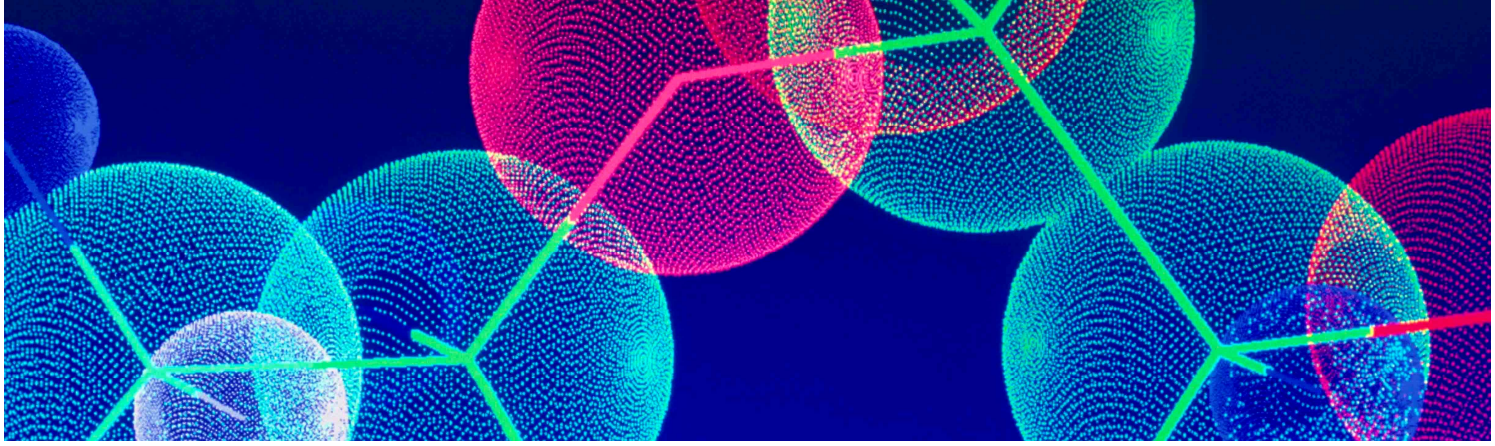
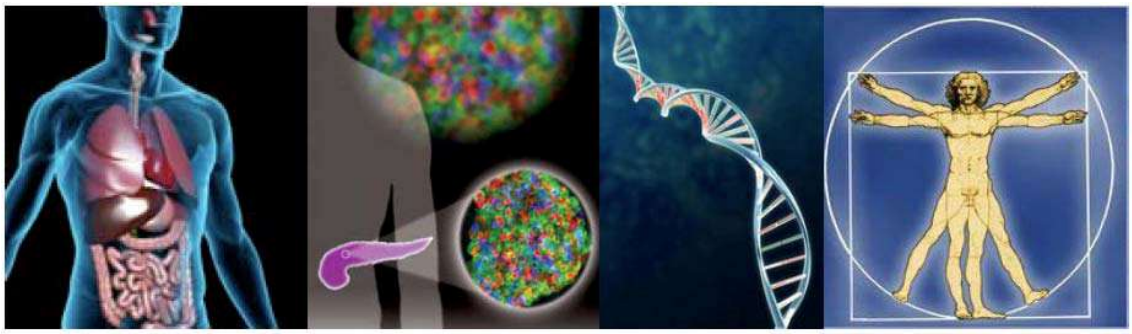


14th SRP Diabetes-EndoMet Retreat



Programme and Abstracts

May 28-29, 2026
Aronsborg Konferenshotell

Karolinska Institutet

UMEÅ UNIVERSITET

SRP DIABETES

Venue Information

Aronsborg Konferenshotell
Helgövägen 12, 746 30 Bålsta
https://aronsborg.se/
info@aronsborg.se
+46 (0) 8-551 201 99

Safety regulations at Aronsborg

Please watch the following clip on YouTube to familiarize yourself with the safety regulations at Aronsborg:
https://www.youtube.com/watch?v=QzKfGoCEg8g

Travel information

Organized bus transport to and from the venue

Hired buses for the participants who signed up will depart at 8:30 am on May 28th from the Central Bus Station (*Cityterminalen*), next to Stockholm Central Train Station. Look for "*SRP Diabetes Retreat*" on the digital screens to find the right gate in the bus terminal.

Please make sure to be there ahead of time as the buses will leave at 8:30 am sharp. The return is scheduled for 16:00 from the venue back to *Cityterminalen*.

Public transport

Take SL or SJ trains to Bålsta Station, and from there, bus 33 towards Viby äng (Bålsta, Håbo). The stop is "Bålsta Aronsborg".

By car

Via E4. Continue on E18 (exit 171) to Södra Bålstaleden in Bålsta. Take exit 147 (Trafikplats Draget) from E18. Continue on Södra Bålstaleden and drive to Kalmarleden

GPS Coordinates

Lat. 59.55353; Long. 17.53359

Poster sessions – Best Poster Awards

The poster session will take place on May 28th between 17:00 and 19:00, and refreshments will be served. The poster session will take place in Vinterträdgården.

Poster numbers can be seen in the program. The poster boards have the following dimensions: 180 cm x 75 cm in portrait orientation. Posters should be mounted before the session. Presenters are expected to stand by their posters at least half of the session (posters with odd numbers between 17:00 and 18:00, posters with even numbers between 18:00 and 19:00)

Best Poster Awards

Poster presenters eligible for poster awards are PhD students and postdocs, for original work that has not been published. A committee will evaluate the eligible abstracts / posters for best posters in two categories:

- Best poster for completed work
- Best poster for most promising project

The Best Poster Award will be announced during dinner.

Best Short Talk Award

Only short talks by postdocs and students are eligible for Best short Talk Award. They are highlighted in the programme as [BSTA]. Scoring by participants will be done directly at the end of each session by scanning the QR code that will be made available on the screen. You will need a smartphone with internet access to score.

Short talks are scored both for content and for delivery of the presentation. Please provide a score (1-5, with 5 being the highest) for each of the 4 questions directly after each eligible talk

1. Level of novelty and interest of the study
2. Quality and rigor in the analysis of the data
3. Engagement and enthusiasm of the speaker about the study
4. Overall design and layout of the slides, easiness to follow

The Best Short Talk Award will be announced at the end of the meeting.

Organizers

Alessandro Furlan
Paul Petrus
Andreas Hörnblad
Emma Ringqvist
Maxence Jollet
Paulina Jonéus
Alfredo Giménez-Cassina

PROGRAMME

THURSDAY 28 MAY

8:30 Bus Departure
9:30 Coffee + sandwiches

* Note: time given for all talks include 5 min. for Q&A

** [BSTA] indicates that this short talk is eligible for "Best Short Talk Award"

SESSION 1: Adipose tissue biology Chairs: Alice Maestri & Leo Westerberg

10:00 – 10:15 Welcome note - Mikael Rydén (chair of SRP Diabetes) + EndoMet representative

10:15 – 10:40 **David Savage** (University of Cambridge): "*A lipodystrophic perspective on insulin resistance*"

10:40 – 10:55 **Katharina Schormair:** "*CRISPRi mediated gene perturbation reveals long non-coding RNAs involved in human adipocyte lipolysis*" [BSTA]

10:55 – 11:10 **Niels Krämer:** "*Single-cell profiling reveals senescent adipocytes as drivers of inflammation in obesity*" [BSTA]

11:10 – 11:25 **Nicole Li:** "*Effects of sex hormones on white adipose tissue immune cells in transgender individuals undergoing gender-affirming treatment*" [BSTA]

11:25 – 11:45 Coffee Break

SESSION 2: Skeletal muscle Chairs: Paola Pinto-Hernández & Emily Shorter

11:45 – 12: 20 **Ben Stocks** (University of Copenhagen – Incoming PI at KI): "*Deciphering Molecular Pathways of Exercise in Type 2 Diabetes Revealed by Multi-Omics*"

12:20 – 12:35 **Zina Riahi:** *"Investigating the effect of NAD+ supplementation in combination with exercise in cardiometabolic disease"* [BSTA]

12:35 – 12: 50 **Tina Gorsek:** *"Cell-type-resolved transcriptomic map of skeletal muscle in women with polycystic ovary syndrome"* [BSTA]

13:00 – 14:30 Lunch + Networking

14:30 – 14:50 **Stefan Nobel** (External Engagement Office)

SESSION 3: Biomarkers of metabolic disease Chairs: Ellen Vercalsteren & Allan Zhao

14:50 – 15:05 **Abigail Dove**: "*Interplay between type 2 diabetes and blood-based biomarkers of Alzheimer's disease on the risk of dementia: a population-based study*" [BSTA]

15:05 – 15:20 **Philipp Valina Allo**: "*cfChIP of H3K4me3 in Plasma: Advancing Noninvasive Biomarkers for liver metabolic disorders"* [BSTA]

15:20 – 15:35 **Cenk Gurdap:** *"Physics of cells as new biomarkers for metabolic diseases"* [BSTA]

15:35 – 16:05 Coffee break + Room check-in

SESSION 4: Round table: Scientific publishing Chairs: Julia Sánchez-Ceinos & Daan Paget

16:05 – 17:00 **Christoph Schmitt** (*Nature Metabolism*) and **Salvatore Fabbiano** (*Cell Metabolism*)

17:00 – 19:00	**Poster session**
19:30	**Dinner and entertainment – Best poster award**

FRIDAY 29 MAY

7:00 – 9:00 Breakfast and room checkout

SESSION 5: The brain and systemic metabolism Chairs: Radhashree Sharma & Tade Idowu

9:00 – 9:35 **Linda Engström Ruud** (University of Gothenburg): "*Semaglutide engages brainstem-hypothalamic circuits controlling feeding and metabolic state*"

9:35 – 9:50 **Davide Rizzato:** "*Systemic circadian coordination arises from tissue-specific clock dependence and direct photic neural signaling*" [BSTA]

9:50 – 10:05 **Clara Sánchez:** "*Caudal Raphe Serotonergic Neurons Regulate Energy Balance and Adipose Tissue-Specific Remodelling*" [BSTA]

10:05 – 10:30 Coffee break

SESSION 6: Islet biology and inflammation Chairs: Fabian Byvald & Ruby Schipper

10:30 – 11:05 **Accalia Fu** (University of Massachusetts): "*Mitochondrial Gatekeeping and Metabolic Tuning of β-Cell Function*"

11:05 – 11:20 **Pere Rehues Masip**: "*Longitudinal* in vivo *evaluation of human β-cell function during GLP-1RA therapy using sensor-engineered islet spheroids*" [BSTA]

11:20 – 11:35 **Joakim Lehrstrand**: "*Optical whole organ 3D characterization of the residual β-cell mass in a long standing T1D pancreas - inversed proportions between extra-islet and islet-associated β-cells*" [BSTA]

11:35 – 11:50 **Emma Ringqvist:** "*JAK inhibitors modulate immune signaling but leave β cell antiviral pathways intact*" [BSTA]

12:00 – 14:00 Lunch + Networking

SESSION 7: Circulating metabolites Chairs: Gustaw Eriksson & Yuyang Miao

14:30 – 15:05 **Cholsoon Jang** (University of Irvine California): "*Inter-organ metabolic communications in healthy, insulin resistance and atherosclerosis*"

15:05 – 15:20 **Timotej Strmen:** *"Effect of type 2 diabetes on plasma and fat tissue lipidomes in human and mouse studies"* [BSTA]

15:20 – 15:35 **Svetlana Michurina:** "*Lipidomic and proteomic signatures of T2D and CVD in the ADIPO-SCAPIS cohort*" [BSTA]

15:05 – 15:30 Concluding remarks + Best oral presentation award

16:00 Bus departure

ORAL PRESENTATIONS

Adipose tissue biology		
O1	***David Savage**	A lipodystrophic perspective on insulin resistance
O2	Katharina Schormair	CRISPRi mediated gene perturbation reveals long non-coding RNAs involved in human adipocyte lipolysis
O3	Niels Krämer	Single-cell profiling reveals senescent adipocytes as drivers of inflammation in obesity
O4	Nicole Li	Effects of sex hormones on white adipose tissue immune cells in transgender individuals undergoing gender-affirming treatment
Skeletal muscle		
O5	***Ben Stocks**	Deciphering Molecular Pathways of Exercise in Type 2 Diabetes Revealed by Multi-Omics
O6	Zina Riahi	Investigating the effect of NAD+ supplementation in combination with exercise in cardiometabolic disease
O7	Tina Gorsek	Cell-type-resolved transcriptomic map of skeletal muscle in women with polycystic ovary syndrome
Biomarkers of metabolic disease		
O8	Abigail Dove	Interplay between type 2 diabetes and blood-based biomarkers of Alzheimer's disease on the risk of dementia: a population-based study
O9	Philipp Valina Allo	cfChIP of H3K4me3 in Plasma: Advancing Noninvasive Biomarkers for liver metabolic disorders
O10	Cenk Gurdap	Physics of cells as new biomarkers for metabolic diseases
The brain and systemic metabolism		
O11	***Linda Engström Ruud**	Semaglutide engages brainstem-hypothalamic circuits controlling feeding and metabolic state
O12	Davide Rizzato	Systemic circadian coordination arises from tissue specific clock dependence and direct photic neural signaling
O13	Clara Sanchez	Caudal Raphe Serotonergic Neurons Regulate Energy Balance and Adipose Tissue-Specific Remodelling
Islet biology and inflammation		
O14	***Accalia Fu**	Mitochondrial Gatekeeping and Metabolic Tuning of β-Cell Function
O15	Pere Rehues	Longitudinal in vivo evaluation of human β-cell function during GLP-1RA therapy using sensor-engineered islet spheroids
O16	Joakim Lehrstrand	Optical whole organ 3D characterization of the residual β-cell mass in a long standing T1D pancreas - inversed proportions between extra-islet and islet-associated β-cells
O17	Emma Ringqvist	JAK inhibitors modulate immune signaling but leave β cell antiviral pathways intact
Circulating metabolites		
O18	***Cholsoon Jang**	Inter-organ metabolic communications in healthy, insulin resistance and atherosclerosis
O19	Timotej Strmen	Effect of type 2 diabetes on plasma and fat tissue lipidomes in human and mouse studies
O20	Svetlana Michurina	Lipidomic and proteomic signatures of T2D and CVD in the ADIPO-SCAPIS cohort

* **Guest Speaker**

POSTERS

P1	Alice Maestri	Aerobic glycolysis drives differentiation of unilocular adipocytes
P2	Ivan Vlassakev	A systemic circadian nicotinic acid riboside (NaR) signal engages the unfolded protein response and adipocyte lipid metabolism via the prefoldin complex.
P3	Ruby Schipper	Adipose Endothelial Cells Lose Fatty Acid Transport Selectivity Under Obesogenic Conditions
P4	Min Cai	Increased LDL uptake to adipocytes impairs local and systemic triglyceride handling
P5	Tiffany Huang	Adipose tissue-derived extracellular histones as novel mediators of endothelial injury in cardiometabolic disease
P6	Claudia Montufar Leon	Inhibition of IMPDH induces cytoskeletal remodeling promoting adipogenic to fibro-inflammatory fate transition
P7	Daan Paget	Extracellular Vesicle associated Glycosylated Sphingolipid Alterations in Type 2 Diabetes Impair Adipocyte Glucose Metabolism
P8	Cheukyau Luk	Depot-Specific Alterations in Adipose Tissue in Type 2 diabetes and the Paracrine Role of Perivascular Adipose Tissue on Vascular Function
P9	Lynn Alaeddine	Glutaminolysis Promotes Inflammation In White Adipose Tissue
P10	Julia Backman	Elucidating the Drivers of Unilocular Adipocytes: A Matched 3D Culture Comparison
P11	Radhashree Sharma	Brain-adipose circuits govern tissue-specific energy homeostasis
P12	Maria Laura Santino	Basally active neurons in the extended amygdala regulate weight gain
P13	Lei Li	Locus coeruleus noradrenergic neurons shape regional adiposity and inflammation
P14	Yuyang Miao	Brain functional MRI study on diabetic retinopathy
P15	Ellen Vercalsteren	Pre-stroke weight loss to improve stroke outcome in diabetes
P16	Aida Collado Sánchez	Myeloperoxidase Delivered by Erythrocyte-Derived Extracellular Vesicles Promotes Oxidative Stress and Endothelial Dysfunction in Type 2 Diabetes
P17	Giovanni Tortorella	Pharmacological inhibition of microplastic uptake prevents endothelial injury
P18	Eftychia Kontidou	Extracellular vesicle-mediated transfer of red blood cell non-coding RNAs induces vascular endothelial dysfunction in type 2 diabetes
P19	Camille Gauthier	Development of a immunodeficient prenatal mouse model of polycystic ovary syndrome for evaluation of new therapeutics
P20	Xueming Zhang	B-lymphopoiesis and humoral immunity in an atherosclerotic environment
P21	Aoxue Li	How do APOB-lipoproteins interact with tissue macrophages in the context of cardiovascular disease?
P22	Georgios Filis	Impairment of circulating metabolites induces endothelial dysfunction in adolescents exposed to gestational diabetes in utero
P23	Álvaro Santana Garrido	Endothelial BUD13 upregulation in type 2 diabetes drives vascular dysfunction through inflammatory pathways
P24	Teodora Piskova	Shift of mechanical homeostasis in the retinal pigment epithelium after cell loss: Relevance to diabetic retinopathy
P25	Gustaw Eriksson	Mapping the Cross-Tissue Cellular Landscape of Polycystic Ovary Syndrome
P26	Ingrid Nilsson	Thrombolysis exacerbates cerebrovascular injury after ischemic stroke via a VEGF-B dependent adipose-brain metabolic axis
P27	Benjamin Heller Sahlgren	Mitochondrial respiration and lipid droplet turnover regulate induced endothelial fatty acid uptake
P28	Tong Jiao	Red blood cell-derived extracellular vesicles impair cardiac post-ischemic recovery in type 2 diabetes: Role of microRNA-210
P29	Rawan Humoud	ATP9A in erythrocytes: A regulator of extracellular vesicle release and endothelial dysfunction in type 2 diabetes
P30	Lukas Cudlman	From silent plaque to clinical events: Mass-spectrometry-based sphingolipid profiling to link subclinical carotid atherosclerosis with overt cardiovascular disease
P31	Xuelei Wang	Cell-type–resolved transcriptomic profiling of human placentas in polycystic ovary syndrome reveals limited effects of gestational metformin treatment
P32	Emmanouella Michaela Xanthopoulou	High-fat diet exacerbates ischemic muscle impairment in the FAL murine model of Peripheral Artery Disease
P33	Haojiang Lu	Spatial and transcriptomic profiling of ovary in PCOS

