKO are also directly bound by ISL1. Taken together, our data indicate that ISL1 functions both as transcriptional activator and repressor during pancreatic endocrine development and maturation. Overall, we demonstrate a novel role for ISL1 in facilitating chromatin transitions to repress progenitor programs and promote endocrine cell maturation. These insights establish ISL1 as a critical regulator of endocrine identity and pancreatic function, with implications for diabetes pathogenesis.

P-319 Lack of β-cell Glutamate Dehydrogenase prevents metabolic rewiring in islets during the onset of prediabetes

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Introduction: Glutamate dehydrogenase (GDH) amplifies insulin secretion through glutamate formation, while being non-essential for glucose homeostasis. However, its deletion in β-cells prevents high-fat diet-induced obesity in adult mice.
Aims: Assessing the role of β-cell GDH in islet metabolic changes during the onset of prediabetes favoured by age plus diet.
Methods: We used 42-week-old male and female mice with β-cell-specific GDH deletion (β*Glud1*$^{-/-}$) and age-matched controls (Ctrl). Both groups were fed a high-caloric diet (HCD; kcal%: 20-40-40 protein-carbohydrate-fat) for 8 weeks, while a subset of β*Glud1*$^{-/-}$ mice received a standard diet (SD). Body weight, food intake, and non-fasting glycemia were measured weekly. At terminal week 9 HCD (1-year-old), we performed ipGTT and ipITT, followed by EchoMRI and tissue collection. We assessed metabolic pathways using flagship dehydrogenase activity through the NBT assay on pancreatic cryosections.
Results: Control male but not female mice fed HCD for 8 weeks increased fat mass and body weight with slightly elevated fasting glycemia. β*Glud1*$^{-/-}$ mice were resistant to such diet-induced changes. All HCD groups developed glucose intolerance versus SD-fed mice, while ITT did not show insulin resistance. In islets of Ctrl-HCD males, activities of glycolytic GAPDH and LDH were increased, although not in β*Glud1*$^{-/-}$, whereas increased mitochondrial SDH activity was observed in both HCD groups. Female mice were partially resistant to HCD diet.

Conclusions: Islets of old HCD-fed male mice exhibited a glycolytic shift, prevented by β*Glud1* deletion, while females showed resistance to diet-induced changes. Our results reveal islet metabolic changes ahead of non-fasting hyperglycemia with sex dimorphisms.

P-320 Glucagon of murine female islets promotes their revascularization after transplantation

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Introduction and aims: The adequate revascularization of transplanted islets during the initial post-transplant phase is crucial for their survival. Studies already reported that angiogenic processes differ between male and female. Therefore, the aim of this study was to investigate sex-related donor and recipient effects on islet revascularization after transplantation.
Methods: Islets were isolated from female and male wild type (WT) mice and cultured for 24 hours. The cellular composition and viability of the islets was analyzed by immunohistochemistry and flow cytometry. The secretion, gene and protein expression of the endocrine hormones and angiogenic determinates were analyzed by enzyme-linked immunosorbent assays (ELISA), quantitative real-time (qRT)-PCR and Western blot. Angiogenic processes were studied by growth curves, tube formation and spheroid sprouting assays. *In vivo*, female and male islets were transplanted into mouse dorsal skinfold chambers of female and male recipient mice, respectively. Subsequently, the revascularization of the grafts was assessed by intravital fluorescence microscopy.
Results: Our *in vivo* analyses revealed an increased revascularized area and higher functional microvessel density of female islets when compared to males. Furthermore, isolated female islets express more VEGF-A and exhibited significantly higher proportion of CD31-positive endothelial cells. Additionally, we detected a higher expression and secretion of glucagon in these islets. Further functional experiments revealed that glucagon significantly triggers proangiogenic processes.
Conclusion: Our findings demonstrate for the first time that the transplantation of female islets markedly improves the revascularization in a glucagon-dependent manner.