P34	Tanja Turunen	Endometrial Cell Dysfunction Across the Menstrual Cycle In Polycystic Ovary Syndrome
P35	Dorothea Theurer	Optimizing siRNA Treatment Targeting AKR1C3 to Reverse PCOS-Associated Androgen Excess in Adipocytes
P36	Antonietta Anatriello	Polypharmacy and potential drug misuse in patients with type 2 diabetes mellitus: a Swedish register-based drug utilization study
P37	Alexander Zadruzny	TARGET TISSUE INSULIN RESISTANCE IN EARLY AND LATE ONSET OF OVERWEIGHT OR OBESITY
P38	Caroline Högardh	Effect of Circadian Rhythm & Physical Exercise on Metabolism in Overweight Type 1 Diabetes Patients
P39	Ana Vankova	Overeating polyunsaturated fat increases the HDL anti-inflammatory activity compared with saturated fat
P40	Lin Chen	Sex-specific KDM6A-HNF4A-CREBH network controls lipoprotein cholesterol metabolism and atherosclerosis via epigenetic reprograming of hepatocytes
P41	Allan Zhao	Impaired gluconeogenesis links maternal diabetes with sex-dimorphic liver disease in offspring
P42	Lohitesh Kovooru	MTARC1 p.A165 ablation reduces hepatocellular carcinoma aggressiveness in vitro and in vivo
P43	Ratish Raman	Nutrient-Driven Remodelling of Liver Splicing Programs in the Development of Insulin Resistance and MASLD
P44	Tanmoy Dutta	Di-lineage human liver spheroids recapitulate steatotic liver disease
P45	Anagha Keshavaprasad	HypoxamicroRNA-210 protects against hepatic steatosis by inhibiting CIDEC expression
P46	Sviatlana Sukhanava	Epigenetic and Transcriptional Remodeling in Human Hepatocytes Under Inflammatory Stress Reveals a Role for NNMT in Inflammation and Fibrosis
P47	Paola Pinto Hernandez	Extracellular vesicles in muscle dysfunction in type 2 diabetes: sex-specific miRNA and lipid profile.
P48	Alesandra Marica	Identification and characterization of novel regulators linking skeletal muscle metabolism and remodeling
P49	Xue Yu	Epoxy fatty acids accumulation enhances skeletal muscle glucose utilization and improves glucose control in female mice
P50	Natalie Norman	DGKδ silencing alters lipidomic profile in human skeletal muscle cells.
P51	Emily Shorter	Single-Nucleus Transcriptomics Uncovers Cell-Type-Specific Remodelling in Gastrocnemius and Vastus Lateralis Muscles in Peripheral Artery Disease
P52	Qi Li	Dissecting the functionality and cardio-metabolic diseases relevance of single- and dual-functional cis-regulatory elements across human tissues/cells
P53	Ziyi Li	Dissecting the functionality and type 2 diabetes metabolic diseases relevance of enhancers/silencers across mouse tissues/cells
P54	Lucia Coppo	Redox Perturbations as Causal Drivers of Insulin Resistance in Obesity
P55	Yue Zhu	Role of Epigenetic Monocyte Alterations in Inflammatory Type 2 Diabetes
P56	Alexander van Deventer	Chronic inflammation in skeletal muscle promotes NOX2 upregulation and drives differential Prdx oxidation in distinct cellular subdomains'
P57	Veijo Salo	Nanoscale architecture of lipid flux at the endoplasmic reticulum
P58	Liying Zhou	A spatiotemporal map of lncRNA in obesity and chronic jet lag
P59	Denise Parreira	Modeling Maternal Stress and Metabolic Exposures Using Term Placenta–Derived Trophoblast Organoids
P60	Chayenne Virginia Tillack	In vivo detection of DNA-based delivery platforms for multivalent targeting of insulin receptors
P61	Tade Idowu	Targeting of Insulin Nanoclusters in Zebrafish for Tailored Insulin Therapies
P62	Mikaela Seoyeon Huh	A co-culture model of patient-derived endometrial stromal and epithelial cells using a recombinant spider silk membrane
P63	Georges Kiriako	Mapping insulin receptor activation states at the cell membrane using super-resolution microscopy
P64	Anja Dekanski	Modelling PCOS adipose dysfunction in vitro using single-layer and adipose spheroid cell culture systems
P65	Stefania Koutsilieri	Molecular dissection of GCGR signaling uncovers pathway rewiring by GCGR-targeted compounds
P66	Ariela Boeder	Gut microbiota-derived dinitrosyl-iron complexes (DNIC): Microbial synthesis and NO-independent signaling pathways
P67	Gaia Picozzi	Gut microbiota generate dinitrosyl-iron complexes with cardiovascular and metabolic benefits
P68	Dimitri Van Simaeys	β-cell–targeted RNA activation of VEGF-A preconditions islet grafts to enhance vascular integration and functional outcomes

P69	Riccardo Lizzani	An in vivo platform to study the endocrine and exocrine pancreas crosstalk in health and disease
P70	Philip Tröster	Stable intracranial imaging of dura mater-engrafted pancreatic islets in awake mice
P71	Kaixuan Zhao	Hyperglycemia deteriorates human β cell exocytosis by exaggerating CaV3 channel-calcineurin-HSF1 signaling
P72	Yue Shi	Maturation of insulin-expressing cells within human embryonic stem cell-derived islets in the anterior chamber of the eye of immunodeficient mice
P73	Fabian Byvald	Proteome profiling of plasma and extracellular vesicles to identify biomarkers for enterovirus induced type 1 diabetes
P74	Bufan Jin	Spatially Resolved Transcriptomic Profiling Identifies Fibrin-Driven Trophoblast Arrest in Placentas of Mothers with Type 1 Diabetes

ORAL PRESENTATIONS

SESSION 1: ADIPOSE TISSUE BIOLOGY

O1. A lipodystrophic perspective on insulin resistance

David Savage

Institute of Metabolic Science, University of Cambridge, UK.

O2. CRISPRi mediated gene perturbation reveals long non-coding RNAs involved in human adipocyte lipolysis

Katharina Schormair [1], Jordi Mengual Marti [1], Laura Daniela Martinez Rico [1], Kanwal Tariq [1], Christopher Thelin [1], Liyang Wu [1], Bingran Xie [1], Alastair George Kerr [1]

[1]Department of Medicine-Huddinge (MedH), Karolinska Institutet, Stockholm, Sweden

Adipose tissue is the major energy storage organ in the human body. In obesity, adipocyte lipolysis regulation dysfunctions. Importantly, basal lipolysis is elevated and the cell becomes resistant to catecholamines, the major stimulators of lipid breakdown. Consequently, metabolic flexibility is blunted and chronic glycerol and fatty acid release into the periphery drives cardiometabolic disease progression. How regulatory RNA molecules, such as long non-coding RNAs (lncRNAs), participate in lipolysis regulation in adipocytes and how this may go awry in adipose tissue dysfunction is not well understood.

To identify lipolysis-associated lncRNAs, we examined subcutaneous adipose tissue biopsies where extensive lipolytic phenotyping and parallel transcriptomics was performed. Filtering for cytoplasmic adipocyte expression, we selected lncRNAs for CRISPRi screening. Each lncRNA was phenotyped for basal and stimulated lipolysis, and selected hits underwent lipid droplet morphology and proteomic profiling for a deeper characterization. We here identify lncRNAs that display significant effects on the adipocyte lipolytic response that are now being investigated for their molecular mechanism of action.

O3. Single-cell profiling reveals senescent adipocytes as drivers of inflammation in obesity

Firoozeh Salehzadeh*,[1,3], Niels Krämer*,[1], Jérémy Dufau*,[1], Yi Ching Esther Wan[1], Leo Westerberg[1], Benjamin Dedic[1], Harry B. Cutler[4,5], Beatrize Rosón Burgo[1,6], Ping Chen[2,7,8], Erik Näslund[9], David E. James[4,5,10], Åsa Björklund[11], Anders Thorell[12], Kirsty L. Spalding[1,#]

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[2] Karolinska Institutet/AstraZeneca Integrated Cardio Metabolic Centre (KI/AZ ICMC), Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden.
[3] Current address: Ramaciotti Centre for Genomics, University of New South Wales, Kensington, Australia.
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[5] Charles Perkins Centre, University of Sydney, Camperdown, New South Wales, Australia.
[6] Current address: Bioengineering department, Universidad Carlos III de Madrid, Madrid, Spain.
[7] Center for Infectious Medicine, Department of Medicine, Karolinska Institute, Stockholm, Sweden.
[8] Current address: Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.
[9] Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden.
[10] Faculty of Medicine and Health, University of Sydney, Camperdown, New South Wales, Australia.
[11] Department of Life Sciences, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Chalmers University of Technology, Gothenburg, Sweden.
[12] Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet and Department of Surgery, Ersta Hospital, Karolinska Institutet, Stockholm, Sweden.

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Obesity-associated diseases are commonly attributed to adipocyte hypertrophy and the resulting production of immune-recruiting inflammatory factors, yet the cellular events that initiate adipocyte dysfunction remain poorly defined. Here we dissect adipocyte phenotype at single-cell resolution in primary human tissue to determine how metabolic decline and chronic inflammation arise within adipose tissue. We generate a dataset with deep gene detection in single whole human adipocytes from a clinically phenotyped cohort, enabling high-resolution analysis of adipocyte gene expression across the metabolic spectrum. We identify distinct transcriptional states that reflect established features of adipocyte dysfunction, including impaired lipid handling, reduced insulin sensitivity and increased matrix remodelling, and show that adipocytes within the same individual span a continuum of metabolic phenotypes. We further define a bona fide population of senescent adipocytes characterised by an expanded inflammatory secretory programme. By disentangling hypertrophy from senescence, and validating these associations at the protein level, we demonstrate that hypertrophy is primarily associated with loss of core metabolic function, whereas senescent adipocytes constitute the dominant source of chemokines and immune-recruiting signals. Together, these findings redefine the cellular basis of adipose tissue dysfunction in obesity and establish senescence as a distinct driver of inflammatory signalling within human metabolic disease.

O4. Effects of sex hormones on white adipose tissue immune cells in transgender individuals undergoing gender-affirming treatment

Nicole Li [1], Laura Miskinyte [1], Kaisa Hofwimmer [1], Jasmin Sag [1], Narmadha Subramanian [1], Anna Wiik [2], Thomas Gustafsson [2], Daniel P. Andersson [1], Jurga Laurencikiene [1]

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[2] Department of Laboratory Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden

Sex differences are well established in metabolic and immune diseases; however, isolating the direct effects of sex hormones on human white adipose tissue (WAT) immune cells is challenging because sex chromosomes, lifetime hormonal exposure, and environmental factors are strong confounding factors. Gender-affirming hormone therapy provides a human model of relatively rapid and well-controlled sex-steroid reprogramming. Subcutaneous WAT biopsies were obtained from 13 trans women (TW) and 14 trans men (TM) at four time points during hormone therapy. In a preliminary subset (3 TM and 3 TW), the WAT stromal vascular fraction (SVF) was FACS-sorted before intervention, after hormone washout, and after 12 months of treatment to quantify SVF composition, and RNA sequencing was performed to assess transcriptional changes. Estrogen treatment in TW was associated with a trend toward a changed M2-to-M1 macrophage ratio, consistent with a shift toward a more pro-inflammatory WAT milieu. Bulk RNA-seq identified more differentially expressed genes (DEGs) in TW than in TM. Principal component analysis showed clustering of TM before treatment with TW after treatment, suggesting a hormone-associated transcriptional shift toward a phenotype typical of the opposite sex. Together, these data support further investigation of this cohort to define the metabolic and immunomodulatory effects of sex hormones in human WAT.

SESSION 2: SKELETAL MUSCLE

O5. Deciphering Molecular Pathways of Exercise in Type 2 Diabetes Revealed by Multi-Omics

Ben Stocks[1,2*], Stephen P Ashcroft[1*], Jeppe Kjærgaard[1], Signe Schmidt Kjølner Hansen[1,2], Kirstin A MacGregor[2], Dimitrius Santiago Passos Simões Fróes Guimaraes[3], Marc Pielies Avelli[1], Simon Wengert[1], Amy M Ehrlich[1], Scott Frendo-Cumbo[1], Simone Jensen[1], Mladen Savikj[2], David Rizo-Roca[2], Roger Moreno-Justicia[1], Håvard Hamarsland[4], Daniel Hammarström[4], Julia Otten[5], Tommy Olsson[5], Simon Rasmussen[1], Kenneth Caidahl[2,6], Harriet Wallberg-Henriksson[3], Anna Krook[3], Atul S Deshmukh[1#], and Juleen R Zierath[1,2#]

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[2]Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden.
[3]Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden.
[4]Section for Health and Exercise Physiology, Department of Public Health and Sport Sciences, Inland Norway University of Applied Sciences, Lillehammer, Norway.
[5]Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden.
[6]Department of Clinical Physiology, Karolinska University Hospital, Stockholm, Sweden. Summary

Exercise is a potent intervention for type 2 diabetes, enhancing glucose uptake and increasing insulin sensitivity even after a single session. Despite these well-documented clinical observations, the underlying molecular mechanisms mediating these metabolic adaptations remain incompletely elucidated. Employing an integrative multi-omics approach, encompassing transcriptomics, proteomics, phosphoproteomics, and metabolomics, we comprehensively investigated the molecular responses of skeletal muscle immediately following exercise and after three hours of rested recovery in a cohort of 92 men and women. Despite a similar exercise stress, individuals with type 2 diabetes displayed an exacerbated immune response, an accumulation of long- and very-long chain fatty acid metabolites, and impaired mitophagy. Conversely, we delineated conserved exercise-responsive signaling pathways involved in glucose metabolism and insulin sensitivity: identifying previously uncharacterized nodes of skeletal muscle insulin sensitization. In particular, we observed an upregulation of transcripts, proteins, and phosphosites involved in RHO GTPase cycling after exercise, including IQGAP1, which we found to regulate insulin-stimulated glucose uptake and glycogen synthesis, as well as insulin-signaling. These multi-omic insights advance the understanding of the molecular pathways underlying the beneficial effects of exercise and identify candidate molecular targets for future therapeutic strategies aimed at replicating the metabolic benefits of exercise in individuals with type 2 diabetes.

O6. Investigating the effect of NAD+ supplementation in combination with exercise in cardiometabolic disease

Zina Riahi [1], Daksh Verma [2], Twisha Dube [3], Elisabeth Moyschewitz [1], Sarah Enzenhofer [1], Jelena Krstic [1], Lilian Lamprecht [1], Sarah Rimser [1], Helene Michenthaler [1], Victoria Zopf [1], Alina Stockner [2], Anila Varghese [2], Subhash L. Khatri [2], Annalisa Filosa [3], Mahmoud Abdellatif [2],Gunter Almer [4], Renate Schreiber [5], Tobias Eisenberg [5], Marion Mußbacher [3], Simon Sedej [2], Andreas Prokesch [1]

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[2] Division of Cardiology; Medical University of Graz; Medical University of Graz, Austria
[3] Department of Pharmaceutical Biosciences; University of Graz, Austria
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Physical activity is an effective strategy to reduce cardiometabolic disease (CMD) risk factors. However, individuals with CMD often display variability in metabolic responses to exercise, which may partly arise from dysregulation of key metabolic pathways, including nicotinamide adenine dinucleotide (NAD^+) metabolism. NAD^+ is an essential coenzyme involved in ATP production and the regulation of metabolic homeostasis and its reduced availability has been implicated in the development of CM dysfunction. Previous work from our group demonstrates that supplementation with the NAD^+ precursor nicotinamide (NAM) alleviates CMD symptoms. In this study, we investigate if combining NAM supplementation with regular exercise training produces additive metabolic benefits in obese rats. Preliminary results indicate that NAM supplementation slightly improves glucose handling for exercising rats. Plasma cholesterol, triglyceride and phospholipid levels were significantly reduced in the combined intervention compared with exercise or NAM alone. Diastolic dysfunction and elevated blood pressure were improved by exercise and further slightly supported by NAM supplementation. Endothelial dysfunction of the aorta was significantly prevented by NAM alone and in combination with exercise. Liver transcriptomics revealed that the combined intervention upregulates gene programs involved in blood vessel- and cardiac muscle tissue remodeling. These findings suggest that restoring NAD^+ availability may enhance benefits of exercise, potentially offering a novel therapeutic approach to target CMD.

O7. Cell-type-resolved transcriptomic map of skeletal muscle in women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is associated with skeletal muscle insulin resistance, fibrosis, and lipotoxicity, yet the cellular origins remain poorly understood.

We performed single-nuclei RNA sequencing analysis on vastus lateralis biopsies from hyperinsulinemic, hyperandrogenic women with PCOS at baseline (n=10), after 16-week metformin treatment (n=5), and controls (n=4). Eight major cell types were identified, and transcriptomic changes were analysed across conditions and correlated with phenotypic data.

Analysis of 72,247 nuclei identified cell-type-specific dysregulations in PCOS. Myofibers displayed fiber-type-specific metabolic impairment, while fibro-adipogenic progenitors (FAPs) were the most dysregulated population displaying pro-fibrotic reprogramming with enhanced TGF-β signalling, altered glutamine metabolism and increased FAPs-myofiber communication. Metformin selectively reversed transcriptional dysregulation in specific cell populations, targeting predominantly FAPs. In vitro, PCOS myotubes retained the metabolic dysfunction, yet show normalized glucose uptake, indicating plasticity despite metabolic memory. Systemic hyperinsulinemia and hyperandrogenemia correlated with transcriptional dysregulation in both myofibers and FAPs, directly linking endocrine imbalance to pro-fibrotic tissue remodelling.

This study identified cell-type specific PCOS alterations and cell-type-specific mechanisms of metformin treatment in skeletal muscle, revealing potential therapeutic targets for novel treatment of skeletal muscle dysfunction.

SESSION 3: BIOMARKERS OF METABOLIC DISEASE

O8. Interplay between type diabetes and blood-based biomarkers of Alzheimer's disease on the risk of dementia: a population-based study

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Introduction: Blood-based biomarkers of Alzheimer's disease (AD) have great potential as a possible screening tool to non-invasively identify AD-related neuropathology. However, it is unclear how the concentration and performance of these biomarkers is influenced by type diabetes, a common comorbidity in older adults.
Methods: The study included 2,dementia-free older adults (≥60y) from the Swedish National Study on Aging and Care, Kungsholmen (SNAC-K) who were followed for up to years. Baseline levels of six biomarkers were measured using Simoa assays: amyloid-β (Aβ) 42/ratio, total tau, p-tau181, p-tau217, NfL, and GFAP. Baseline diabetes was diagnosed based on medical history, medication use, and/or glycated hemoglobin (HbA1c) ≥6.5%. Dementia was clinically diagnosed following DSM-IV criteria.
Results: At baseline, (8.6%) participants had diabetes. Diabetes and/or higher HbA1c levels were cross-sectionally associated with higher levels of p-tau and p-tauin multi-adjusted models. In joint effect analyses, the highest risk of dementia was observed among participants with both diabetes and unfavorable levels of total tau (HR = 2.[1.30, 3.51]), p-tau(HR = 2.[1.74, 4.29]), NfL (HR = 2.[1.73, 4.84]), p-tau(HR = 3.[2.30, 5.63]), or GFAP (HR = 4.[2.49, 6.53]), compared to their counterparts with neither exposure.
Conclusions: Older adults with both diabetes and elevated levels of total tau, p-tau181, p-tau217, NfL, or GFAP may constitute an especially high-risk group for developing dementia.

O9. cfChIP of H3K4me in Plasma: Advancing Noninvasive Biomarkers for liver metabolic disorders

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Metabolic dysfunction–associated steatotic liver disease and metabolic associated steatohepatitis represent a major global health burden. Current diagnostics largely rely on FibroScans and invasive liver biopsies, as well as scores such as FIB-4, lacking reliable minimally invasive biomarkers to monitor disease progression. Circulating cell-free DNA retains nucleosomal and epigenetic information reflective of its tissue of origin, offering a promising opportunity for noninvasive disease profiling. Here, we explore the potential of cfDNA chromatin immunoprecipitation sequencing targeting the active promoter mark H3K4meas a source of epigenetic biomarkers for MASLD and MASH.
We analyzed publicly available ENCODE datasets to characterize the genomic regions and tissue specificity of cfH3K4mepeaks detected in plasma. Peaks were found among multiple tissue types rather than limited to liver specific regions, indicating diverse origins of circulating nucleosomal fragments. By mapping these promoter regions, we created a matrix to identify the tissue sources of cfH3K4mesignals in plasma.

This study provides a foundation for future cfChIP-seq analyses aimed at deconvoluting the tissue composition of circulating chromatin and identifying disease associated patterns in MASLD and MASH patients. Our findings highlight the potential of cfH3K4meprofiling as a minimal invasive tool to trace tissue specific transcriptional activity and monitor epigenetic changes in plasma. Further studies using patient samples from the KaLIB biobank are essential to identify potential biomarkers.

O10. Physics of cells as new biomarkers for metabolic diseases

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Physical features (e.g., membrane order, tension, mitochondrial potential) are tightly regulated and directly influence intracellular trafficking, signaling, and energy metabolism. Alterations in cellular biophysics are especially relevant in disorders characterized by dysregulated lipids, including diabetes and metabolic syndrome. Such physical remodeling holds strong potential as a functional diagnostic and prognostic biomarker.

Despite technological advances, most tools for measuring biophysical properties remain low throughput and low dimensional, limiting their clinical applicability. Consequently, we lack a comprehensive understanding of how collective biophysical properties shift across metabolic disease states. Here, we present a high-throughput and multi-parametric platform with single-cell resolution that enables measurement of biophysical properties in patient-derived cells. We apply this approach to Niemann-Pick disease type C, a rare inherited lipid storage disorder due to impaired intracellular cholesterol transport.

We establish a pipeline to: (1) biophysically phenotype patient cells and identify systemic signatures of biophysical remodeling; (2) predict disease state from blood-derived cells using machine learning; and (3) evaluate therapeutic interventions using quantitative physical measurements as functional readouts. This strategy positions cellular biophysics as a complementary biomarker layer to current protein or nucleic acid markers for metabolic diagnostics.

SESSION 5: THE BRAIN AND SYSTEMIC METABOLISM

O11. Semaglutide engages brainstem-hypothalamic circuits controlling feeding and metabolic state

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Semaglutide induces profound weight loss, yet the neural substrates underlying its effects on energy balance remain incompletely defined. Using activity-dependent genetic targeting (TRAP2), we selectively manipulated semaglutide-responsive neurons in the dorsal vagal complex (DVC), including the area postrema (AP) and nucleus of the solitary tract (NTS). Chemogenetic reactivation of this TRAPed population recapitulates key features of semaglutide treatment, including reduced food intake, weight loss, increased fat utilization, and conditioned taste aversion. Within this broader semaglutide-responsive DVC ensemble, a substantial subset of neurons expresses *Adcyap1* mRNA. Functional interrogation reveals that *Adcyap1*+ neurons in the AP/NTS are required for full semaglutide-induced suppression of feeding and body weight, as their ablation attenuates these effects in both lean and obese mice. These neurons preferentially contribute to semaglutide-induced fat mass loss, with minimal impact on lean mass or conditioned taste aversion. Circuit mapping further suggests that selective activation of projections from *Adcyap1*+ NTS neurons to hypothalamic targets suppresses fasting-induced and hedonic feeding while sparing dark-phase chow intake, and promotes a catabolic state characterized by increased fat utilization. Together, these findings support a model in which semaglutide recruits a distributed DVC ensemble, within which *Adcyap1*+ neurons form a key functional node linking brainstem signaling to hypothalamic feeding circuits. Delineating these pathways may help guide the development of more selective and better tolerated anti-obesity strategies.

O12. Systemic circadian coordination arises from tissue specific clock dependence and direct photic neural signaling

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Circadian physiology depends on coordinated oscillations across tissues, yet the mechanisms that organize systemic rhythmicity remain incompletely understood. Here, we combined multi-organ circadian transcriptomics with genetic and neural perturbations to dissect the systems-level organization of temporal gene regulation. Across brain regions and peripheral metabolic organs, rhythmic transcription formed distinct coordination domains, with neuronal plasticity programs dominating the brain and lipid metabolic pathways emerging as prominent spatiotemporal features of peripheral tissues. While many rhythmic transcripts required the molecular circadian clock, a substantial fraction persisted in its absence. Restoring clock function selectively in the brain reinstated rhythmic expression of many metabolic genes in peripheral tissues, demonstrating a dominant role for central timing signals. However, anatomical tracing and neural perturbation revealed an additional pathway linking intrinsically photosensitive retinal ganglion cells (ipRGCs) to metabolic organs through thalamic nuclei and the celiac ganglia. Together, these findings show that systemic circadian coordination arises from interactions between central clocks, peripheral clocks, and light-driven neural circuits, revealing a route by which environmental light bypasses the clock system and contributes to metabolic gene oscillations.

O13. Caudal Raphe Serotonergic Neurons Regulate Energy Balance and Adipose Tissue-Specific Remodelling

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Obesity arises from disruption of energy homeostasis, and body fat distribution critically determines metabolic disease risk, with visceral adiposity linked to insulin resistance and type diabetes. However, the central mechanisms controlling regional adiposity remain unclear.

Using trans-synaptic viral tracing from fat, we identified serotonin-expressing neurons in the caudal raphe nuclei (CRn) as a key output node of brain–adipose circuits projecting to retroperitoneal, inguinal and mesenteric white adipose tissues (rpWAT, iWAT and mWAT, respectively). To test the function of these pathways, we injected a Cre-dependent virus expressing tetanus toxin in the CRn of SERT-Cre mice. These mice were protected from diet-induced obesity. Strikingly, this lean phenotype occurred without major changes in food intake or energy expenditure, but was associated with a lower respiratory exchange ratio, indicating enhanced lipid utilization. In line with this, WATs were reduced in mass and had smaller adipocytes. However, mWAT showed increased autonomic innervation and mounted a catabolic program, while iWAT and rpWAT showed reduced inflammatory signalling.

Our findings reveal fundamental principles of brain–body communication and a role for serotonin signalling in tissue-specific adiposity regulation. Given that several serotonin-targeting antidepressants are associated with unexplained weight changes, understanding these pathways may help explain and mitigate drug-induced metabolic dysfunction.

SESSION 6: ISLET BIOLOGY AND INFLAMMATION

O14. Mitochondrial Gatekeeping and Metabolic Tuning of β-Cell Function

Accalia Fu

University of Massachusetts

Pancreatic β-cell function is tightly coupled to metabolic state, yet how mitochondrial pathways directly control insulin secretion is incompletely understood. Our work uncovers several pathways that act to gate and tune cellular function. Using multi-omics analyses, we recently defined the molecular responses of purified human β-cells to physiologic glucose. These data reveal acute mitochondrial cristae remodeling as a structural mechanism controlling β-cell function and further identify coordinated regulation of proteins and metabolites with functional relevance. In a distinct study, we find that mitochondrial arginine metabolism is essential for glucose homeostasis and the β-cell's response to glucose *in vivo*, with molecular findings pointing to a previously unrecognized role. Our findings support a model in which mitochondrial gatekeeping and metabolic tuning are central to β-cell function and highlight new metabolic dependencies that may be leveraged to preserve islet health in diabetes.

O15. Longitudinal in vivo evaluation of human β-cell function during GLP-1RA therapy using sensor-engineered islet spheroids

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Understanding how human pancreatic β-cells respond to metabolic stress and therapies in vivo remains a major challenge in diabetes research. To address this, we used an intravital imaging platform with the anterior chamber of the eye (ACE) as a transplantation site to enable longitudinal, single-cell resolution monitoring of islet function.
Rag1-/- mice were metabolically transplanted with human islets into the ACE of one eye and ten reporter human islets in the other eye. After ablation of endogenous murine β-cells with streptozotocin, fluorescent Ca^2+ sensor islet spheroids expressing GCaMPwere co-transplanted into the reporter eye to enable longitudinal in vivo imaging of β-cell Ca^2+ dynamics. Mice were then challenged with a high-fat, high-sucrose diet (HFHSD) to induce obesity-associated T2D and subsequently treated with the GLP-1RA liraglutide or placebo. Ca^2+ dynamics, body weight, blood glucose, glucose tolerance and plasma human C-peptide were monitored longitudinally.
Human grafts preserved normoglycemia after murine β-cell ablation. HFHSD induced obesity, hyperglycemia and impaired glucose tolerance. Liraglutide reduced body weight, restored normoglycemia and glucose tolerance and increased human C-peptide, indicating enhanced human β-cell function. Amplitude and periodicity of Ca^2+ oscillations were also increased, showing improved β-cell responsiveness and coordination.
This humanized in vivo platform enables longitudinal assessment of β-cell function, providing a robust system to study dysfunction and validate diabetes therapies directly in human islets.

O16. Optical whole organ 3D characterization of the residual β-cell mass in a long standing T1D pancreas - inversed proportions between extra-islet and islet-associated β-cells

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Residual β-cell function can positively affect diabetes regulation in T1D, but details of residual β-cell mass distribution in T1D is largely lacking. We implemented an optical 3D imaging pipeline to generate a first account of the 3D-spatial and volumetric distribution of the remaining β-cells throughout the volume of an entire human late onset T1D pancreas, at a microscopic resolution.

As expected, β-cell mass was dramatically lower than in non-diabetic pancreas and the exocrine volume was greatly reduced as previously described., The pancreatic head displayed a morphology and size resembling the non-diabetic pancreas and displayed a times higher β-cell density compared to the rest of the organ. However, only a fraction of these residual β-cells were located within islet structures. Instead, the absolute majority were present as extra-islet β-cells, either as scattered individual cells or as punctated clusters of β-cells, spatially separated from all other endocrine cell-types. Strikingly, these extra-islet β-cells comprised the absolute majority of the residual β-cell mass, completely inverting the proportional relationship of extra-islet vs islet associated β-cells in non-diabetic pancreas.
This 3D whole organ depiction of a long standing, late onset, T1D pancreas shows that individual β-cells may be preserved in a highly regionalized manner, and that further investigation of residual β-cells cells could give clues to developing potential strategies for β-cell preservation in T1D.

O17. JAK inhibitors modulate immune signaling but leave β cell antiviral pathways intact

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Type 1-diabetes (T1D) arises from an autoimmune destruction of the pancreatic β cells. Type III interferons (IFNLs) are expressed in the human pancreas at the onset of T1D and IFNLs exert immunostimulatory effects on β cells including upregulating HLA-I, while simultaneously providing antiviral protection against T1D associated viruses (Ringqvist, manuscript in preparation). IFNs signal through JAK and TYKkinases and the BANDIT trial demonstrated that Baricitinib (a JAK1/inhibitor) can preserve C peptide levels in individuals with recent onset T1D.

Here, we analysed if JAK/TYKinhibitors (JAKis) modulate IFNL signaling in β cells. Human islets, stem cell–derived islets and EndoC βHcells were treated with JAKis (Baricitinib/Ruxolitinib/Deucravacitinib) and exposed to IFNLor IFNL2. Cells were also infected with a GFP expressing Coxsackievirus-B(CVB3). Surface HLA I expression was quantified by flow cytometry, and viral replication was monitored by live cell imaging (Incucyte).

We show that IFNL-induced HLA-I upregulation and antiviral activity are blocked by JAKis at supraphysiological concentrations. In contrast, at clinically relevant concentrations most JAKis reduce IFNL-induced HLA-I expression, while preserving the antiviral capacity of β cells.

Our result indicates that JAKis, commonly used to limit autoimmune activity driven by type I IFNs, also modulate signaling downstream type III IFNs. Despite this modulation, the antiviral activity mediated by type III IFNs is preserved in β cells under both JAK1/and TYK2-selective inhibition.

SESSION 7: CIRCULATING METABOLITES

O18. Inter-organ metabolic communications in healthy, insulin resistance and atherosclerosis

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Homeostasis is fundamental for organismal health and survival. Disrupted homeostasis causes various human diseases, such as elevated blood glucose in diabetes, cholesterol in cardiovascular diseases, and misfolded proteins in neurodegenerative diseases. However, the mechanisms by which diverse organ systems coordinate delicate homeostasis are poorly understood. Using innovative analytical chemistry techniques and unconventional animal models, my lab has identified novel biochemical mechanisms of metabolic homeostasis mediated by organ-specific metabolism and their crosstalk through circulating metabolite exchange.

O19. Effect of type 2 diabetes on plasma and fat tissue lipidomes in human and mouse studies

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Sphingolipids are bioactive lipids implicated in insulin resistance (IR) and type diabetes, yet their comprehensive analysis remains challenging because of extensive structural heterogeneity and ion suppression by more abundant lipids in complex biological matrices. To improve detection of low-abundance sphingolipids, we implemented a hydrolysis-assisted extraction workflow, together with an optimized LC-MS method, enabling enhanced coverage of minor and atypical sphingolipid species.
Using this approach, we identified 1-deoxyceramides as the most discriminant sphingolipids in subcutaneous adipose tissue from obese individuals with T2D. In a paired human remission cohort (N=23), adipose and plasma deoxyceramides were elevated at baseline relative to obese controls and declined after weight-loss-induced remission. These findings indicate that adipose deoxyceramides are not only markers of obesity but may reflect reversible sphingolipid remodelling during restoration of insulin sensitivity.
To further validate these findings, we studied a high-fat diet mouse model with or without a mitochondrial uncoupler ATX-304, which preserves obesity while preventing IR. IR was associated with selective accumulation of medium-chain deoxyceramides (C16-C20). Furthermore, adipose transcriptomics revealed remodelling of sphingolipid pathway, including reduced expression of SLC7aand increased CerSin IR mice, all normalized by ATX-304. These data implicate reduced serine availability and altered ceramide synthesis leading to deoxyceramide accumulation in IR adipose tissue.

O20. Lipidomic and proteomic signatures of T2D and CVD in the ADIPO-SCAPIS cohort

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Cardiovascular disease (CVD) risk is markedly elevated in individuals with type diabetes (T2D), yet conventional clinical parameters often fail to detect atherosclerotic changes. In the ADIPO-SCAPIS cohort (n=120), stratified by T2D status and presence of coronary atherosclerosis, we applied integrative plasma lipidomics, adipose tissue proteomics and ex vivo metabolic characterization to identify molecular pathways and candidate biomarkers associated with cardiometabolic disease.

Targeted plasma lipidomics quantified lipid species and revealed that cardiometabolic disease is characterized predominantly by structural and species-level lipid remodeling rather than changes in total lipid abundance. T2D was associated with reduced plasmalogens and accumulation of hydroxylated acylcarnitines, consistent with mitochondrial dysfunction, impaired fatty acid β-oxidation and oxidative stress. CVD showed enrichment of rare d20-backbone ceramides that increased with disease severity. Incorporating lipidomic variables into multivariable linear models improved CVD discrimination compared with conventional clinical parameters.

In adipose tissue proteomics, we developed BloodScore to correct for blood contamination, enabling detection of >5 proteins. Pathway analysis linked CVD to inflammatory and immune-remodeling pathways, whereas T2D showed suppression of metabolic pathways. Proteins strongly correlated with adipose tissue function, particularly lipolysis and lipogenesis, were selected as potential biomarkers for CVD and T2D.

POSTERS (P1-74)

P1. Aerobic glycolysis drives differentiation of unilocular adipocytes

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White adipocytes are defined by their single, large lipid droplet (LD), yet recreating this unilocular phenotype in vitro has been difficult. We engineered the Human Unilocular Vascularized Adipocyte Spheroid (HUVAS), a 3D niche incorporating endothelial sprouts and extracellular matrix that enhances preadipocyte differentiation, enabling full adipocyte maturation, unilocular LD formation, and physiologic adipokine secretion. Using HUVAS, we found that metabolic reprogramming is the central driver of unilocularity in human 3D adipocyte cultures. Transcriptomic and metabolomic analyses revealed that unilocularity is defined by elevated aerobic glycolysis and reduced mitochondrial content, mirroring the metabolic profile of freshly isolated human white adipocytes, while being distinct from preadipocytes and 2D cultures. Aerobic glycolysis activates AMPK, boosting CD36-dependent fatty acid uptake. Inhibiting glycolysis lowers fatty acid uptake and shifts cells toward a multilocular morphology, an effect reversible by AMPK re-activation. Conversely, reducing mitochondrial activity or stimulating AMPK in multilocular 3D cultures is sufficient to increase their unilocularity. These results establish aerobic glycolysis as a key driver of LD morphology and size in white adipocytes and highlight the powerful influence of the 3D microenvironment on adipocyte metabolism and function, especially fatty acid uptake. Collectively, this work provides new mechanistic insight into LD biology and offers improved modeling of human adipocyte biology for the study of metabolic health.

P2. A systemic circadian nicotinic acid riboside (NaR) signal engages the unfolded protein response and adipocyte lipid metabolism via the prefoldin complex.

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Daily light–dark cycles impose predictable environmental fluctuations that require coordinated temporal regulation of cellular physiology. This coordination is mediated by the circadian clock, which operates as a network of tissue oscillators; however, the molecular signals that convey circadian information between organs remain incompletely defined. Here, we identify nicotinic acid riboside (NaR) as a circulating metabolite whose rhythmicity depends on the liver clock. In differentiating 3T3-Ladipocytes, NaR engages unfolded protein response (UPR) gene programs and modulates adipogenic competence. Proteome-wide stability profiling implicates the prefoldin complex as a molecular target of NaR signalling, linking NaR exposure to altered proteostasis. Functionally, NaR-induced UPR signalling converges on the adipogenic transcription factor CEBPA, which is a central regulator of adipogenesis. Importantly, sustained NaR exposure suppresses adipocyte lipid deposition, whereas temporally restricted NaR stimulation enhances adipogenesis, indicating that NaR acts in a time-dependent manner. Together, these findings identify NaR as a liver clock– controlled circulating metabolite that couples systemic circadian metabolism to adipocyte proteostasis and differentiation, revealing a mechanism by which temporal metabolic signals shape tissue-specific physiological outcomes.

P3. Adipose Endothelial Cells Lose Fatty Acid Transport Selectivity Under Obesogenic Conditions

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Lipid storage in white adipose tissue (WAT) requires crossing its microvascular barrier, formed by endothelial cells (ECs) that act as metabolic gatekeepers. However, how dyslipidemia impacts WAT microvascular transport remains unclear.
We developed a dual-tracer mix to measure fatty-acid (FA) transport using BODIPY-FA and barrier integrity using FITC-Dextran, applicable in mice and primary ECs. Human adipose-derived ECs (AdipoECs) and cardiac ECs (CardiacECs) were exposed to an obesogenic mix and analyzed by tracer assays, imaging, and RNA-seq. High fat diet–fed or caveolae-altered mice received the tracer mix or Evans Blue to evaluate FA flux and transcytosis.
High fat diet increased non-specific tracer uptake in subcutaneous WAT but not in heart tissue, revealing depot-specific endothelial sensitivity to lipid overload. Mouse models with modified caveolae confirmed their role in WAT transcytosis. In vitro, obesogenic exposure caused AdipoECs to lose FA selectivity and shift toward non-specific vesicular transport, while CardiacECs remained stable. Transcriptomic changes appeared mainly in CardiacECs, suggesting AdipoECs adapt through post-translational mechanisms of reduced SFK activity and downregulated CAV[1], resulting in lipid overload mediated caveolar remodeling.
Overall, AdipoECs show organ-specific adaptation, shifting from efficient FA transport to less selective transcytosis during obesity. This reduced specificity may worsen lipid imbalance. Endothelial transcytosis in WAT emerges as a tissue-specific target for regulating lipid flux in metabolic disease.