P-321 IFNα negatively affects intracellular Ca^{2+} signalling and hormonal secretion in pancreatic alpha- and beta-cells

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Background and Aims: Type 1 diabetes (T1D) is characterized by progressive loss of beta-cells. During disease progression, pancreatic alpha- and beta-cells are exposed to the same stress stimuli, such as interferon-α (IFNα) and other cytokines. However, the immune system only targets beta-cells, while alpha-cells survive. Since IFNα is a key mediator of the early stages of insulitis and a driving factor in T1D development, we aimed to characterize the alterations induced by IFNα in alpha- and beta-cell Ca^{2+} signalling and secretory function in different models.
Materials and Methods: AlphaTC1-9, MIN6 cells, and mouse islets were exposed to 1000 U/ml IFNα for 24 h. Intracellular Ca^{2+} was measured using FURA-2/AM under different glucose conditions and maximal stimuli. Insulin

and glucagon secretion were assessed by ELISA, including an additional low glucose + insulin condition for glucagon secretion. Viability was evaluated by Hoechst/propidium iodide staining.
Results: In MIN6 cells, IFNα reduced Ca^{2+} oscillations in response to glucose and KCl ($p < 0.001$) but did not alter insulin secretion. AlphaTC1-9 cells exhibited a diminished oscillatory Ca^{2+} response ($p < 0.05$) and impaired suppression of glucagon secretion by high glucose or insulin (p<0.05) after IFNα treatment. In primary islets, IFNα decreased Ca^{2+} response in beta-cells ($p < 0.05$), while insulin secretion remained unchanged. Moreover, IFNα reduced glucagon release at low glucose ($p < 0.01$) and weakened high glucose-induced inhibition of glucagon secretion. IFNα did not induce apoptosis in any model.
Conclusions: IFNα impairs glucose-induced Ca^{2+} signalling and hormone secretion in both alpha- and beta-cells, highlighting its role in pancreatic dysfunction during T1D progression.
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P-322 cGMP-cAMP cross talk in the primary cilium of islet cells

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Background and aim Primary cilia, although continuous with the plasma membrane and cytosol, act as specialized signaling hubs for integrating external cues. Cyclic nucleotides, cAMP and cGMP, shape the ciliary signaling landscape and influence pathways such as Hedgehog. Although cilia contain both cGMP-sensitive phosphodiesterase and effector proteins (e.g., CNG channels), it is unclear how cGMP signals are shaped and to what extent they crosstalk with cAMP and Ca^{2+}. This study aimed to elucidate the dynamics of cyclic nucleotide signaling in the primary cilia of pancreatic islet β-cells.
Material and methods TIRF microscopy of intact mouse, human and MIN6 pseudo-islets expressing cilia-targeted sensors for cGMP, cAMP and Ca^{2+} were used to record ciliary dynamics.
Results In human islets, both atrial natriuretic peptide and GLP-1 stimulated cytosolic cGMP production in a glucose and Ca^{2+}-dependent manner. Similar to cAMP, cGMP entered the cilium via diffusion, but in contrast to cAMP elevations it triggered localized Ca^{2+} influx via CNG3A channel activation. Intriguingly, somatostatin-induced reductions in ciliary cAMP also triggered ciliary Ca^{2+} signaling, indicating opposing but converging effects of the two cyclic nucleotides. Consistent with such opposing roles, both cAMP lowering and cGMP elevation induced nuclear translocation of the cilia-dependent transcription factor GLI2 through a Ca^{2+}-dependent mechanism.
Conclusion We describe a novel cGMP-mediated signaling mechanism in islet β-cells that involve localized Ca^{2+} influx and GLI2 activation, indicating that primary cilia are major target of cGMP action.

P-323 Adeno-associated virus capsid variant screen for diabetes research: building a scalable methodology for modification of gene expression in primary human islets