P4. Increased LDL uptake to adipocytes impairs local and systemic triglyceride handling

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Obesity is strongly associated with development of an atherogenic lipoprotein profile, including hypertriglyceridemia. The white adipose tissue is one of the major peripheral tissues responsible for triglyceride-uptake, which becomes reduced in obesity. The underlying mechanism behind impaired triglyceride-clearance by the obese adipose tissue remains poorly understood.

Bulk RNA-seq was performed in lean and obese human subcutaneous isolated adipocytes. Low-density-lipoprotein (LDL) and fatty acid uptake were studied in human adipocyte spheroid. A transgenic mouse strain was created by conditionally over-expressing the human LDL receptor only in adipocytes (Adipo-LDLR). Plasma lipids profile, oral lipid tolerance and adipocyte size were assessed in Adipo-LDLR mice and their littermate controls fed HFD for weeks.

Adipocyte expression of the LDLR was increased only in obese insulin-resistant individuals and related with higher plasma triglycerides. In vitro, obese adipocyte spheroids displayed increased uptake of LDL, which both reduced their fatty acid uptake and lipid droplet size. In vivo, Adipo-LDLR mice showed higher adipocyte LDL uptake to subcutaneous fat, led to increased cholesteryl ester proportion while decreased adipocyte size and fatty acid uptake ability. Moreover, impaired post-prandial oral lipid tolerance and a triglyceridemic lipoprotein profile were observed.

Increasing LDL-cholesterol uptake implicates dampened triglyceride handing in the development of obesity-induced adipocyte dysfunction, and subsequent development of hypertriglyceridemia.

P5. Adipose tissue-derived extracellular histones as novel mediators of endothelial injury in cardiometabolic disease

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Obesity and type diabetes increase atherosclerotic cardiovascular disease risk. Altered adipose tissue (AT) secretory profile impairs vascular health. Circulating histones are elevated in obesity and predict cardiometabolic outcomes. However, whether AT is a source of circulating histones that contribute to vascular damage is unknown. To address this question, we analyzed the secretome of multiple AT depots from WT and db/db mice. The secretion of histones Hand Hwas increased across several depots in db/db mice, with the strongest elevation observed in gonadal visceral AT (goVAT). Proteomics identified histones in the goVAT secretome, of which were increased in db/db mice, with histone H3.showing the largest fold change. Circulating H3.levels were elevated in db/db mice and positively correlated with goVAT secretion. Functionally, histone H3.impaired endothelial function in isolated mouse aortas. In human aortic endothelial cells, H3.reduced NO production, increased ROS generation, and induced pro-inflammatory gene expression. Analyses of human transcriptomics datasets suggest leukocytes and dysfunctional (pre)adipocytes as potential sources of extracellular histones within AT. Moreover, multiple histones were upregulated in VAT from patients with coronary artery disease (STARNET) and their expression was associated with genes involved in chromatin remodeling in arteries. Collectively, our results identify AT as a source of extracellular histones and indicate that AT-derived histones may contribute to endothelial dysfunction in cardiometabolic disease.

P6. Inhibition of IMPDH induces cytoskeletal remodeling promoting adipogenic to fibro-inflammatory fate transition

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Aging progressively diminishes the adipogenic potential of mesenchymal stem cells (MSCs) in subcutaneous white adipose tissue (SAT). However, the relationship between metabolic remodeling and transcriptional regulation underlying the age-associated decline in MSC adipogenic capacity remains unclear. Here, bulk RNA-seq analysis revealed that inhibition of inosine-5′-monophosphate dehydrogenase (IMPDH) enhances TGF-βsignaling-associated fibrogenic programs while suppressing adipogenesis. These findings suggest that inhibition of nucleotide biosynthesis not only disrupts adipogenic regulatory networks but also promotes a transition from an adipogenic to a fibro-inflammatory cell fate. We studied the mechanisms underlying this shift in differentiation potential. Pharmacological inhibition of IMPDH induced its polymerization into filamentous structures known as cytoophidia. Our results further demonstrate that cytoophidia strongly associate with intermediate filaments. Cytoophidia were observed in primary stromal vascular fraction cells isolated from the SAT of 45-week-old mice, and guanosine supplementation restored their adipogenic capacity. Future studies will determine whether cytoophidia regulate cell fate through cell stiffening mediated by guanine nucleotide–dependent cytoskeletal remodeling. Collectively, my studies reveal a previously unrecognized mechanism by which nucleotide availability modulates cell differentiation outcomes and contributes to the adipogenic-to-fibroinflammatory transition during aging.

P7. Extracellular Vesicle associated Glycosylated Sphingolipid Alterations in Type Diabetes Impair Adipocyte Glucose Metabolism

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Dysregulated metabolism in type diabetes is underlined by a disrupted interorgan signalling. Extracellular vesicles (EV) facilitate intercellular communication through their cargo. However, the lipid composition of EV and its functional implications in type diabetes are incompletely understood. This study aims to characterize the lipid species alterations in EV from individuals with vs. without type diabetes and investigate how these lipid changes influence metabolism in target tissues.

EV were isolated from age and BMI-matched men and women with or without type diabetes (n=11-per group). In total, sphingolipids were detected in serum-derived EVs by targeted sphingolipid profiling by LC-MS/MS. Principal component analysis indicated that the EV sphingolipidome was altered in both men and women with vs. without type diabetes. Linear modelling indicated a significant reduction in glycosylated sphingolipids in EV from individuals with vs. without type diabetes. Glycosylated sphingolipid treatment lowered basal glucose uptake in both 3T3-Land human adipocytes. These findings were recapitulated when cells were treated with liposomal formulations mimicking the incorporation in EV (21±13%, p=0.04).

These findings demonstrate that glycosylated sphingolipids were lower in serum EVs from individuals with vs. without type diabetes and could modulate glucose metabolism in adipocytes. This study reveals a novel role for EV-associated lipids in glucose metabolism and suggest that such lipid species constitute functional components of inter-tissue communication.

P8. Depot-Specific Alterations in Adipose Tissue in Type diabetes and the Paracrine Role of Perivascular Adipose Tissue on Vascular Function

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Background: Individuals with type 2 diabetes (T2DM) present residual cardiovascular risks, partly linked to dysregulated lipid handling with higher non-HDL-C and triglycerides. As adipose tissue (AT) regulates systemic lipid metabolism and releases vasoactive adipocytokines, we hypothesize that AT dysfunction contributes to T2DM-linked cardiovascular events. To study this, we generated a biobank of adipose biopsies from patients with coronary artery disease, complemented with a murine diabetes model.

Methods: Epicardial AT (EAT), chest subcutaneous AT (cSAT), and perivascular AT (PVAT) adjacent to the internal mammary artery (IMA) were collected from patients ±T2DM undergoing coronary artery bypass grafting. Adipocyte size and extracellular matrix collagen content were assessed using hematoxylin and eosin or Masson's trichrome staining. To assess the paracrine role of diabetic PVAT in vascular dysfunction, lean murine aortic rings were exposed to PVAT-conditioned media from lean or db/db mice, followed by wire myography.

Results: In T2DM, adipocyte hypertrophy was observed in EAT and IMA-PVAT, but not in cSAT. In IMA-PVAT, adipocyte size positively correlates with glycated hemoglobin but not body mass index and shows no association with fibrosis scoring. Conditioned media from db/db PVAT impaired endothelium-dependent relaxation in lean murine aortic rings.

Conclusion: We observed depot-specific AT alterations in T2DM and a paracrine role of diabetic PVAT in endothelial dysfunction. Future studies will explore the paracrine role of human adipose depots in vascular function.

P9. Glutaminolysis Promotes Inflammation In White Adipose Tissue

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Karolinska Intitutet

Obesity is characterized by WAT inflammation. We showed that glutaminolysis is enhanced in obese WAT and that TNF-α stimulates glutaminolysis in adipocytes. Here, we investigated whether TNF-α driven glutaminolysis is required to sustain inflammatory signaling in adipocytes. Human adipocytes were stimulated with TNF-α and subjected to pharmacological inhibition of GLS. Mitochondrial function was assessed and downstream metabolic nodes were targeted to dissect glutaminolysis dependent mechanisms. TNF-α stimulation increased mitochondrial (mt) OCR and the availability of metabolites linked to downstream regulatory pathways. Disruption of glutamine metabolism attenuated CCL expression and reduced mitochondrial respiration. TNF-α also altered the subcellular localization of metabolic enzymes and increased H3K27Ac at inflammatory gene loci. Mechanistically, TNF-α enhanced citrate availability for ACLY, and inhibition of mt citrate export or ACLY suppressed CCL2. TNF-α promoted nuclear translocation of total and phosphorylated ACLY, which was abolished by inhibition of glutaminolysis or ACLY. Reduced nuclear ACLY was associated with decreased H3K27Ac at the CCL locus, linking glutaminolysis-derived citrate metabolism to epigenetic regulation of inflammatory gene expression. Our results show that TNF-α induced glutaminolysis sustains inflammation in adipocytes by promoting mitochondrial metabolism that fuels nuclear signaling and histone acetylation to drive inflammation. Targeting GLS or ACLY attenuates WAT inflammation, highlighting it as a potential therapeutic target.

P10. Elucidating the Drivers of Unilocular Adipocytes: A Matched 3D Culture Comparison

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In vivo, white adipocytes contain a single unilocular lipid droplet that dynamically changes in size in response to energy availability. As traditional 2D adipocyte culture models form multilocular droplets, interest in more physiologically relevant 3D adipose tissue models has grown. Our lab has developed a 3D adipocyte culture model, Human Unilocular Vascularized Adipocyte Spheroids (HUVAS), that successfully recapitulates the unilocular phenotype seen in vivo. Stromal vascular fraction (SVF) cells from the same donor were cultured under three conditions: 2D cultures, scaffold-free 3D cultures, and 3D cultures with a scaffold for vascularization, the HUVAS model (Ioannidou et al., 2022). To identify the features that distinguish HUVAS from the other culture systems, we performed bulk RNA-seq on all three models and conducted time-course experiments comparing the differentiation and maturation of HUVAS to scaffold-free 3D spheroids. Bulk RNA-seq revealed that 2D cultures more closely resemble preadipocytes, while both 3D models show greater similarity to native adipocytes and adipose tissue. Throughout differentiation and maturation, HUVAS exhibited stronger unilocular adipocyte characteristics including larger lipid droplets and higher expression of lipid-droplet–associated genes compared to scaffold-free 3D spheroids. These findings demonstrate that HUVAS more accurately captures adipogenic differentiation and maturation trajectories compared to scaffold-free 3D cultures, establishing HUVAS as a physiologically relevant model for studying human white adipocytes in vitro.

P11. Brain-adipose circuits govern tissue-specific energy homeostasis

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While endocrine control of body weight and energy balance is well established, the role of neuronal innervation in these processes remains poorly defined. An outstanding question is whether homeostasis is governed by a unified central autonomic program or by anatomically and functionally specialized circuits that differentially innervate individual organs. To define the central constituents of these hypothesized brain-body circuits, we injected pseudorabies virus into white adipose tissue, brown adipose tissue, the tibialis anterior muscle, the liver, and the pancreas of wild-type mice. PRV propagates retrogradely in a time-dependent manner, labelling synaptically connected neurons with green fluorescent protein (GFP). This strategy revealed a tissue-dependent modular organization of premotor neurons in the brain. Using complementary viral tracing approaches, we complemented these findings by mapping the full architecture of brain-adipose circuits and found that individual WATs are innervated by largely non-overlapping pathways. Amongst these, we discovered a non-canonical “hybrid” autonomic circuit innervating visceral, but not subcutaneous WATs, via the dorsal motor nucleus of the vagus and the celiac-superior mesenteric ganglion complex. Disruption of this pathway in high-fat diet–fed mice induced profound depot-specific remodelling of white adipose tissue at both cellular and molecular levels, without detectable alterations in behavior. These findings support a modular, circuit-based organization of autonomic control that underlies regional tissue homeostasis.

P12. Basally active neurons in the extended amygdala regulate weight gain

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Basal neuronal activity is a well-established feature of brain circuits and is increasingly recognized as functionally meaningful and not overlooked as mere “noise”. Most studies investigating the circuits regulating feeding and homeostatic regulation, however, focuses on the role played in these processes by genetically defined neuronal populations. This leaves unexplored the possibility that basally active neuronal ensembles may critically contribute to body weight and energy balance.

Here, we used an activity-dependent transgenic approach (TRAP mice) to express Cre recombinase in basally active neurons within the bed nucleus of the stria terminalis (BNST), a key hub controlling consummatory behaviors and involved in circuits mediating the effects of anti-obesity medications. We used adeno-associated viruses (AAVs) to manipulate the activity of these neurons and assess their contribution to energy homeostasis. Our preliminary results show that inactivation of basally active BNST neurons reduces body weight gain in mice fed a high-fat diet, with only minimal effects on feeding. In our next experiments, we will investigate the role of these neurons on energy balance using metabolic cages, assess their electrophysiological properties and interrogate their molecular phenotype.

Although preliminary, our findings suggest that basally active neurons are required for energy homeostasis and may provide insight into the mechanisms of existing anti-obesity drugs while informing the development of new therapeutic strategies.

P13. Locus coeruleus noradrenergic neurons shape regional adiposity and inflammation

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Energy-dense diets promote overeating, obesity, and metabolic dysfunction, yet the central circuits involved remain unclear. Using retrograde polysynaptic tracing, we identified the locus coeruleus (LC), a noradrenergic nucleus, as part of the brain–adipose circuits that innervate white adipose tissue (WAT).

To interrogate the role of the LC in behavior and metabolism, we bidirectionally manipulated the activity of tyrosine hydroxylase (TH)-expressing neurons in transgenic mice. Although AAV-mediated activation and inactivation of LC neurons had opposite effects on locomotion, each reduced feeding on a high-fat diet without changes in energy expenditure or substrate utilization. In line with this, both groups were protected from weight gain, with a stronger effect after inactivation. Strikingly, excess energy was not uniformly stored across WATs: in both experimental groups, inguinal and mesenteric fat mass were reduced, while the mass of retroperitoneal WAT was unchanged, compared to controls. Of note, RNA-seq revealed that diet-induced inflammation was more pronounced in the mWAT despite its smaller size, whereas the rpWAT was less inflamed than controls, despite similar mass.

Our findings reveal a previously unrecognized role for the locus coeruleus in regulating adipose tissue homeostasis and demonstrate that brain–adipose circuits can independently control adiposity and inflammation, dissociating both from feeding behavior and from each other. These findings also suggest that drugs targeting central noradrenergic systems may have unappreciated metabolic consequences.

P14. Brain functional MRI study on diabetic retinopathy

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Diabetic Retinopathy (DR), as a severe microvascular complication of diabetes, seriously threatens the visual health of patients. DR is limited to ocular lesions previously, however, with the development of neuroimaging techniques, evidence indicates a close connection between DR and brain function and structure.
This study, based on multi - center data, aims to reveal the reproducible patterns of gray matter and white matter damage in the brain in diabetic retinopathy, explore the similarities and differences in brain structural damage between DR and simple diabetes mellitus (DM), to clarify the origin of DR related brain damage.
The discovery set of this study comes from the UK Biobank (UKB), including three groups of data: DR, DM, and healthy controls (HC), collecting VBM and DTI data from brain MRI, as well as data from eye OCT. The validation set comes from DR patients and their HC groups collected by Tianjin Medical University General Hospital (TMUGH).
The study found that there are brain regions with abnormal GMV repeatedly discovered in the UKB and TMUGH data sets, suggesting that these brain regions may be the brain targets involved in DR specific damage. white matter fiber tracts affected by DR were discovered, emphasizing that these brain regions may be the white matter pathways of DR specific damage.
This study contributes to a complex understanding of the pathophysiological process of DR and lays a foundation for developing more effective treatment strategies and improving patient prognosis.

P15. Pre-stroke weight loss to improve stroke outcome in diabetes

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INTRODUCTION
Type diabetes (T2D) significantly worsens stroke outcome, often resulting in chronic disability and to date, no effective treatments exist.
Here, we investigated whether pharmacologically targeting obesity, highly prevalent in T2D, before stroke improves stroke outcome in T2D using a clinically relevant murine model.

METHODS
To induce weight loss, obese/T2D mice were treated for weeks with the glucagon-like peptide receptor (GLP-1R) agonist Semaglutide. Weight loss was potentiated using the Neuropeptide Y receptor Y(NPY2R) agonist BI8271. Since GLP-1R and NPY2R could exert direct brain effects in addition to their weight-reducing properties, a diet-based weight-matched group was included. After weight loss, stroke was induced and functional recovery after stroke was assessed via grip strength test and corridor task.

RESULTS
Pre-stroke pharmacological weight loss using Semaglutide significantly improved functional recovery after stroke. Potentiated weight loss using Semaglutide + BIenhanced this effect. Moreover, this improved stroke outcome was entirely driven by weight loss, as weight-matched and pharmacological weight loss groups recovered similarly.

CONCLUSIONS
The T2D/obesity pandemic is increasing the need for treatments to improve stroke outcome. Here, we show the potential of clinically used weight loss pharmacotherapy to improve stroke outcome in T2D, opening new perspectives for a large patient population in need of care.

CONFLICTS OF INTEREST
This study was partly funded by Boehringer Ingelheim.

P16. Myeloperoxidase Delivered by Erythrocyte-Derived Extracellular Vesicles Promotes Oxidative Stress and Endothelial Dysfunction in Type Diabetes

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Background: Red blood cells from individuals with type diabetes (T2D-RBCs) act as mediators of endothelial dysfunction via their extracellular vesicles (EVs). Myeloperoxidase (MPO), a pro-inflammatory enzyme linked to oxidative stress and cardiovascular injury, is present in RBC membranes, but its role in EV-mediated endothelial dysfunction in T2D remains unknown. This study investigates whether RBC-derived MPO contributes to endothelial dysfunction via EVs in T2D.
Methods: RBCs and their EVs were isolated from individuals with T2D and age-matched healthy controls. Mouse aortas were incubated ex vivo with RBCs or their EVs for 18h, and endothelium-dependent relaxation (EDR) was assessed using a wire myograph. A selective MPO inhibitor was used to evaluate functionality. MPO activity and expression were measured in RBCs, EVs, and aortas, and reactive oxygen species (ROS) formation was studied in human endothelial cells exposed to RBC-derived EVs.
Results: MPO activity and protein expression were higher in T2D-RBCs and their EVs compared to healthy controls. T2D-RBCs and their EVs impaired EDR, increased vascular MPO levels and oxidative stress, and enhanced ROS formation in endothelial cells; all attenuated by MPO inhibition in T2D-RBCs or their EVs. MPO inhibition in aortas attenuated EDR impairment and reduced vascular oxidative stress induced by T2D-RBCs and their EVs.
Conclusion: T2D-RBCs and their EVs promote endothelial dysfunction by transferring MPO and inducing MPO-driven oxidative stress, revealing a novel mechanism of RBC-mediated vascular injury in T2D.

P17. Pharmacological inhibition of microplastic uptake prevents endothelial injury

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Emerging evidence suggests that micro- and nanoplastics (MNPs) may contribute to cardiovascular disease. Recent clinical studies have detected plastic particles in human blood and arteries, with higher prevalence reported in patients with diabetes, suggesting a potential link between particle exposure and vascular injury. Polystyrene (PS) particles have been shown to impair endothelial function, although the mechanisms leading to endothelial injury remain unclear. This study investigates whether inhibition of PS internalization could mitigate endothelial dysfunction. Human aortic endothelial cells (HAECs) and aortas isolated from wild-type mice were exposed to PS particles with or without pretreatment with amiloride, an inhibitor of macropinocytosis. Oxidative stress, inflammatory signaling, nitric oxide (NO) bioavailability, and monocyte adhesion were assessed with molecular assays. Vascular reactivity was measured in isolated mouse aortic rings. Circulating PS were measured in peripheral blood samples; in these patients the endothelial function was assessed by flow-mediated dilation (FMD). Plastics accumulated in HAECs, increasing oxidative and inflammatory signaling. PS impaired endothelium-dependent vasodilation in mouse aortic rings. Circulating PS was detected in human blood, and higher PS levels were inversely associated with the FMD. Inhibition of PS uptake with amiloride reduced intracellular particle accumulation, attenuated oxidative and inflammatory responses, restored NO bioavailability, decreased monocyte adhesion, and improved endothelial vasodilation.

P18. Extracellular vesicle-mediated transfer of red blood cell non-coding RNAs induces vascular endothelial dysfunction in type diabetes

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Type diabetes (T2D) is a major risk factor for cardiovascular disease, with endothelial dysfunction as an early event. Red blood cells (RBCs) and their extracellular vesicles (EVs) contribute to endothelial impairment in T2D and contain abundant non-coding RNAs (ncRNAs), including circular RNAs (circRNAs) and microRNAs (miRNAs). Here we investigated how RBC-derived ncRNAs regulate endothelial function in T2D. The ncRNA profile was identified by RNA sequencing and validated by qPCR and Sanger sequencing. Target protein expression was assessed by immunohistochemistry. Endothelium-dependent relaxation (EDR) was measured in rodent aortas incubated with human RBCs or RBC-derived EVs using wire myography. RNA sequencing identified a novel RBC circRNA, circZC3H7A. qPCR showed reduced circZC3H7A levels in RBCs from individuals with T2D compared with healthy controls. The circZC3H7A back-splicing junction was confirmed by Sanger sequencing. Silencing circZC3H7A in healthy RBCs increased vascular oxidative stress and impaired EDR. miR-591, predicted as a circZC3H7A target, was elevated in T2D RBCs and enriched in RBC-derived EVs, which were efficiently delivered to endothelial cells. Aortas incubated with T2D RBCs or their EVs displayed reduced levels of the miR-target protein ZEB1. Inhibition of miR-in T2D RBCs, or in EVs from these RBCs, improved EDR and restored vascular ZEBlevels. These findings suggest that reduced circZC3H7A in T2D RBCs promotes miR-expression and EV-mediated delivery to the endothelium, leading to ZEBsuppression and endothelial dysfunction.

P19. Development of an immunodeficient prenatal mouse model of polycystic ovary syndrome for evaluation of new therapeutics

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Polycystic ovary syndrome (PCOS) affects ~10% of women and is the main cause of anovulatory infertility, defined by hyperandrogenism, irregular cycles, and/or polycystic ovarian morphology, and is linked to cardiometabolic comorbidities. Current treatments are symptom-based, with no targeted options. A key barrier is immune response causing anti-drug antibodies that neutralize drugs. We aimed to establish a PCOS mouse model on an immunodeficient background for preclinical testing without immune interference.
Wild type (WT) and B- cell deficient mice (KO) were used to generate prenatal androgenised (PNA) models via daily dihydrotestosterone injections in pregnant dams at E16.to 18.5. Reproductive traits included anogenital distance, puberty onset and estrus cyclicity. Serum sex steroids, AMH and FSH, were measured. Metabolic traits were assessed by EchoMRI, and with glucose and insulin tolerance tests in Fmice.
PNA mice on both backgrounds showed delayed puberty onset, increased AGD, disrupted estrous cyclicity. Androgen levels were unchanged, but AMH was elevated in WT and KO PNA mice, and FSH was lower in WT PNA mice. Body weight and composition were similar across groups, though KO-PNA mice had increased subcutaneous and visceral fat at P186. Glucose and insulin tolerance did not differ significantly.
In conclusion, B-cell-deficient PNA mice recapitulate key reproductive PCOS features despite lacking adaptive immunity. This PCOS-like model provides a robust platform to evaluate therapeutics while limiting immune-mediated drug clearance.

P20. B-lymphopoiesis and humoral immunity in an atherosclerotic environment

Xueming Zhang[1], Jiawen Wang[1], Yuyang Zhang[1], Sanna Hellberg[1], Giada Di Nunzio[1], Aoxue Li[1], Peder Olofsson[1], Christoph Binder[2], Meinrad Busslingerand Stephen G. Malin[1]

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Antibody responses to infection or recognition of auto-antigens in inflammatory conditions are controlled at two critical stages: the immunoglobulin repertoire constructed in the bone marrow and the coordination of immune responses in secondary lymphoid organs or tissues. B cells are critical mediators of atherosclerotic cardiovascular disease in pre-clinical models and are currently being targeted in clinical trials. Mechanistically, how B cells can directly or indirectly influence atherosclerosis remains largely unknown. We are attempting to disentangle the variables underlying atherosclerosis. Using a selection of novel mouse models, we have addressed whether dyslipidemia or the inflammatory nature of plaques drives B cell responses. We demonstrate that germinal centre formation occurs independent of an atherogenic dyslipidemia, resulting in fulminant auto-antibody production that drives plaque growth, in an APOE-dependent manner. In contrast, the absolute number of pro-B cells decreased significantly in mice lacking the LDL receptor, as pro-B cells are unique amongst B cell populations in being competent in taking up apolipoprotein-B containing lipoproteins. Mechanistically, the interleukin-7/JAK-STAT/PI3K pathway regulated the expression of the LDLR, indicating that ApoB-lipoproteins may be important for B-cell progenitor growth and potentially subsequent immunoglobulin repertoire development. Our results will now allow for a redefinition of atherosclerosis initiation in the context of B cell responses.

P21. B-lymphopoiesis and humoral immunity in an atherosclerotic environment

Xueming Zhang[1], Jiawen Wang[1], Yuyang Zhang[1], Sanna Hellberg[1], Giada Di Nunzio[1], Aoxue Li[1], Peder Olofsson[1], Christoph Binder[2], Meinrad Busslingerand Stephen G. Malin[1]

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Antibody responses to infection or recognition of auto-antigens in inflammatory conditions are controlled at two critical stages: the immunoglobulin repertoire constructed in the bone marrow and the coordination of immune responses in secondary lymphoid organs or tissues. B cells are critical mediators of atherosclerotic cardiovascular disease in pre-clinical models and are currently being targeted in clinical trials. Mechanistically, how B cells can directly or indirectly influence atherosclerosis remains largely unknown. We are attempting to disentangle the variables underlying atherosclerosis. Using a selection of novel mouse models, we have addressed whether dyslipidemia or the inflammatory nature of plaques drives B cell responses. We demonstrate that germinal centre formation occurs independent of an atherogenic dyslipidemia, resulting in fulminant auto-antibody production that drives plaque growth, in an APOE-dependent manner. In contrast, the absolute number of pro-B cells decreased significantly in mice lacking the LDL receptor, as pro-B cells are unique amongst B cell populations in being competent in taking up apolipoprotein-B containing lipoproteins. Mechanistically, the interleukin-7/JAK-STAT/PI3K pathway regulated the expression of the LDLR, indicating that ApoB-lipoproteins may be important for B-cell progenitor growth and potentially subsequent immunoglobulin repertoire development. Our results will now allow for a redefinition of atherosclerosis initiation in the context of B cell responses.

P22. Impairment of circulating metabolites induces endothelial dysfunction in adolescents exposed to gestational diabetes in utero

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Offspring born to women with gestational diabetes (GD) are at increased risk of atherosclerotic cardiovascular disease (ASCVD), though the mechanisms remain unclear. We hypothesized that an altered circulating environment in GD offspring impairs vascular function, promoting ASCVD risk. Vascular function was assessed in mouse aortas incubated with plasma from adolescents born to control or GD pregnancies. Aortas exposed to GD plasma showed reduced endothelium-dependent relaxation (EDR), indicating endothelial dysfunction. This impairment was accompanied by endothelial accumulation of the oxidative stress marker 4-HNE and was rescued by antioxidant treatment (Tempol, NAC). In human aortic endothelial cells, GD plasma increased pro-oxidant and inflammatory gene expression, ROS production, and monocyte adhesion. Metabolomic profiling revealed dysregulated plasma metabolites in GD adolescents, including increased N-monomethylarginine (NMMA), a nitric oxide synthase inhibitor, and reduced 3-hydroxyanthranilic acid (3-HAA), a metabolite with anti-inflammatory properties. Supplementation of GD plasma with L-arginine restored NO production and reversed NMMA-driven effects, while addition of 3-HAA reduced inflammatory gene expression. THP-monocytes exposed to GD plasma also showed altered expression of genes involved in 3-HAA metabolism. These findings indicate that adolescents exposed to GD in utero exhibit an altered circulating metabolome that promotes endothelial dysfunction through oxidative stress and inflammation, potentially contributing to increased ASCVD risk.

P23. Endothelial BUD upregulation in type diabetes drives vascular dysfunction through inflammatory pathways

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Background: Type diabetes (T2D)-associated vascular complications contribute to increased morbidity and mortality. BUD13, a nuclear RNA-binding protein, plays a crucial role in RNA splicing and is involved in metabolic syndrome. However, its role in cardiovascular disease is unclear. This study investigates the contribution of BUD to T2D-associated vascular injury.

Methods: BUD expression was evaluated in mouse aortas (macrovasculature) and ocular vascular tissues (retina and choroid, microvasculature). Endothelium-dependent relaxation (EDR) was measured in aortas isolated from mice using wire myograph. In human carotid arterial endothelial cells (HCtAECs), reactive oxygen species (ROS) and nitric oxide (NO) production, together with cell migration were measured after high glucose (HG) treatment. RNA-and protein-omics analyses were also performed. BUD siRNA was applied in these models to study its function.

Results: BUD was highly expressed in the endothelium of aortic, retinal, and choroidal vessels of db/db mice compared to wild-type mice. EDR was impaired in db/db aortas but was attenuated by the BUD siRNA. Both mRNA and protein levels of BUD were elevated in HG-treated HCtAECs. BUD siRNA not only reduced its levels but also restored the ROS/NO balance and cell migration in these cells. Omics analyses revealed that BUD regulates key pro-inflammatory pathway.

Conclusion: Our findings indicate that endothelial BUD drives T2D-related vascular injury via oxidative and inflammatory pathways, suggesting it as a potential therapeutic target.

P24. Shift of mechanical homeostasis in the retinal pigment epithelium after cell loss: Relevance to diabetic retinopathy

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In diabetes, early retinal pigment epithelium (RPE) dysfunction contributes to diabetic retinopathy (DR) progression, yet the role of mechanical homeostasis remains unexplored. Postmitotic RPE monolayers face cell density loss in DR, similar to cell loss in ageing, which is exacerbated by hyperglycemia and may challenge force balance within the tissue. We hypothesize this triggers cytoskeletal remodelling that impairs vision-supporting phagocytosis, promoting debris accumulation and neurodegeneration in DR.

Using a reductionist in vitro model of density reduction via apoptosis in stem cell-derived RPE monolayers, we recapitulate aged/DR-like features: reduced cell height, shortened microvilli, and actin reorganization. Nanoindentation reveals tissue stiffening, reinforced junctions, and heightened contractility. Functionally, monolayers show defective photoreceptor outer segment (POS) phagocytosis of fewer, larger internalized fragments, connected to diminished apical ruffling and apicolateral deformation. Transcriptomics highlights altered expression of actin regulators (nucleators, bundlers, linkers), while Arp^2/and formin inhibition during POS phagocytosis differentially rescue particle number and fragment size.

These data establish density-driven mechanical reinforcement as a DR-relevant mechanism redirecting actin dynamics from phagocytic plasticity to tissue reinforcement, independent of glucose stress. This RPE platform unveils mechanobiology as an early therapeutic target to restore homeostasis and prevent retinopathy progression.

P25. Mapping the Cross-Tissue Cellular Landscape of Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a multisystem disorder characterized by hyperandrogenism, insulin resistance, adipose tissue dysfunction, and impaired endometrial receptivity, resulting in implantation failure, miscarriage and type diabetes. Despite affecting 10–13% of women of reproductive age, the underlying mechanisms remain poorly understood.

We aim to perform cross-organ profiling to define molecular and cellular drivers of reproductive and metabolic dysfunction in PCOS, and how first-line treatment with lifestyle modification and metformin modulates them.

We will integrate our three single-nuclei RNA-sequencing (snRNA-seq) atlases from paired endometrium, subcutaneous white adipose tissue (scWAT), and skeletal muscle samples from the same women with and without PCOS, including after a 16-week treatment with metformin and/or lifestyle modification.

Datasets will be integrated by benchmarking via scIB, differential gene expression will be performed using MAST, followed by over-representation and gene set enrichment analysis. Cell–cell communication will be inferred using CellChat or LIANA. Findings will be complemented by immune plasma proteomic profiling (Olink Inflammation Panel) from three independent cohorts to identify circulating immune and inflammatory mediators of cross-tissue signaling.

This multi-tissue atlas will resolve cell type–specific PCOS alterations, define cross-organ molecular subtypes aligned with clinical phenotypes, and identify biomarker signatures predictive of therapeutic response, enabling precision stratification in PCOS.

P26. Thrombolysis exacerbates cerebrovascular injury after ischemic stroke via a VEGF-B dependent adipose-brain metabolic axis

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Obesity, metabolic syndrome, and diabetes can significantly impact stroke outcomes, increasing the risk of edema and hemorrhage after ischemic stroke. Diabetic patients also have higher risk of symptomatic intracerebral hemorrhage following thrombolysis with recombinant tissue plasminogen activator (rtPA). The mechanisms underlying this increased risk are largely unknown. Herein we demonstrate the presence of a previously unrecognized metabolic axis connecting activation of white adipose tissue (WAT) lipolysis to the risk of intracerebral hemorrhage following ischemic stroke and thrombolysis with rtPA. In both human stroke patients and murine models, intravenous rtPA induced a rapid rise in circulating fatty acids. This was due to rtPA-mediated increased WAT lipolysis and ectopic cerebrovascular lipid accumulation, leading to destabilization of the ischemic vasculature after stroke. Inhibition of vascular endothelial growth factor-B (VEGF-B) signaling reduced WAT lipolysis and cerebrovascular lipid accumulation resulting in improved vascular integrity and importantly, in enhanced efficacy and safety of thrombolytic rtPA treatment. This suggests a new strategy to reduce hemorrhagic complications and expand the therapeutic window of thrombolysis with rtPA.

P27. Mitochondrial respiration and lipid droplet turnover regulate induced endothelial fatty acid uptake

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Endothelial cells (ECs) rely on anaerobic glycolysis for energy yet require mitochondrial ATP for fatty acid (FA) uptake. However, which energy substrates contribute to mitochondrial ATP-dependent FA uptake is unknown. We investigated if inducers of endothelial FA uptake promote mitochondrial respiration and whether FA and lipid droplet (LD) metabolism are involved. Using cultured ECs, inducers of long-chain FA uptake (3-HIB, lactate and VEGF-B), metabolic inhibitors, siRNA-mediated knockdown of lipolysis enzymes, and live-cell metabolic analyses, we show that 3-HIB, lactate and VEGF-B act directly or indirectly as substrates for mitochondrial respiration. We identify oleic acid and β-adrenergic agonists as novel inducers of endothelial FA uptake and demonstrate that oleic acid, β-adrenergic agonists and VEGF-B promote FA uptake via lipolysis, enhancing β-oxidation and ATP production. Supporting these findings, fasting-induced lipolysis increased FA uptake and retention in the brain. Notably, despite available mitochondrial ATP, induced FA uptake requires a functional cycle of FA esterification, LD synthesis and subsequent lipolytic degradation. These findings highlight how different substrates drive endothelial FA uptake and connect LD metabolism to FA uptake.

P28. Red blood cell-derived extracellular vesicles impair cardiac post-ischemic recovery in type diabetes: Role of microRNA-^{210}

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Red blood cells (RBCs) from patients with type diabetes (T2D) impair cardiac post-ischemic recovery through an unclear mechanism. RBCs and RBCs-derived extracellular vesicles (EVs) contribute to endothelial dysfunction via loss of protective RBC-microRNA (miR)-210. We hypothesize that T2D-RBCs impair cardiac function through EVs-mediated signaling associated with reduced RBCs-miR-210. H-RBCs and T2D-RBCs, as well as their EVs were administered to isolated Langendorff-perfused rat hearts to assess the post-ischemic recovery. Left ventricular developed pressure/end-diastolic pressure and infarct size were measured. RBC miR-levels and uptake of RBCs-derived EVs in the hearts were evaluated. T2D-RBCs and their EVs impaired cardiac recovery and increased infarct size compared to healthy controls. This impairment was abolished by co-incubation of EVs with heparin, suggesting EV-mediated effects. Despite producing fewer EVs, EVs from T2D-RBCs were taken up to a greater extent by endothelial cells in the left ventricle and apex of the heart. miR-expression was reduced in T2D-RBCs and restoration of miR-levels improved cardiac recovery, whereas inhibition of miR-in H-RBCs induced the impairment. Administration of EVs from T2D-RBCs with miR-mimic attenuated cardiac dysfunction and limited infarct size. T2D-RBCs impair cardiac post-ischemic recovery through EV-mediated mechanisms associated with reduced RBC miR-and enhanced EV uptake in the heart. Restoration of miR-levels in T2D-RBCs confers cardioprotection, highlighting a potential therapeutic target.

P29. ATP9A in erythrocytes: A regulator of extracellular vesicle release and endothelial dysfunction in type diabetes

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Background

Red blood cells (RBCs) drive endothelial dysfunction in type diabetes (T2D) through release of extracellular vesicles (EVs). ATP9A, a lipid flippase shown to suppress EV release in a few cell types, may regulate release of RBC-EVs. Here, we explore the role of RBC ATP9A in regulating EV release and endothelial function in T2D.

Methods

EVs were isolated with the exoEasy kit, and concentrations were measured by NTA. RNA-sequencing, qPCR, and immunostainings were used for expression analysis. RBCs and EVs were incubated with mouse aortas for endothelium-dependent relaxation (EDR) using wire myography. GapmeR was used to inhibit ATP9A.