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Translating therapeutic strategies from animal models to humans remains challenging and isolated human islets, the experimental gold standard for translational diabetes research, exhibit limited amenability to genetic manipulation. Here we describe an adeno-associated virus (AAV)-mediated transduction methodology to effectively modify gene expression in reaggregated human islets.
To identify the AAV capsid with highest affinity for islet transduction, we screened nine variants encoding eGFP by

reaggregating dispersed islet cells with AAVs. Transduction efficiency, transgene expression, and cellular proliferation were assessed via 3D-confocal microscopy. ATP levels, insulin secretion and content were analyzed to assess potential impact on islet function and viability.
Among the screened variants, AAV2.7m8 exhibited highest transduction efficiency and eGFP expression with minimal effects on insulin and ATP levels. However, it significantly reduced cellular proliferation. AAV9 preserved cellular proliferative capacity, but delivered lower transduction efficiencies and moderate eGFP expression. At lower titers, AAVKP1 displayed superior transduction efficiency and similar eGFP expression compared to AAV2.7m8. Using AAV2.7m8, we knocked down dual-specificity tyrosine phosphorylation-regulated kinase 1A (*DYRK1A*) and compared its effects on β-cell proliferation to harmine, a known *DYRK1A* inhibitor. *DYRK1A* knockdown increased non-β-cell proliferation but not β-cell proliferation, likely due to AAV2.7m8's negative effects on proliferation. Harmine effectively induced both β- and non-β-cell proliferation.
In summary, we developed a scalable platform for AAV-mediated gene expression modification in human islets, achieving high transduction efficiencies while maintaining stable islet function. Our platform can enable the study of putative therapeutic targets directly in human islets, offering a translational platform for novel therapeutic strategies.

P-324 Helios$^+$ T-cells destruct human stem cell-derived islets

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β cells, leading to insulin deficiency and hyperglycemia. Evidence suggests T1D is mediated by T-cells; however, a definitive auto-reactive T-cell marker has not been identified. In preclinical autoimmune models, Helios-expressing effector T-cells have been suggested as potential autoreactive cells.
Aim: This study aims to investigate the role of Helios$^+$ T-cells in clinical T1D and preclinical models of T1D.
Methods: We analyzed the frequency of Helios$^+$ T-cells in the peripheral blood of T1D patients and matched healthy controls. Additionally, we examined these cells in preclinical T1D models, including multiple low-dose streptozotocin (MLDSTZ)-treated and non-obese diabetic (NOD) mice, using flow cytometry. To evaluate the direct impact of Helios$^+$ T-cells on β cell destruction, we co-cultured human stem cell-derived islets (SC-islets) with Helios-overexpressing Jurkat T-cells, GFP-expressing Jurkat T-cells, or standard Jurkat T-cells. We then assessed the number of apoptotic β cells using confocal microscopy. Herein, GFP-expressing Jurkat T-cells and standard Jurkat T-cells were control groups.
Results: Helios$^+$ T-cells were significantly elevated in T1D patients, MLDSTZ-treated mice, and NOD mice. These cells exhibited an IL-17 and IFN-γ proinflammatory signature. Furthermore, Helios-overexpressing Jurkat cells induced higher β-cell apoptosis in SC-islets compared to both GFP-expressing and standard Jurkat T-cells, indicating their destructive potential.
Conclusion: Helios$^+$ T-cells exhibit a strong proinflammatory response and contribute to increased β-cell apoptosis. Thus, Helios may serve as a surrogate marker for detecting T1D and its progression.

P-325 The glucagon-like peptide-1 receptor antagonist, Exendin (9-39)NH_2, improves the impaired glucagon response to hypoglycaemia from Type 1 diabetic islets.

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Introduction: In Type 1 diabetes (T1D), counterregulatory glucagon secretion from the islet α-cells becomes impaired, contributing to potentially fatal hypoglycaemia. Normally, glucagon-like peptide-1 (GLP-1) is not produced by α-cells due to the selective expression of prohormone convertase-2, which processes proglucagon to glucagon. Recent observations suggest that prohormone convertase-1/3, an enzyme that processes proglucagon to GLP-1, is increased in T1D α-cells.
Aims: To investigate the contribution of intra-islet GLP-1 signalling on glucagon secretion during hypoglycaemia in islets from the autoimmune non-obese diabetic (NOD) mouse and human T1D donors.
Methods: Islet hormone secretion was assessed in adult diabetic (blood glucose $\geq$ 25 mmol/l) and normoglycaemic (blood glucose 5–8 mmol/l) pre-diabetic control NOD/ShiLtJ mice by *in situ* pancreas perfusion and from human T1D and non-diabetic islets by *in vitro* static secretion.
Results: While hypoglycaemia increased glucagon secretion from the perfused NOD control pancreas, this response was abolished from the diabetic pancreas (10 vs 1 mmol/l glucose: control, 9.17 ± 1.36 vs 21.5 ± 4.32 pg/min, $n = 7$; diabetic, 3.99 ± 1.78 vs 6.03 ± 0.22 pg/min, $n = 5$). The GLP-1R antagonist, exendin (9-39)NH_2, significantly improved hypoglycaemia-induced glucagon secretion from the diabetic NOD pancreas by 3.5-fold ($*p < 0.05$, $n = 5$ mice) and from human T1D islets by 1.9-fold ($*p < 0.05$, $n = 2$ T1D donors).
Conclusion: An increase in intra-islet GLP-1 signalling plays a significant role in the defective glucagon response to hypoglycaemia in T1D and provides a rationale for GLP-1R antagonists as a promising therapy for reducing the risk of hypoglycaemia in individuals with T1D.