Results

RNA-sequencing revealed ATP9A as a top upregulated mRNA in RBCs from T2D donors (T2D-RBCs) compared to healthy controls. Gene ontology revealed changes in EV-related pathways. qPCR and immunofluorescence confirmed elevated ATP9A expression, and NTA revealed decreased EV-release in both T2D-RBCs and diabetic db/db mouse RBCs compared to healthy controls. Human RBC ATP9A expression inversely correlated with EV release. In vivo ATP9A knockdown in db/db mice increased vascular oxidative stress and further impaired EDR, while GapmeR transfection in isolated db/db aortas had no effect. RBCs from these mice worsened EDR and released 60% more EVs. These EVs caused further impairment in EDR and showed increased uptake by endothelial cells.

Conclusion

Our findings indicate ATP9A upregulation in RBCs as a compensatory mechanism to limit EV release and mitigate the extent of EV-driven endothelial dysfunction in T2D.

P30. From silent plaque to clinical events: Mass-spectrometry-based sphingolipid profiling to link subclinical carotid atherosclerosis with overt cardiovascular disease

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Atherosclerosis is a major cause of heart attacks and strokes. It often progresses silently but can lead to severe cardiovascular complications. Sphingolipids play an important role in atherosclerosis pathogenesis and are linked to early signs of cardiovascular dysfunction and plaque formation. Structural diversity of sphingolipids, driven by variations in fatty acyl chains and sphingoid bases, reflects their biological activity. LC-MS is a powerful method for their high-accuracy initial screening (QTOF) and targeted quantification (QQQ).
This workflow will be applied to plasma from participants in the VIPVIZA trial to explore associations between sphingolipid profiles and carotid intima-media thickness, and longitudinally plaque progression over three years. Candidate sphingolipid markers will be validated in the Umeå cohort of the SCAPIS study, where carotid ultrasound and coronary CT angiography allow assessment of multi-bed plaque burden.
Based on our preliminary data and evidence that specific circulating sphingolipids (ceramides) can improve cardiovascular risk prediction beyond Framingham risk score, we will assess whether sphingolipid-based indices from VIPVIZA and SCAPIS enhance prediction of myocardial infarction and stroke in the NSHDS study. Using pre-diagnostic samples, we will compare models with established risk factors and Framingham-type scores with and without sphingolipid markers. This project will determine whether targeted sphingolipid profiling can refine current cardiovascular risk assessment and support the development of ceramide-based risk scores.

P31. Cell-type–resolved transcriptomic profiling of human placentas in polycystic ovary syndrome reveals limited effects of gestational metformin treatment

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Polycystic ovary syndrome (PCOS) is associated with pregnancy complications and increased cardiometabolic risk in offspring. Metformin is often prescribed despite limited evidence for preventing obstetric complications. The placenta responds to maternal metabolic conditions and can be used to understand PCOS and metformin exposure during pregnancy. We therefore performed single-nucleus and spatial transcriptomic analyses on the placentas from the PregMet trial.

PCOS placentas showed predominant downregulation of gene expression across several cell types. Moreover, intercellular communication networks were globally weakened with pronounced reductions in WNT, BMP and VEGF signaling, some of which related with maternal serum growth factor levels. Notably, metformin failed to prevent most PCOS-associated molecular alterations, with limited effects on certain gene expression, including PAPPA in cytotrophoblasts, endothelial cells, and fibroblasts, and ELMO in syncytiotrophoblasts. Spatial transcriptomics localized major cell types and ligand expression within the villi structure, showing no regional specificity of key differentially expressed genes in each cell type.

Our findings defined a PCOS-associated placental transcriptional signature and revealed limited responsiveness to metformin, prompting a reassessment of current therapeutic strategies and highlighting potential long-term consequences for offspring. Importantly, the disrupted signaling pathways identified here could serve as further mechanistic and interventional studies.

P32. High-fat diet exacerbates ischemic muscle impairment in the FAL murine model of Peripheral Artery Disease

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Peripheral artery disease (PAD) affects over 200 million people worldwide and often co-occurs with type 2 diabetes. PAD arises from reduced limb perfusion causing ischemia and progressive skeletal muscle impairment, further aggravated by diabetes-associated metabolic stress. Current treatments aim to restore perfusion but do not fully recover muscle function, highlighting the need to better understand mechanisms driving PAD related myopathy. The femoral artery ligation (FAL) mouse model is widely used to induce ischemia, yet its muscle phenotype under diabetic-like metabolic conditions remains uncharacterized.

We examined skeletal muscle responses to FAL in mice on a normal diet (ND) or high fat diet (HFD), the latter modelling diabetes-related metabolic stress. ND mice showed substantial recovery of muscle force by 30 days post FAL, whereas HFD mice displayed persistent deficits. Mitochondrial respiration was markedly reduced in HFD FAL muscles, indicating impaired oxidative capacity. Histology revealed smaller myofibers, increased lipid accumulation and elevated macrophage infiltration post FAL, all worsened by HFD. These findings show that ischemia combined with metabolic stress reproduces key features of PAD myopathy in the FAL mouse model.

Ongoing bulk-RNA-sequencing analysis of muscles at 3 days, 1, 2 & 4 weeks after FAL in ND and HFD mice aims to define transcriptional programs underlying divergent recovery. Analyses examine time and diet dependent gene trajectories and pathways controlling, among others, mitochondrial function, inflammation and adipogenic signaling.

P33. Spatial and transcriptomic profiling of ovary in PCOS

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Polycystic ovary syndrome (PCOS) affects 11-13% of reproductive age women worldwide and is associated with women's reproductive, cardiometabolic and psychological complications. The ovary sits in the center of PCOS pathophysiology, driving excessive androgen production, irregular cyclicity and subfertility. Due to difficulties in obtaining ovarian biopsies, current research mostly focused on granulosa cells and follicles, leaving the other ovarian cell types largely unexplored. As a result, despite the importance of the ovary in PCOS etiology, the transcriptomic and metabolomic landscape of the PCOS ovary remains poorly characterized.
In our project, we collected ovarian cortex biopsies from healthy women and women with PCOS. We performed spatial transcriptomics and mass spectrometry imaging (MSI) on ovarian cortex biopsy sections. With these methods, we aim to achieve integrated characterization of the cellular architecture, metabolomic signatures and spatial localization of the ovarian cortex, especially in the context of PCOS.
The analysis of spatial transcriptomics and MSI are still ongoing, but preliminary results suggest a fibrosis like feature in the ovarian cortex. Our preliminary results from MSI suggested altered glycosylation patterns and tryptic peptides in PCOS ovaries, which may be attributed to fibrosis-like changes in PCOS ovaries. These findings suggest distinct molecular and structural alterations in the PCOS ovarian cortex and highlight the value of combining spatial transcriptomics with MSI to advance the understanding of PCOS ovarian biology.

P34. Endometrial Cell Dysfunction Across the Menstrual Cycle In Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) affects up to % of reproductive-aged women and is characterized by hyperandrogenism, polycystic ovarian morphology, and menstrual irregularities. It is a major cause of anovulatory infertility, and affected women have an increased risk of implantation failure and miscarriage, likely due to endometrial dysfunction. Our recent PCOS Endometrial Cell Atlas (PECA 1.0) identified cell-type–specific disease signatures in proliferative phase endometrium. Here, we establish PECA 2.to define cell-type and transcriptomic changes across the menstrual cycle in PCOS.
Single-nuclei RNA-seq libraries were prepared using Chromium GEM-X technology vand v(10X Genomics) from frozen endometrial biopsies obtained from women with and without PCOS. Combined with PECA 1.0, our samples cover proliferative, early secretory, mid-secretory, and late secretory phases, as well as the anovulatory state.
PECA 2.0 comprised 998, high-quality nuclei (625, PCOS; 373, control), identifying epithelial, stromal, immune, and endothelial cell types. Further analysis revealed cell subpopulations. PCOS endometrium showed a lower proportion of stromal cells in the proliferative phase and a lower proportion of decidualized stromal cells in the late secretory phase compared to controls.
PECA 2.0 maps heterogeneity across the menstrual cycle and reveals transcriptomic signatures of PCOS-associated endometrial dysfunction, providing a resource for developing diagnostic tools, targeted therapies, and preventive strategies to improve endometrial health.

P35. Optimizing siRNA Treatment Targeting AKR1C to Reverse PCOS-Associated Androgen Excess in Adipocytes

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Androgen excess and metabolic comorbidities are characteristic features of polycystic ovary syndrome (PCOS), affecting ~10–13% of women worldwide. AKR1Ccontributes to androgen excess and altered adipocyte function in adipose tissue by converting androstenedione to testosterone. Uptake-optimized siRNAs targeting AKR1Cmay overcome limitations of unmodified siRNAs and ameliorate PCOS adipose changes. Here, we aim to determine whether our uptake-optimized siRNA is efficiently internalized in 2D and 3D adipocyte models without transfection reagents.

Fluorescently labeled, uptake-optimized inactive siRNA was applied to healthy differentiating (1) preadipocytes, (2) adipose spheroids, and (3) patient-derived mature adipocytes. siRNA was administered via free uptake at 10, 50, and nM or with Lipofectamine to enhance uptake at various time points. Cellular uptake was assessed at the end of differentiation by measuring fluorescence using confocal microscopy. Uptake of fluorescent siRNA was successful at all time points and concentrations, and Lipofectamine did not improve uptake. The signal appeared weaker at 10 nM but persisted in cells for several days across all concentrations, suggesting that all adipose models are receptive to the siRNA. As a next step, we will apply active AKR1CsiRNA to our 2D and 3D adipose models to knock down AKR1C expression and potentially restore normal adipocyte functionality. This study represents an initial step toward developing a therapeutic siRNA treatment for adipose tissue dysfunction and metabolic alterations characteristic of PCOS.

P36. Polypharmacy and potential drug misuse in patients with type diabetes mellitus: a Swedish register-based drug utilization study

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Background: type diabetes mellitus (T^2DM) is often associated with multiple comorbidities, particularly in elderly, leading to polypharmacy and increased risk of adverse drug events and inappropriate medication use. The exposure to commonly misused drug classes (opioids, CNS depressants, stimulants) is an additional concern, but data in this population remain limited.
Objective: to assess the prevalence of polypharmacy among T^2DM patients and, within the polypharmacy subgroup, to assess the prevalence of potential drug misuse, to identify the most involved pharmacological classes and to characterize patient profiles at higher risk of inappropriate medication use.
Methods: ongoing observational register-based cohort study using Swedish national health data, population registers and the National Diabetes Register. Adult T^2DM patients (≥years) with ≥antidiabetic prescription between ^{2007}–will be included. Polypharmacy is defined as concurrent use of ≥medications for ≥months. Potential for drug misuse will be evaluated using a proxy indicator defined as exposure to drug classes commonly associated with misuse among patients with polypharmacy. Prevalence estimates will be calculated, and multivariable logistic regression models will be used to identify factors associated with polypharmacy and potential drug misuse.
Expected results: this ongoing study will provide population-based evidence on the burden of polypharmacy and exposure to medications that could lead to drug misuse among T^2DM patients, contributing to drug safety in this population.

P37. Target tissue insulin resistance in early and late onset of overweight or obesity

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Overweight and obesity are associated with insulin resistance. However, the importance of the time of onset of excess body fat is unknown and was presently examined.
We included 339 adults having information about their body mass index (BMI) at 18 years of age. Insulin action was determined as homeostasis model assessment (HOMA-IR) reflecting liver, hyperinsulinemic euglycemic clamp reflecting skeletal muscle, Adipo-IR reflecting adipose tissue in vivo, and insulin action on lipogeneses reflecting fat cells. The subjects were divided into never overweight with BMI always <25 kg/m^2 (NO), having BMI ≥25 kg/m^2 already at 18 years of age (EO), and late onset of overweight when BMI was ≥25 kg/m^2 only at current examination (LO). The groups were compared by regression, unpaired t-test and analysis of variance or covariance.
EO had 5 kg/m^2 higher BMI and was 10 years younger than LO at examination ($p<0.0001$). EO was more insulin resistant than LO for both HOMA-IR and Adipo-IR but not Clamp ($p=0.01$; 0.02; and 0.11 respectively). However, when the different measures of insulin resistance were corrected for current BMI or age there were no significant differences between EO and LO for any of the measured values for insulin action ($F\leq3.7$, $p\geq0.055$). Furthermore, in all subjects current BMI ($p<0.0001$) but not BMI when 18 years old ($p\geq0.13$) correlated with different insulin resistance measures.
When current BMI or age is considered, there is no difference between early or late onset of overweight/obesity for the level of insulin resistance in the different target tissues of the hormone.

P38. Effect of Circadian Rhythm & Physical Exercise on Metabolism in Overweight Type Diabetes Patients

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Patients with type diabetes (T1D) are at increased risk of cardiovascular disease (CVD). Regular physical exercise is a powerful modulator of energy homeostasis, improving cardiometabolic health and is advised for T1D patients. The circadian clock governs metabolic homeostasis and is tightly interconnected with gene regulatory pathways that mediate responses to exercise. Physical activity can synchronize the clock in skeletal muscle and thus have favorable effect on metabolic regulation.

The study aims to investigate systemic and organ-specific metabolic responses to high-intensity interval training (HIIT) and whether exercise timing modulates these adaptations in individuals with overweight and T1D. We hypothesize that HIIT interacts with the circadian clock and drives metabolic responses in overweight subjects with or without T1D.

We recruited healthy controls and individuals with T1D (men and women, BMI 25-kg/m2). Participants completed an initial VO2max test on a cycle ergometer to determine exercise load and subsequently performed one HIIT session in the morning (09.00) and one in the afternoon (16.00) separated by a minimum of seven days. Blood samples and skeletal muscle biopsies were obtained pre- and post-exercise, with an additional blood sample collected 1h post workout. All patients consumed standardized meals throughout the study.

Data collection is completed and analysis ongoing. Preliminary results will be presented at the conference. This study hopes to clarify whether circadian timing influences metabolic responses to HIIT in patients with T1D.

P39. Overeating polyunsaturated fat increases the HDL anti-inflammatory activity compared with saturated fat

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Introduction:

High-density lipoprotein cholesterol (HDL-C) is associated with reduced cardiovascular disease (CVD) risk, yet raising HDL-C levels has not improved outcomes. Current research focuses on HDL function, including its anti-inflammatory activity, which predicts CVD events in the general population. Diet quality is a major CVD risk factor. Guidelines advocate replacing saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA). Their impact on HDL anti-inflammatory function remains unknown.

Aim:

To assess the effect of 8-week dietary overfeeding of SFA or PUFA on HDL anti-inflammatory activity and the HDL lipidome.
Methods:Sixty overweight subjects were randomized to receive muffins with PUFA or SFA (n=each) to gain ~3% body weight. HDL anti-inflammatory activity was assessed by its ability to suppress tumor necrosis factor α (TNFα)-induced vascular cell adhesion molecule-(VCAM-1) mRNA expression in endothelial cells in vitro. HDL profiling used nuclear magnetic resonance (NMR) spectroscopy and targeted lipidomics via liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results:

Despite weight gain, PUFA diet increased HDL anti-inflammatory activity, HDL particle number and HDL-C. No such effects were seen with SFA. HDL lipidomics showed increased linoleic and arachidonic acid-containing species after PUFA diet, while eicosapentaenoic acid (EPA)-containing species decreased.

Conclusion:

Short-term PUFA intake enhances HDL anti-inflammatory function and induces distinct lipidome remodelling, offering a potential mechanism for CVD reduction.

P40. Sex-specific KDM6A-HNF4A-CREBH network controls lipoprotein cholesterol metabolism and atherosclerosis via epigenetic reprograming of hepatocytes

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The liver is a central organ in the maintenance of lipid metabolism. It coordinates cholesterol uptake, biosynthesis, excretion and clearance through intricated transcriptional networks defined in the epigenetic level by transcription factors and coregulators. These networks differ between males and females, resulting in marked sex differences in lipoprotein patterns and risk of developing atherosclerosis. How sex chromosome-linked epigenetic modulators contribute to such sex-specific metabolism remains unclear. Here, we have demonstrated that the X-linked histone demethylase 6A (KDM6A) is a crucial coregulator involved in liver cholesterol regulation. KDM6A knockdown in human liver cells induces transcriptional changes annotated to lipoprotein and cholesterol metabolic pathways linked with cardiovascular disorders. Consistently, hepatocyte specific Kdm6a knockout (LKO) female, but not male, mice display substantial atherogenic circulating lipoprotein profiles and are prone to developing atherosclerosis upon genetic and dietary challenges. Mechanistically, KDM6A is recruited to chromatin by Hepatocyte Nuclear Factor Alpha (HNF4A). This creates an active epigenetic landscape essential for the binding of cAMP-responsive element-binding protein H (CREBH), which subsequently activates the transcription of lipoprotein and cholesterol metabolic genes in hepatocytes. Therefore, our study uncovers a novel mechanistic link of KDM6A with atherosclerosis using both human liver cells and mouse models.

P41. Impaired gluconeogenesis links maternal diabetes with sex-dimorphic liver disease in offspring

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Growing evidence suggests that prenatal exposure to maternal diabetes increases the risk of developing fatty liver disease. However, the mechanistic understanding remains limited.

Here, we investigate the long-term effects of mild maternal hyperglycemia on offspring liver function using an optimized streptozotocin (STZ)-induced model. After mating with healthy males, their offspring were followed longitudinally on three different diets: chow, high-fat diet (HFD) and the Gubra Amylin NASH diet (GAND).

Both male and female offspring of STZ-induced mice (STZ offspring) developed insulin resistance on a chow diet. However, only female STZ offspring exhibited early adiposity, signs of hepatic lipid accumulation and later glucose intolerance, with corresponding transcriptomic alterations primarily in the liver. When challenged with a HFD or GAND, a subset of female STZ offspring developed hepatic fibrosis, with activation of pro-fibrotic and pro-inflammatory transcriptomic pathways. Surprisingly, the female STZ offspring with fibrotic livers showed preserved systemic glucose homeostasis. However, these mice had impaired gluconeogenic capacity, highlighting dysfunctional gluconeogenesis as a potential driver of disease.

In summary, mild maternal hyperglycemia programs liver disease in female offspring, which can be further exacerbated by a HFD or GAND challenge. The hepatic phenotype is not associated with development of other metabolic alterations, decoupling overall metabolic health from liver function. Further studies are ongoing to understand the underlying mechanisms.

P42. MTARCp.A ablation reduces hepatocellular carcinoma aggressiveness in vitro and in vivo

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Hepatocellular carcinoma (HCC) is a leading cause of cancer death, driven by metabolic reprogramming that fuels tumor growth. The missense variant rs(p.A165T) in Mitochondrial amidoxime reducing component (MTARC1) is protective against liver disease and linked to lower risk of steatosis, cirrhosis, and HCC. However, MTARC1's mechanistic role in HCC remains unclear, prompting investigation into its function in tumor development and progression.

We investigated the role of MTARCi n HCC using siRNA knockdown in human HCC cell lines homozygous for the risk allele (p.A165) and generated stable CRISPR-Cas knockout (KO) models. We then assessed MTARC loss on proliferation, migration, lipid metabolism, and fatty acid oxidation in vitro, and tumor aggressiveness in subcutaneous xenografts. Global proteomics was also performed in both in vitro and xenograft models.

Transient knockdown of MTARCp.A reduced proliferation in HCC cell lines. CRISPR-Cas9-mediated stable MTARCp.A KO in Hep3Bcells led to decreased neutral lipid intracellular accumulation, enhanced β-oxidation and reduced cell migration. A MTARCKO xenograft model had reduced tumor volume. Proteomic analyses of both in vitro HCC cells and xenograft tumors revealed inhibition of oncogenic pathways and activation of anti-proliferative proteins.