P-326 Glucose-derived redox equivalents preserve alpha cell function during hypoglycemia

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Background and aim: Glucagon, the primary counterregulatory hormone, is secreted from pancreatic apha cells. Though ATP production in alpha cells is thought to be derived from fatty acid oxidation, glycolytic activity is similar to that of the beta cells. Since islet cells can metabolize glucose via the pentose phosphate pathway and elevated glucose levels lead to increased cytosolic nicotinamide adenine dinucleotide phosphate (NADPH) in alpha cells, we hypothesized that alpha cells utilize glucose metabolism to maintain cytosolic redox potential. Furthermore, given the redox sensitivity of protein kinase A (PKA), which is key for regulating alpha cell secretion, we propose that PKA activation mediates these effects.
Methods: To test this hypothesis, we infected whole islets with an alpha cell-specific redox sensor (Grx1-roGFP) and monitored cytosolic redox changes using live-cell imaging. We then pre-incubated whole islets in 1 or 5 mM glucose for 1 hour and subsequently measured glucagon release. Additionally, we examined the impact of the antioxidant N-acetylcysteine (NAC) on redox potential, glucagon secretion, and PKA substrate phosphorylation.
Results: Increasing extracellular glucose from 1 to 5 mM elevated the reductive potential. Interestingly, prior exposure to 5 mM glucose elevated glucagon release at 1 mM. Similarly, pre-incubating islets with NAC at 1 mM glucose enhanced

the reductive potential and increased glucagon secretion. Treating alphaTC1-6 cells with NAC at 1 mM glucose increased PKA-substrate phosphorylation. However, blocking L-type Ca^{2+} channels countered these effects on glucagon secretion. **Conclusion:** These findings highlight a novel glucose-dependent regulatory mechanism in alpha cells, suggesting that enhancing cytosolic reductive capacity potentiates glucagon secretion through PKA during hypoglycaemia.

P-327 Establishing the African pancreas tissue resource for promoting islet research in uncharted territory: insights from the PAN-PATH study in Uganda

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Introduction In sub-Saharan Africa (SSA), type 2 diabetes (T2D) presents uniquely, particularly in lean individuals, where beta cell insufficiency rather than insulin resistance is thought to be the primary defect. However, understanding the processes contributing to this form of diabetes is limited due to lack of islet cell research in Africa. The PAN-PATH study aims to establish a long term African pancreas tissue resource to enable histopathological studies (to complement metabolic characterisation) to unravel the underlying molecular mechanisms.
Methods In a prospective case-control study, anonymized post-mortem pancreatic tissue samples are collected from individuals with T2D and non-diabetic age- and sex-matched controls. Histo-chemistry analyses are undertaken at the Larry Hillblom Islet Research Center at UCLA, with parameters of interest including beta cell mass, immune infiltration (insulitis), markers of beta cell differentiation, and misfolded protein stress.
Results Pancreatic tissue processing is ongoing, with samples stored in both fixed and frozen formats, prepared according to standard protocols. Preliminary data reveal that individuals with T2D have significant beta cell deficits, abnormal islet architecture, and evidence of misfolded protein stress, with no evidence of insulitis.
Conclusions The early findings support that beta cell dysfunction is a major feature of diabetes in lean individuals with T2D in SSA. The PAN-PATH pancreas tissue resource has the potential to enhance our understanding of T2D mechanisms in underrepresented African populations, and to inform development of targeted interventions, as well as enable international comparative studies.

P-328 Highly accurate real shape-based volume assessment of isolated pancreatic islets

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Introduction: Volume is a fundamental descriptor of isolated pancreatic islets. The current standard for islet volume assessment from 2D microscopic projections was developed by Camilio Ricordi in 1990. The limitations include the assumption of improbable islet geometry and the lack of rigorous validation against actual volumes.
Aims: We aimed to assess the accuracy of the current standard, identify morphological reasons for inaccuracies, and ultimately develop and validate a more accurate model with equivalent practicality.
Methods: Islets isolated from four rat strains (19 donors, 3 datasets) and humans (7 donors, 1 dataset) were used. 2D microscopic projections were acquired by stereo, inverted, and widefield microscopy. Volumes were measured in digitized islets generated from high-quality images acquired by light sheet microscopy. Islet 3D geometry was characterized using Zigg plot (compactness, oblateness).

Results: Validation of the standard method on 166 rat islets revealed 1.48-fold volume overestimation. Zigg plot analysis of 1732 rat islets revealed that $< 10\%$ spherical shape conformity. Using the same data set, a new model was developed and implemented, based on spherical extrusion of islet contours with size-related flattening function, reflecting closely the real shapes. Validation of the model against 273 rat and 246 human islets confirmed high accuracy of new model (Relative bias -0.04 and -0.02; MRSE 0.16 and 0.18, respectively).
Conclusions: The inherent inaccuracy of the current model was quantified and explained. A highly accurate alternative is proposed for both rat and human islets, including a Fiji plugin and the corresponding conversion table for manual assessment. (Grants: NU22-01-00141, LX22NPO5104).

P-329 Sex differences in islet cell type composition contribute to phenotypic differences in publicly available human islet datasets

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Introduction Sex differences exist in islet function and gene expression. The recent development and expansion of publicly available human islet datasets offer a valuable resource for the discovery of sex differences in islets.
Aim We sought to identify sex differences in multiple islet phenotypes using publicly available data.
Methods We performed sex-based analysis of scRNAseq, bulk RNAseq, oxygen consumption, intracellular calcium imaging, and perifusion data from PancDB and Humanislets.com.
Results Among non-diabetic young donors (aged 15–39), female islets had greater alpha-cell and lower β-cell contents as a proportion of endocrine cells, compared to male islets, based on CyToF (PancDB) and deconvolution from proteomics (Humanislets.com). In oxygen consumption assays, female islets had lower spare respiratory capacities than male islets. This difference was no longer apparent when normalised to beta-cell proportion. Female islets also showed stronger calcium responses following amino acid treatment, but this difference was no longer observed when normalised to alpha-cell proportion. However, despite their lower beta-cell proportion, female islets had higher neutral solution-soluble insulin content, suggesting greater content of semi-mature insulin prior to hexamer formation with zinc. Female beta-cells from young donors also showed enriched expression of protein synthesis-related genes. Islets from female donors therefore show attributes consistent with a greater capacity for insulin biosynthesis.
Conclusions Overall, sex differences in the islet endocrine cell proportions account for important phenotypic differences. This highlights the importance of cell composition as a key determinant of islet attributes and underscores the need to distinguish differences due to cell type composition versus cellular processes.

P-330 Primary cilia regulate glucagon secretion in alpha cells through c-Kit signalling

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Introduction and Aims Over the past decade, primary cilia have emerged as key regulators of hormone secretion in pancreatic islets. While ciliary signalling in beta cells and delta cells is known to influence insulin release, the role of primary cilia in alpha cell function and glucagon secretion remains poorly understood. Therefore, our aim was to identify novel ciliary proteins in alpha cells that may regulate glucagon secretion.