Downregulation of MTARCp.A inhibits lipid accumulation, dampens tumor-promoting pathways and restricts tumor growth, highlighting MTARC as a promising therapeutic target for HCC.

P43. Nutrient-Driven Remodelling of Liver Splicing Programs in the Development of Insulin Resistance and MASLD

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Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most common chronic liver disease worldwide and is strongly associated with metabolic syndrome and T2D. Insulin resistance is a fundamental driver of MASLD and its more severe form, metabolic dysfunction-associated steatohepatitis (MASH). The liver is a central regulator of glucose and lipid homeostasis, and hepatic insulin resistance contribute to increased glucose production, impaired glycogen storage as well as lipid dysregulation. Liver dysfunction associated with MASLD thus become a key driver of T2D progression.

Recent findings shows that splicing programs are disrupted upon overnutrition and metabolic disease, and several studies have demonstrated splicing alterations specifically in both MASLD/MASH and T2D. Emerging evidence also suggest that isoform usage provide a distinct regulatory layer for metabolic adaptation to nutritional input. However, it is not known how distinct macronutrient composition reshape genome-wide splicing programs in the liver, how these changes contribute to MASLD development, and how metabolic states and signalling interface with splicing decisions.

The aim of this study is to determine the effect of macronutrient composition on alternative splicing programs in the liver, its relation to insulin resistance and disturbed glucose homeostasis, and how they influence onset and progression of MASLD. The study also aims to investigate the interplay between splicing patterns, chromatin remodelling and 3D genome organisation under distinct nutritional conditions.

P44. Di-lineage human liver spheroids recapitulate steatotic liver disease

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common comorbidity of type diabetes, affecting nearly 2/3rd individuals and contributing to adverse hepatic and cardiometabolic outcomes. Diabetes and MASLD share key mechanisms, including insulin resistance, altered lipid metabolism, inflammation, and fibrogenesis. Physiologically relevant human in vitro models are needed for better understanding. Here we show a di-lineage human hepatic spheroid model that recapitulates key features of MASLD.
Methods: Spheroids were generated using primary human hepatocytes and primary human hepatic stellate cells from several donors in a predefined 24:ratio. Steatotic and fibrotic-like conditions were induced using oleic acid and palmitic acid (2:1; total µM) and TGFB(ng/mL). Spheroids were characterized by transcriptomics and proteomics.
Results: The model reproduced major hallmarks of MASLD, including increased neutral lipid accumulation, elevated COL1Aexpression, and reduced ApoBsecretion. Transcriptomic and proteomic analyses showed altered genes involved in extracellular matrix remodeling and metabolic pathways, consistent with MASLD. Findings were validated against RNA-seq data from individuals across the full fibrosis spectrum. Resmetirom and obeticholic acid reversed steatosis and fibrosis-associated phenotypes, consistent with clinical observations.
Conclusions: This spheroid model is physiologically relevant and scalable platform for mechanistic and therapeutic discoveries in MASLD and diabetes-related liver disease.

P45. HypoxamicroRNA-protects against hepatic steatosis by inhibiting CIDEC expression

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Metabolic dysfunction–associated steatotic liver disease (MASLD) is a major global health burden characterized by excessive hepatic lipid accumulation, yet the contribution of hypoxia-responsive pathways to this process remains incompletely understood. Hypoxia-inducible microRNA-(miR-210) is a central mediator of cellular responses to hypoxia, but its role in hepatic lipid homeostasis has not been fully defined. We observed that serum miR-levels were significantly reduced in individuals with MASLD. In human hepatic spheroids, fatty acid - induced intracellular hypoxia failed to appropriately induce miR-expression, which was associated with increased lipid accumulation. Consistently, loss of miR-in mice resulted in enhanced hepatic lipid deposition. Mechanistically, CIDEC, a lipid droplet-associated protein, was identified as a direct target of miR-mediating its effects on hepatic lipid storage. Restoration of miR-with mimics suppressed CIDEC and reduced hepatic lipid accumulation in both human hepatic spheroids and mouse models of hepatic steatosis. Collectively, these findings demonstrate that hypoxia-responsive miR-limits hepatic lipid accumulation by repressing CIDEC expression, indicate circulating miR-as a potential biomarker of hepatic steatosis, and suggest that modulation of the miR-- CIDEC axis may represent a therapeutic strategy to reduce hepatic lipid accumulation.

P46. Epigenetic and Transcriptional Remodeling in Human Hepatocytes Under Inflammatory Stress Reveals a Role for NNMT in Inflammation and Fibrosis

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Metabolic and inflammatory signals shape the epigenetic and transcriptional landscape of hepatocytes and contribute to the pathogenesis of metabolic-associated steatotic liver disease (MASLD), metabolic-associated steatohepatitis (MASH), hepatocellular carcinoma (HCC), and fibrosis. These regulatory changes involve cis-regulatory elements, non-coding RNAs, transcription factors (TFs), coregulators, and histone/DNA modifications. However, transcriptomic and epigenomic responses of hepatocytes to inflammatory stress remain incompletely understood, particularly in human metabolic liver disease.

To address this, HuH-cells and freshly isolated primary human hepatocytes were treated with the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. Using RNA-seq, ATAC-seq, and ChIP-seq, we investigated cytokine-induced transcriptomic and epigenomic alterations and identified gene expression changes and regulatory elements linked to metabolic pathways. Among the affected factors, nicotinamide N-methyltransferase (NNMT), a metabolic enzyme, emerged as a potential regulator of hepatocyte responses.

NNMT knockdown and overexpression experiments were performed to assess its role in hepatocyte regulatory programs. Integrative chromatin and transcriptional analyses suggested associations between NNMT-dependent regulatory programs and inflammatory and fibrotic transcription factor networks and gene expression programs. These findings provide a framework for understanding inflammation-driven transcriptional and chromatin remodeling in metabolic liver disease.

P47. Extracellular vesicles in muscle dysfunction in type diabetes: sex-specific miRNA and lipid profile.

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Background: Skeletal muscle regulates metabolic homeostasis via extracellular vesicles (EVs). Their miRNA/lipid cargo are key mediators of tissue crosstalk in type diabetes (T^2D). We aim to characterize the abundance, size, and cargo of EVs secreted by primary human skeletal muscle cells in T^2D, identifying sex-specific differences and exercise-like stimulation effects.
Materials and methods: Primary skeletal muscle cells were obtained from donors (T^2D n=^{10}; NGT n=^{10}). Small and large EVs were collected from media at ^{3}h, ^{16}h, and ^{24}h. Isolation was performed via differential ultracentrifugation. miRNA and lipid profiles were analyzed using a targeted miRNA panel and mass spectrometry. Exercise was mimicked in vitro via acute (^{3}h) and chronic (^{24}h) electrical pulse stimulation (EPS).
Results: Pilot data confirmed the isolation of distinct small and large EV populations. We hypothesize that T^2D-derived muscle cells display dysregulated miRNA and sphingolipid signatures with sex-specific patterns, explaining dimorphic disease progression. EPS is expected to induce adaptations in EV cargo, with chronic stimulation potentially reversing T^2D-associated dysregulations.
Conclusion: Characterizing the muscle-derived EV profile in T^2D provides insights into impaired crosstalk. Sex-specific signatures and exercise-induced adaptations may reveal novel biomarkers and therapeutic targets to restore metabolic health.

P48. Identification and characterization of novel regulators linking skeletal muscle metabolism and remodeling

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Background and Aims

One of the earliest detectable signs of insulin resistance preceding type diabetes (T2D) is reduced glucose uptake in skeletal muscle. T2D is also closely associated with impaired skeletal muscle regeneration. However, the molecular mechanisms underlying this relationship remain incompletely understood. Here, we seek to characterize the role of novel myogenic regulators, including myotubularin-related protein (MTMR3), in skeletal muscle metabolism and remodeling.

Materials and Methods

Ten publicly available single-cell RNA sequencing datasets comprising cells from human skeletal muscle were integrated using standardized bioinformatic pipelines. Primary cells isolated from vastus lateralis skeletal muscle biopsies were transfected with siRNA to target MTMR3. The expression profile of myogenic gene markers was assessed by qPCR.
Results. Our meta-analysis of 71 quality-filtered cells segregated six major clusters spanning myogenesis from quiescent skeletal muscle stem cells to fully mature myotubes. We identified MTMR as a novel regulator of myogenesis. Upon MTMR silencing, we detected a reduction in the expression of MKI67, MSTN and MYF5. Conversely, silencing of MTMR upregulated the expression of CKM, DES, MYH and MYH7.

Conclusion

Our results suggest that MTMR coordinates the progression of human skeletal muscle differentiation, acting as a brake on myogenic maturation. These findings provide a foundation for future studies investigating whether MTMR modulation influences skeletal muscle mass and function in T2D.

P49. Epoxy fatty acids accumulation enhances skeletal muscle glucose utilization and improves glucose control in female mice

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Background and aims

Dysregulated inflammation contributes to cardiometabolic complications in obesity and type diabetes . Soluble epoxide hydrolase (sEH) converts anti-inflammatory epoxy fatty acids into diols, potentially modulating inflammatory and metabolic regulation. We investigated whether pharmacological sEH inhibition alters glucose control in obese mice and examined the metabolic effects of epoxy fatty acids in skeletal muscle cells.

Method

Plasma epoxy fatty acids and their diols were quantified in people with type diabetes and healthy volunteers before and after acute exercise. Primary human myotubes were used for metabolic assays and signaling analyses. Whole-body glucose homeostasis and plasma insulin were assessed in high fat diet mice of both sexes treated with or without the sEH inhibitor TPPU.

Results

Exercise transiently increased circulating 9(10)-EpOME and its diol, 9(10)-DiHOME, in men, regardless of disease status, whereas women with type diabetes showed a sustained elevation in 9(10)-EpOME/9(10)-DiHOME ratio, suggesting altered sEH activity. In myotubes, 9(10)-EpOME enhanced glucose utilization and glycogen accumulation, accompanied by increased phosphorylation of PKA substrates, while attenuating palmitate oxidation. sEH inhibition improved glucose tolerance in obese female mice without affecting insulin.

Conclusions

9(10)-EpOME is an exercise-responsive metabolite that promotes glucose utilization in skeletal muscle, and modulating its levels via sEH inhibition may improve glucose control in obesity in a sex-dependent manner.

P50. DGKδ silencing alters lipidomic profile in human skeletal muscle cells.

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Reduced Diacylglycerol Kinase δ (DGKδ) protein abundance and activity have been observed in skeletal muscle from people with type diabetes. DGKδ catalyzes the phosphorylation of diacylglycerol (DAG) to phosphatidic acid (PA). The accumulation of DAG leads to insulin resistance through attenuation of the insulin signaling cascade. Hence, DGKδ may serve as an essential molecular switch that has major consequences for downstream lipid signaling and cellular metabolism. We hypopothesize that silencing of DGKδ leads to an increase in DAG species and affects downstream lipid synthesis. Skeletal muscle myotubes isolated from vastus lateralis were differentiated to mature myotubes and transfected with DGKδ siRNA (siDGKδ) or scramble and harvested hours after transfection, under basal and insulin-stimulated (120nM) conditions. Lipidomic analyses were performed for DAGs, triglycerides (TG), ceramides, sphingolipids and PA. Lipidomic analysis showed limited differences between scramble and DGKδ-silenced cells in basal conditions. However, insulin stimulation revealed alterations in multiple diacylglycerol (DG) species, which were consistently reduced in DGKδ-deficient cells. This suggests that there may be a compensatory effect from other DGK isoforms that would preserve the phosphorylation of DAG to PA.

P51. Single-Nucleus Transcriptomics Uncovers Cell-Type-Specific Remodelling in Gastrocnemius and Vastus Lateralis Muscles in Peripheral Artery Disease

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Comorbidities of type II diabetes (T2D), including peripheral artery disease (PAD), are major global health concerns. PAD involves reduced blood flow to the lower limbs, causing muscle ischemia, dysfunction, and weakness that can progress to necrosis if untreated. Current interventions, such as bypass surgery and endovascular procedures, restore blood flow but often fail to correct skeletal muscle abnormalities. Despite this, the molecular mechanisms underlying intramuscular dysfunction in PAD remain poorly understood.

To define cellular and transcriptional alterations in PAD muscle, we generated single-nucleus RNA-sequencing data from approximately 100,000 nuclei isolated from paired gastrocnemius and vastus lateralis biopsies obtained from 6 patients with PAD and 6 healthy controls.

Our analysis identified marked cell type-specific changes in PAD muscle. Fibro-adipogenic progenitor cells had an increased proportion in both muscles of PAD patients, indicating broad remodelling of the stromal niche. The ischemic gastrocnemius showed a higher proportion of immune cells, consistent with an altered inflammatory landscape. At the myofiber level, type I fibers exhibited downregulation of mitochondrial pathways, implicating mitochondrial dysfunction in PAD-associated muscle pathology.

Together, these data establish a single-nucleus transcriptomic atlas of human skeletal muscle in PAD, strengthened by paired distal and proximal sampling alongside controls, providing a foundation for mechanistic insight into PAD-associated myopathy.

P52. Dissecting the functionality and cardio-metabolic diseases relevance of single- and dual-functional cis-regulatory elements across human tissues/cells

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Over-nutritional and inflammatory cues drive chromatin remodeling that contributes to cardiometabolic diseases (CMDs), including obesity, and tyepe diabetes (T2D). Cis-regulatory elements in non-coding regions, particularly enhancers and silencers, coordinate tissue-specific transcription through interactions with transcription factors and coregulators, yet their genome-wide functional architecture across human tissues and cell types remains unclear.
Here we systematically map functional enhancers and silencers by integrating multi-omics datasets from public resources. Regulatory regions were linked to target genes using expression quantitative trait loci analyses across tissues and primary cell types. Most regions regulating a given gene show consistent effects across tissues, whereas about one-third of multifunctional regions regulating gene pairs display opposing directions.
We classify these elements into single- and dual-functional categories and compile them into the Human Single or Dual Functional Enhancers and Silencers in Cardio-Metabolic Diseases database. Integration of ATAC profiles with clinical measurements (HbA1c, HOMA2-B and HOMA2-IR) from T2D patients and curated T2D-associated SNVs reveals strong associations between CD14+ monocyte regulatory elements and T2D phenotypes. Links with more than cardiometabolic traits are supported by UK Biobank data, and CRISPR knockout experiments in Huhand THP-cells validate representative elements.
Together, this study provides a framework and resource for investigating enhancers and silencers in CMDs.

P53. Dissecting the functionality and type diabetes metabolic diseases relevance of enhancers/silencers across mouse tissues/cells

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CCL is a key chemokine driving chronic inflammation in obesity and T2D. Elevated CCL levels are linked to T2D. We previously identified two regulatory regions that coordinatively regulate *Ccl* transcription in macrophages with H3K27ac enrichment. However, CRISPR knockout revealed that one region functions as an enhancer whereas the other acts as a silencer. We hypothesize that silencers are often misclassified as enhancers based on active chromatin marks.
To evaluate tissue specificity of the *Ccl* enhancer and silencer, we checked chromatin accessibility and H3K27ac profiling across mouse tissues. We find that the enhancer is consistently active, whereas the silencer is restricted to adipose tissue and macrophages. Interestingly, enhancer deletion decreases *Ccl* expression across tissues under basal conditions, LPS stimulation, and high–fat diet–induced inflammation. In contrast, silencer deletion increases *Ccl* expression only in macrophages and visceral adipose tissue, indicating broader enhancer function and a more tissue-restricted silencer.
We next assessed their roles in obesity induced metabolic dysfunction. Enhancer knockout mice show improved glucose tolerance and insulin sensitivity, whereas silencer knockout mice exhibit only minor metabolic changes. These findings identify a tissue-conserved enhancer as a dominant regulator of *Ccl* expression, metabolic inflammation, and T2D progression. CCL regulatory elements in human monocytes will be mapped in THP cells, establishing a framework for identifying enhancers and silencers regarding T2D pathogenesis.

P54. Redox Perturbations as Causal Drivers of Insulin Resistance in Obesity

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Obesity-associated low-grade inflammation disrupts insulin signaling and contributes to type diabetes. Obesity is also linked to increased oxidative stress, but whether disrupted redox homeostasis is merely correlative or causative in insulin resistance remains unclear. Reanalysis of public human and murine adipose transcriptomics reveals strong, selective correlations between key redox-regulatory genes (TXNRD1, NQO1, NADPH-oxidase family) and clinical markers of obesity and insulin resistance. Contradicting patterns, such as positive correlations of TXNRDand negative correlations of CAT, indicate pathway-specific redox rewiring rather than a uniform oxidative-stress response.
We hypothesise that thiol-based redox switches act as causal drivers of early metabolic dysfunction. Using human adipocytes and hepatocytes exposed to nutrient overload and low-grade inflammation, we will map alterations in the thioredoxin and glutaredoxin systems and identify redox-modified targets such as PTP1B, glycolytic enzymes, and mitochondrial regulators. Genetic and pharmacological perturbations will define how these redox shifts reshape inflammatory signaling and impair insulin responsiveness, aiming to reveal enzyme-specific mechanisms and actionable targets to restore metabolic health.

P55. Role of Epigenetic Monocyte Alterations in Inflammatory Type Diabetes

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T2D is a heterogeneous disease with inflammatory subtypes suggested more recently. Circulating blood monocytes are the suspected major drivers of inflammatory T2D. Monocytes secrete cytokines that act systemically, and they differentiate into tissue macrophages to manifest T2D progression towards complications. In our study we aim to overcome a current knowledge gap by identifying and functionally characterizing inflammatory T2D-associated epigenetic alterations that affect gene expression in human monocytes. Our focus is on inflammation-induced epigenetic alterations affecting cis-regulatory elements (CREs) such as enhancers and silencers, and on the CRE-interacting transcription factors and coregulators that dynamically remodel chromatin and determine which genes are turned on or off. We will present our current progress in genome-wide profiling of PBMC-derived monocytes from T2D patients and in the functional characterization of candidate CREs, along with regulatory proteins and inflammatory signals, in the human THP-monocyte cell line.

P56. Chronic inflammation in skeletal muscle promotes NOX upregulation and drives differential Prdx oxidation in distinct cellular subdomains'

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Reactive oxygen species (ROS) regulate many intracellular processes. At physiological levels, ROS can activate redox sensitive proteins and initiate downstream signaling. However, excessive ROS, as seen in metabolic diseases, disrupt redox homeostasis and impair these pathways. How chronic inflammation elevates ROS and affects specific subcellular compartments remains unclear. This project aims to define mechanisms underlying inflammation-associated ROS upregulation and characterize its effects across cellular subdomains.

Inflammation was induced in female C57Bl/6JRj mice (12 to 16 weeks) by intra-articular injection of Complete Freund's Adjuvant (CFA; 5 µL, 10 mg/mL) into the right ankle joint. The contralateral leg received saline (SAL; 5 ul, 0.9% NaCl) as control. Gastrocnemius muscles were collected 14 days post injection (dpi) and analyzed by LC MS/MS proteomics and redox proteomics, complemented by reducing and non-reducing Western blot.