Methods We generated a stable αTC1-6 cell line expressing the ciliary membrane marker ARL13B, tagged with the proximity labelling enzyme TurboID and performed mass spectrometry and used immunofluorescence, static secretion and western blotting to test whether candidate proteins affected alpha cell function.
Results We identified 3,515 proteins, of which 270 were enriched in primary cilia. We focused on c-Kit, a receptor tyrosine kinase linked to cell proliferation and differentiation. We confirmed c-Kit localisation to primary cilia in both αTC1-6 cells and isolated mouse islets. In mouse islets, 73% of alpha cells expressed c-Kit, and 57% of the cells which display ciliary c-Kit were alpha cells. Interestingly, when whole islets were stimulated with c-Kit ligand, stem cell factor (SCF), glucagon secretion was reduced at 1 mM glucose and 7 mM glucose no longer suppressed glucagon secretion. We next wondered whether the primary cilia were required for c-Kit signalling and in mouse embryonic fibroblasts (MEFs) with impaired cilia formation ($Tg737^{\text{ORPK}}$), SCF reduced the activation of AKT and ERK.
Conclusions These findings suggest that signalling through c-Kit in primary cilia suppress glucagon secretion at low glucose.

P-331 Mitochondrial Fission Process 1 (MTFP1) in β-cells: A Mitochondrial Driver of Insulin Secretion

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Introduction and aims. β-cell mitochondria play a crucial role in coupling glucose metabolism to insulin secretion, with their dysfunction contributing to type 2 diabetes (T2D) progression. Mitochondrial fission process 1 (MTFP1) is an inner mitochondrial membrane protein that regulates mitochondrial fission and/or function in a cell-specific manner. In β-cells, MTFP1 is directly silenced by miR-125b, a glucose-regulated miRNA that negatively impacts insulin secretion. This study explores the previously uncharacterized role of MTFP1 in β-cell function both *in vitro* and *in vivo*.
Methods. We used CRISPR/Cas9 and viral vectors expressing MTFP1-targeting shRNAs or human MTFP1 to achieve MTFP1 loss- and gain-of-function in EndoC-βH3 cells and human islets. We assessed glucose stimulated insulin secretion (GSIS), mitochondrial morphology and respiration using time-resolved fluorescence, MitoTracker staining and Seahorse assays. Glucose tolerance and insulin secretion were assessed in mice with β-cell specific MTFP1 elimination (*MTFP1*-βKO).
Results. MTFP1 overexpression in EndoC-βH3 cells and human islets increased GSIS ($15-40\%, p<0.01$) and induced mitochondrial shortening ($\approx 30\%, p<0.01$). Conversely, *MTFP1* knockout/knockdown in these cells decreased GSIS ($30-50\%, p<0.05$) and led to mitochondrial elongation ($\approx 40\%, p<0.01$). Oxygen consumption rates (OCR) in response to oligomycin and FCCP were significantly altered following MTFP1 dysregulation ($p<0.01$). *MTFP1*-βKO mice displayed glucose intolerance (p<0.01) and impaired GSIS *in vivo*.
Conclusion. MTFP1 is a key regulator of β-cell insulin secretion and glucose homeostasis, acting through the regulation of mitochondrial morphology and function. Further studies will investigate its mechanism and role in hyperglycaemia and T2D progression.

P-332 Tardigrade Damage Suppressor Protein (DSUP) for Protecting Pancreatic Beta-Cells Against Immune-Mediated Cell Death

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Introduction Type 1 diabetes (T1D) is an autoimmune disease characterized by a progressive destruction of pancreatic beta-cells by autoreactive T cells through inflammation, oxidative stress and/or direct cell cytotoxicity. *Rammazzottius varieornatus* (Tardigrade) is a metazoan showed high resistance to several adverse conditions through synthesizing DSUP (Damage Suppressor protein), responsible for the protection effects.
Aims We aim to investigate whether DSUP protects beta-cells from inflammatory and/or oxidative stress.
Methods MIN6 murine beta-cells were stably transfected with His-Tagged DSUP-expressing plasmid or CTR-plasmid, and expression verified using immunofluorescence staining and confocal microscopy. Stably transfected, CTR and not-transfected (NT) cells were subjected to inflammatory stress (IL-1β+TNFα+IFNγ) for 24h and 48h, or to oxidative stress (100–250 μM H_2O_2 – 90min, 4h and 12h). Apoptosis rate [pyknotic nuclei (PN+) and Propidium Iodide (PI+)], cell viability (MTT assay) and Glucose Stimulated Insulin Secretion (GSIS) were evaluated to assess DSUP-mediated protection effect on survival and function.
Results In MIN6 cells, DSUP was successfully expressed and localized to the nucleus. Overexpression of DSUP reduced inflammation-induced apoptosis (Untreated: CTR = 2.6 ± 0.9, DSUP = 2.31 ± 0.6; Cytokine-treated: CTR = 6.98 ± 1.8, DSUP = 3.06 ± 0.6; $p = 0.04$, CTR vs. DSUP). Moreover, DSUP-overexpressing cells were protected from oxidative stress, displaying increased viability (Fold change vs. NT = 1.4, $p = 0.04$; vs. CTR = 1.7, $p = 0.03$). GSIS analysis revealed no significant differences in the functional response of DSUP-overexpressing MIN6 cells.
Conclusions DSUP protects beta-cells from both inflammatory and oxidative stresses, thus opening a new frontier for a potential therapeutic strategy against T1D.

P-333 Novel METTL3/14 methyltransferase activators for protecting pancreatic beta cells in diabetes mellitus

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RNA methylation of N6-adenosine (m^6A) is a common reversible RNA modification, involved in various biological processes, e.g. RNA stability, splicing, translation. Recent research show that dysregulated m^6A mRNA levels in islets is associated with diabetes, suggesting increasing m^6A mRNA levels as a valuable target for beta-cell protection.
The aim of this project is to elucidate a) mRNA m^6A and their regulating proteins levels in healthy beta-cells and diabetes pathology, and b) the effect of METTL3/14 activator compounds on the proliferation and survival of cytokine- or palmitate treated beta-cells *in vitro*.
We use islets from NOD and *db/db* mice at different stages of diabetes development, EndoC-βH1, WT mouse islets and MIN6 cells treated with cytokines or palmitate *in vitro*. m^6A levels are analyzed with m^6A RNA methylation Quantification kit, and their regulators with RT-qPCR and immunocytochemistry. Proliferation and survival are assessed with Click-It proliferation and CCK-8 viability assays.
Preliminary data detected first changes in expression of beta-cell markers in db/db mice as early as 4 weeks of age while *ad libitum* blood glucose levels are still normal. The first signs of increased UPR and inflammation were also seen. However, no differences in m^6A regulators levels were observed. Further analysis of islets from older db/db and NOD mice are in progress. Pharmacological METTL3/14 inhibitors dose-dependently

reduced the viability of EndoC-βH1 and mouse MIN6 cells. The protective role of METTL3/14 activators are under investigation.
Current project is at the beginning of unveiling m^6A role in diabetes and exploring its potential as therapeutic target.

P-334 LMO1 interacts with PDX1, ISL1 and NEUROD1 to regulate insulin transcription and secretion in human beta cells

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Introduction LMO1 is an oncogene and transcriptional regulator. Its role in type 2 diabetes or beta cells is unknown. Differential gene coordination network analysis on human scRNAseq data (n=32), revealed LMO1 in a network with most known beta cell transcription factors (TFs). We hypothesized that LMO1 may regulate insulin transcription and secretion, potentially via interactions with beta cell TFs.
Aims INS-1 832/13 and isolated islets from humans and mice were used to investigate the role of LMO1 on insulin secretion and interactions with key beta cell TFs.
Methods We studied insulin transcription, secretion and interaction of LMO1 and beta cell TFs using siRNA knockdown, immunoprecipitation and proximity ligation assay (PLA) in INS-1 cells and human and mouse islets.
Results Immunohistochemistry confirmed expression in human beta cells. LMO1 correlated with *PDX1* and *ISL1* in human islet bulk and scRNAseq data. *Lmo1* KD in INS-1 cells increased *Pdx1*, *Nkx2.2*, *Neurod1*, *Isl1* and *Mafa* mRNA. PLA and co-IP revealed interactions with *PDX1*, *ISL1* and *NEUROD1*. *Lmo1* KD yielded a 1.4-fold increase in insulin secretion at high glucose with IBMX, increased proinsulin-to-insulin ratio and increased *Pcsk1/3* and *Pcsk2* mRNA. Insulin secretion in mouse islets was decreased at high glucose after *Lmo1* KD. In human islets, *LMO1* KD decreased insulin secretion at low glucose and at high glucose. Finally, *Lmo1* KD decreased apoptosis in INS-1 cells under glucolipotoxic conditions.
Conclusions LMO1 is a novel T2D-affected regulator of insulin transcription, potentially via interactions with *PDX1*, *ISL1* and *NEUROD1*.

P-335 Cellular mediators of pancreatic islet morphogenesis

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New therapies for diabetes will emerge through a better understanding of islet development. Pancreatic islet morphogenesis requires differentiating cells to sort out from ductal cells and reorganize, to generate the precisely structured arrangement of endocrine and supporting cell types. Due to the inaccessible location of the pancreas in mammals, little is known about factors directing endocrine cell assembly. Previously, we performed high resolution *in vivo* visualization of islet morphogenesis in the vertebrate model organism zebrafish, and documented highly motile behaviors similar to those reported in *ex vivo* mammalian studies.
We now aim to define the role of membrane phospholipid signaling and mechanotransduction in islet morphogenesis. Fluorescent biosensors are expressed within pancreatic islet cells of transparent zebrafish larvae, followed by high-resolution time-lapse imaging. Sensor activity is correlated with protrusion formation and cytoskeletal rearrangements. We furthermore applied bioinformatics approaches to published mouse single cell RNA sequencing datasets which

distinguish the stepwise progression from early progenitors to differentiated islet cells. Cell-cell communication between these endocrine cell-forming populations has been examined to identify candidate membrane-bound and secreted ligands guiding pancreatic islet cell assembly. Potential mediators have previously defined roles in β cell function or cell migration in other systems, but have not been well characterized for their role in islet formation. We examine spatial patterns of gene expression during development before continuing to functional investigations. Overall, these studies will uncover novel factors acting during islet development and contribute insight into pathways that may promote optimal cell organization during *in vitro* differentiation.
We focus on signals being sent and/or received by ductal cells, endocrine progenitors, and differentiated islet cells. Critical cell-cell interactions contribute to forming the three-dimensional islet structure by providing not only biochemical, but also biomechanical signals that are also essential for establishing full β cell function. Due to the inaccessible location of the pancreas during development in mammalian model systems, the membrane-localized and biophysical cues that direct islet cell fate determination, assembly and maturation remain poorly defined.
We utilize fluorescent sensors for membrane tension and reveal localization of phospholipid species with reported roles in cell motility to delineate molecular regulators directing dynamic protrusive behaviors, and to define mediators of cell-cell recognition.

P-336 Synthetic Peptide Growth Factors featuring KGF Alternative Peptide

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Introduction
Keratinocyte Growth Factor (KGF, also known as FGF7) is a member of the FGF family that binds to FGFR2b and promotes the proliferation and differentiation of various endoderm-derived cells, including epidermal cells and pancreatic islet cells. It plays a key role in inducing the differentiation of pluripotent stem cells into islet-like cells, making it a vital factor in regenerative approaches for diabetes. However, like other FGFs such as FGF2, KGF suffers from poor stability at 37°C, rapidly losing activity and requiring frequent medium changes or high dose usage, posing significant operational and cost challenges.
Aim To develop a stable peptide-based alternative to KGF with equivalent biological activity.
Methods Using PeptiDream's Peptide Discovery Platform System (PDPS), we identified cyclic peptides that bind human FGFR2b. These were chemically linked to form dimeric structures designed to mimic KGF's agonistic activity. Peptide sequences and linkers were optimized to generate the lead compound PG-012.
Results PG-012 activated ERK signaling downstream of FGFR2b and promoted the proliferation of FGFR2b-expressing BaF3 cells. It also supported the differentiation of pluripotent stem cells into islet-like cells at levels comparable to KGF. Unlike recombinant KGF, PG-012 maintained full activity after prolonged incubation at 37°C, demonstrating superior thermal stability.
Conclusion PG-012 is a promising KGF alternative with high stability and KGF-equivalent activity. It may reduce medium change frequency and reagent use, offering practical advantages for stem cell research and regenerative medicine.

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