Proteomics and Western blot showed upregulation of NOX and Prdxin CFA muscle at 14 dpi. Redox proteomics revealed increased oxidation of peptides and decreased oxidation of 72 peptides. Cysteine-154 of malate dehydrogenase (Mdh1) was among most oxidized peptides, whereas many less oxidized peptides originated from immune related proteins. Non-reducing Western blot showed increased sarcoplasmic Prdx4 dimerization in CFA muscle, while cytosolic Prdx2 dimerization was reduced. As Prdx4 dimerization reflects hydrogen peroxide levels, this could suggest increased oxidation in the SR and a more reduced cytosol during inflammation.

P57. Nanoscale architecture of lipid flux at the endoplasmic reticulum

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The endoplasmic reticulum (ER) serves as a central hub where neutral lipids are partitioned between storage in lipid droplets (LDs) and secretion in lipoproteins. How the ER physically organizes this decision at the nanoscale remains poorly understood. Using in situ cryo-electron tomography and complementary functional analyses, we show that the key assembly factor seipin undergoes a pronounced conformational transition from a compact to an open state that stabilizes a stereotyped ~^{20}-nm ER–LD neck and promotes neutral lipid accumulation. Disrupting this transition impairs LD formation, demonstrating that regulated seipin opening functions as a structural checkpoint in lipid storage. We further identify a liver-enriched ER microprotein that modulates this conformational transition, suggesting a mechanism by which lipid storage capacity can be tuned in a tissue-specific manner. Together, these findings provide a structural framework towards understanding how nanoscale ER architecture shapes lipid flux between storage and secretion.

P58. A spatiotemporal map of lncRNA in obesity and chronic jet lag

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The circadian clock drives daily rhythms in gene expression and physiology which is coordinated across multiple tissues. Long non-coding RNAs (lncRNAs) have been increasingly recognized as important regulators of molecular and cellular functions. Although advances in next-generation sequencing technologies have provided extensive insights into transcriptome-wide RNA expression, the circadian regulation of lncRNAs in time and space remains incompletely understood. In this study, we generated a comprehensive circadian lncRNA atlas by analyzing tissues, including peripheral tissues and brain regions, in mice with diet induced obesity and exposed to chronic jet lag. In total, we profiled 2 samples and detected 32 lncRNAs and 21 protein-coding genes. This multi-tissue circadian lncRNA atlas provides a fundamental resource for understanding the spatiotemporal regulation of lncRNA expression and offers new insights into the potential roles of lncRNAs in circadian biology and systemic physiology.

P59. Modeling Maternal Stress and Metabolic Exposures Using Term Placenta–Derived Trophoblast Organoids

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The placenta regulates developmental programming by mediating maternal environmental signals that influence fetal development and long-term offspring health. However, placental tissue is inaccessible during pregnancy, limiting studies of placental responses. Human trophoblast organoids (TOs) are a relevant physiological in vitro model that mimics placental architecture and can differentiate into all trophoblast cell types while preserving key tissue features. TOs can be derived from term placentas, which are accessible and enables integration of maternal and offspring clinical data. As term placentas originate from vaginal or cesarean deliveries, it is unclear whether delivery mode influences TOs properties.
To address this, TOs from both sources were compared molecularly and functionally. Transcriptomic differences from placental tissues were not preserved in the derived TOs. Organoids from both delivery types exhibited comparable growth rates, trophoblast marker expression and differentiation towards extravillous trophoblasts, indicating functional equivalence regardless of delivery mode.
Using this platform, we explored trophoblast responses to maternal stress by exposing TOs to dexamethasone. Short-term exposure induced enrichment of mitotic spindle and downregulation of oxidative phosphorylation, mTORCsignaling, interferon response and reactive oxygen species pathways, suggesting a transient adaptive state marked by metabolic restraint and increased trophoblast plasticity. Ongoing experiments aim to establish a physiological in vitro model of maternal hyperglycemia.

P60. In vivo detection of DNA-based delivery platforms for multivalent targeting of insulin receptors

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DNA-based nanomedicine forms a powerful platform for biomedical applications and therapeutic targeting strategies. Multivalent presentation of insulin ligands on DNA nanostructures can effectively modulate the activation of insulin receptors (IR) whose clustering has been shown to be impaired in insulin-resistant phenotypes. Despite this potential, accurately measuring the in vivo biodistribution of DNA-based delivery systems remains challenging. Fluorophore quenching and unwanted interactions with biomolecules can obscure reliable detection.
Addressing these limitations, we utilize an alternative strategy to determine the biodistribution of DNA-based nanocarriers in mammalian tissues. DNA nanostructures are synthesized with pre-incorporated alkyne groups that act as chemical handles for copper-catalyzed click chemistry after tissue processing. This enables covalent attachment of fluorophores post-fixation, reducing signal loss and improving detection specificity. Using fluorescence microscopy, we have visualized the biodistribution of DNA nanocarriers without insulin ligands with high specificity and sensitivity. Future work aims to characterize biodistribution of multivalent insulin nanostructures and test whether ligand multivalency influences tissue targeting and IR clustering, particularly in insulin-resistant contexts. Ultimately, this work will provide functional insight into how multivalent insulin presentation affects in vivo targeting and support the development of future DNA-based nanomedicine strategies for metabolic disease therapy and precision drug delivery.

P61. Targeting of Insulin Nanoclusters in Zebrafish for Tailored Insulin Therapies

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Insulin receptors (IRs) organize into nanoclusters on the cell membrane, as demonstrated by our group and others using super-resolution microscopy. The nanocluster arrangements of IRs provide the ideal environment for multivalent interactions with insulin. We designed an insulin-DNA nanostructure that offers precise control over the number and spatial distribution of insulin molecules within each nanostructure. Our group found that certain multivalent insulin configurations significantly increased IR activation, extended receptor engagement, and improved glucose regulation in a zebrafish model of diabetes.

Our ongoing work investigates the biodistribution and functional activity of insulin-DNA origami nanostructures in zebrafish. Using light-sheet microscopy, we observe distinct differences between insulin-functionalized nanostructures and control treatments, indicating specific regions targeted by the insulin nanostructures.

Future work will incorporate single-cell sequencing to precisely identify the sites of insulin interaction. Understanding tissue-specific IR expression patterns may enable the development of novel insulin therapies selectively targeting key tissues, thereby fine-tuning insulin efficacy at the cellular level.

P62. A co-culture model of patient-derived endometrial stromal and epithelial cells using a recombinant spider silk membrane

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Spider silk membranes provide a promising alternative to rigid plastic counterparts in transwell systems, as their reduced thickness and flexibility more closely mimic the native basement membrane's flexibility and dimensions. This membrane system enables the co-culture of different cell types on opposite sides, and its porous structure allows crosstalk and signaling in proximity. This project uses FN-silk to co-culture donor-matched human primary endometrial epithelial cells (EC) and stromal cells (SC) from both women with polycystic ovary syndrome (PCOS) and controls on FN-silk. While these cells naturally exist in the endometrium, their cell communication remains poorly studied. In FN-silk, these cells are in a significantly more physiologically relevant microenvironment compared to monoculture on hard plastic and 3D co-culture techniques. Unlike co-culture of epithelial organoids, where the apical side of the epithelium is often inaccessible and the organization fails to reflect the natural monolayer, our model accurately replicates the in vivo architecture. As the endometrium is a highly dynamic tissue, the EC and SC undergo drastic changes upon menstrual hormone fluctuations. Here, we optimize cell culture conditions for mono- and co-culture of endometrial EC and SC. Afterwards, we will perform a 14-day hormone treatment on mono- and co-cultures to differentiate the cells and mimic mid-secretory phase endometrium. We expect to observe differential crosstalk between endometrial EC and SC in PCOS and control samples, and present the preliminary data at the conference.

P63. Mapping insulin receptor activation states at the cell membrane using super-resolution microscopy

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Most membrane proteins are organized in dynamic nanoscale domains that regulate signaling and can become dysregulated in disease. Understanding membrane protein function, therefore, requires not only mapping their spatial organization but also resolving their activation states in the native cellular context. However, the activation states of individual receptors within membrane nanodomains remain poorly understood due to technical limitations in resolving protein conformations at the cell membrane.
Here, we aim to characterize activation states of individual insulin receptor dimers using Resolution Enhancement by Sequential Imaging (RESI), a recently developed super-resolution microscopy method capable of Ångström-scale resolution. Structural studies have shown that insulin binding induces a conformational change in the receptor dimer, reducing the distance between FnIII domains from ~Å to ~Å. By introducing tags into the extracellular FnIII domain of the endogenous insulin receptor using CRISPR gene editing, we will be able to determine the activation states of insulin receptor dimers. Using differentiated mouse adipocytes, we will examine how insulin stimulation and insulin-resistant conditions affect the distribution of receptor activation states at the plasma membrane.
This work will provide the first nanoscale map of insulin receptor activation states at the cell membrane and may reveal how receptor spatial organization and activation state contribute to insulin resistance.

P64. Modelling PCOS adipose dysfunction in vitro using single-layer and adipose spheroid cell culture systems

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Metabolic disorders in hyperandrogenic women with polycystic ovary syndrome (PCOS) are common, including adipose tissue dysfunction with enlarged adipocytes and decreased adiponectin. An in vitro adipocyte system mirroring the tissue of origin is crucial to investigate PCOS-specific adipose dysfunction and to test potential new therapies. We collect white subcutaneous adipose tissue from hyperandrogenic and insulin resistant women with PCOS and controls. From isolated cells, we established free-floating adipose spheroids (3D) and single-layer (2D) adipocytes by in vitro differentiation.

To characterise PCOS-specific disease signatures in vitro, we performed bulk RNA sequencing and lipidomics, and assessed differences in lipid accumulation, sex steroid secretion and glucose uptake. Despite being cultured identically to controls, both 2D and 3D PCOS cultures preserve a larger lipid droplet size. The 2D adipocytes from women with PCOS exhibit differential expression of genes related to cellular respiration. Spheroids derived from PCOS have lower glucose consumption when compared to spheroids derived from healthy controls. In addition, estrone and estradiol levels secreted by adipocytes were lower in PCOS spheroid-conditioned media. Lipidomics analysis of adipose spheroids also showed higher levels of atypical ceramides in PCOS.

Next, we plan to profile the secretome and extracellular vesicles in the media, and compare the transcriptomic data with freshly isolated mature adipocytes as well as with our single nuclei RNA sequencing data to cross-validate our models.

P65. Molecular dissection of GCGR signaling uncovers pathway rewiring by GCGR-targeted compounds

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The glucagon receptor (GCGR) is increasingly recognized as a promising target for the pharmacotherapy of metabolic diseases. However, realizing therapeutic benefits requires a detailed understanding of the underlying molecular and structural pharmacology. Here, we present a comprehensive, multi-level characterization of signaling elicited by clinically relevant drugs and drug candidates that include mono-, dual-, and triple GCGR agonists. Specifically, we integrate receptor-proximal signaling profiling at subcellular resolution using enhanced bystander bioluminescence resonance energy transfer (ebBRET) with downstream secondary messenger assays. We identify substantial cross-reactivity of GCGR ligands at the GLP-[1]R, underscoring an important consideration for the design of multivalent secretin therapeutics. Beyond receptor selectivity, our results demonstrate that GCGR-targeting ligands with near-physiological intrinsic activity preferentially bias signaling toward the canonical Gs pathway. We further show that promiscuous agonists exhibit distinct subcellular signaling patterns characterized by a relative shift toward inhibitory Gi/o pathway engagement, highlighting the importance of signaling analyses with high spatial subcellular resolution. These compartment-resolved analyses further reveal that enhanced Gi/o signaling can override stimulatory Gs activity, thereby shaping the downstream second messenger responses.

P66. Gut microbiota-derived dinitrosyl-iron complexes (DNIC): Microbial synthesis and NO-independent signaling pathways

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The intricate relationship between gut bacteria and host physiology is a rapidly expanding field of research. Dinitrosyl-iron complexes (DNIC), stable nitric oxide-related species, are potent signaling molecules with significant cardiometabolic implications. Our initial findings revealed DNIC in conventional, but not germ-free, mouse tissues, strongly implicating gut microbiota. Therefore, our study aimed to elucidate how gut microbiota generate DNIC from inorganic nitrate and non-heme iron, confirm host-derived nitrate's contribution, and characterize DNIC's NO-independent sGC-cGMP signaling. Using EPR, we showed in vitro that mixed fecal microbiota and wild-type E. coli generated DNIC from nitrate/non-heme iron, and this process was dependent on bacterial nitrate reductase. Furthermore, pharmacological inhibition of host nitric oxide synthase activity in vivo significantly reduced liver DNIC levels and plasma nitrate/nitrite, indicating that host NOS-derived nitrate contributes to fueling microbiota-dependent DNIC formation. Also, DNIC-thiosulfate activated cGMP signaling and released only minimal amounts of free NO. In conclusion, nitrate reductase-expressing gut bacteria generate bioactive DNIC from dietary and host NOS-derived nitrate/non-heme iron. These microbiota-derived DNIC, acting as distinct $Fe(NO)_2$ entities, activate sGC-cGMP signaling independently of free NO. This novel understanding highlights their potential as a therapeutic target for cardiometabolic health, with direct implications for diabetes and metabolic disorders.

P67. Gut microbiota generate dinitrosyl-iron complexes with cardiovascular and metabolic benefits

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Gut bacteria influence host physiology through interactions with endogenous and dietary compounds, yet the underlying mechanisms remain poorly understood. Here, we uncover a previously unrecognized metabolic pathway in which gut bacteria convert endogenous and dietary inorganic nitrate and non-heme iron into bioactive, mobile dinitrosyl-iron complexes (DNIC), which are absorbed and accumulate in host tissues in a protein-bound form. Using electron paramagnetic resonance, DNIC were detected in tissues of conventional but not germ-free mice. In vitro, mouse and human faeces and E. coli produced DNIC from nitrate and iron-citrate, whereas a nitrate reductase–deficient mutant failed to do so. Dietary supplementation with nitrate plus iron-citrate, or with synthetic DNIC, increased tissue DNIC levels and ameliorated cardiometabolic dysfunction in Western diet-fed mice. In HepG cells and human hepatocyte spheroids, DNIC attenuated free fatty acid-induced steatosis. Our data suggest that DNIC bioactivity is mediated by the Fe(NO)entity, but not free NO. DNIC bioactivity involves activation of sGC, inhibition of leucine uptake, and normalization of mTORC signaling. Dietary modulation of microbiota-derived DNIC formation may represent a novel strategy to support cardiovascular and metabolic health.

P68. β-cell–targeted RNA activation of VEGF-A preconditions islet grafts to enhance vascular integration and functional outcomes

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Background and Aims

Islet transplantation is constrained by a post-engraftment avascular window that exposes the islet core to hypoxia. We investigated whether pre-transplant transcriptional VEGF-A activation, decoupled from stress signalling, improves graft vascular and functional outcomes.

Methods

Aptamer–VEGF-A saRNA chimeras were applied ex vivo to mouse and human islets (24h) before syngeneic transplantation. Outcomes included intravital vascular imaging, LC3 autophagic profiling, marginal-mass glucose restoration, and STZ challenge.

Results

Chimera treatment induced robust, transient VEGF-A upregulation without engaging hypoxia or stress programmes. Primed grafts achieved perfused vasculature one week earlier than controls, with faster autophagic stress resolution and unchanged final vascular density. In marginal-mass models, primed grafts restored normoglycemia more effectively and delayed STZ-induced hyperglycemia with preserved islet architecture. Efficacy was confirmed across multiple human donor preparations.

Conclusions

Ex vivo RNA priming accelerates vascular integration and reduces engraftment stress in mouse and human grafts. Requiring no genomic modification and applicable across donors and species, this strategy may reduce islet requirements and improve scalability of cellular diabetes therapy.

P69. Pancreatic exocrine spheroids engrafted in the anterior chamber of the eye (ACE) - development of a novel platform to study pancreatic intra-organ crosstalk

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The endocrine and exocrine pancreas exert distinct functions despite cohabiting the same organ. For this reason, they have been long studied separately by different medical fields. Accumulating evidence shows that these two tissues strongly influence each other, also due to their vascular interconnection. However, longitudinal studies on the pancreas are limited by lack of valuable in vitro models, the fast loss of acinar phenotype upon isolation, and optical inaccessibility of the organ in vivo. In our work, we mimic the exocrine-endocrine crosstalk outside the pancreas by transplanting acinar-derived spheroids together with pancreatic islets into the anterior chamber of the mouse eye. By transplanting exocrine spheroids after days in culture, they get vascularized, regain their acinar morphology, and can be non-invasively monitored overtime by confocal microscopy. Characterization performed on explanted grafts with immunofluorescence and TEM shows amylase expression, cell granularity and polarization, and presence of a common lumen in the acinus center. Overall, this novel approach has the potential to overcome major limitations on pancreatic research by enabling longitudinal and non-invasive studies in an in vivo setup, refining animal usage and allowing for microvasculature and functionality studies by implementation of fluorescent probes. Additionally, morphological and functional changes may be detected upon administration of xenobiotics in toxicological studies or upon induction of pathological conditions in the experimental animal, i.e. diabetes or pancreatitis.

P70. Stable intracranial imaging of dura mater-engrafted pancreatic islets in awake mice

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Traditional intravital imaging of pancreatic islets is often limited by restricted optical access and the physiological effects of anesthesia. To address these constraints, we developed a microscopy platform in which pancreatic islets are engrafted onto the mouse dura mater. Combined with a cranial window, an air-cushioned floating arena, and stable head fixation, this configuration provides mechanical stability compatible with continuous single-cell calcium (Ca^{2+}) imaging for up to minutes in awake mice.

Intracranial islet grafts integrated with the host vasculature and displayed morphological associations with neural structures. Engrafted human islets secreted C-peptide in response to glucose stimulation, demonstrating functional integration into systemic glucose metabolism.

Using this preparation, we quantified anesthesia-associated alterations in capillary blood flow and islet Ca^{2+} dynamics. In awake mice, the system enabled monitoring of physiological responses to subcutaneous glucose administration. Within insulin-secreting β-cells, intracellular Ca^{2+} oscillations showed dynamic changes in signal amplitude, oscillation period, and plateau fraction, while overall cellular network coordination remained stable.

This dura mater engraftment model provides a stable optical interface for in vivo imaging of endocrine tissue. The approach enables high-resolution analysis of islet physiology in awake animals while minimizing confounding effects of anesthesia.

P71. Hyperglycemia deteriorates human β cell exocytosis by exaggerating CaVchannel-calcineurin-HSFsignaling

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Mechanisms whereby hyperglycemia and impaired glucose-stimulated insulin secretion (GSIS) reciprocally aggravate each other in diabetes development have been poorly understood. We now show that human islets survived with satisfactory vascularization and light scattering signals when grafted into the anterior chamber of the eye (ACE) of immunodeficient mice. Streptozotocin induced hyperglycemia by destroying β cells in recipient pancreata without influencing intracameral human β cells. The CaVchannel blocker NNC 55-alleviated hyperglycemia-induced events including in vivo reduction in backscattered light from intracameral grafts, exaggeration of CaVchannels and impairment of capacitance responses in β cells within these grafts retrieved intact. NNC 55-and/or the calcineurin inhibitor tacrolimus similarly counteracted hyperglycemia-induced HSFcytoplasmic retention and VAMP-downregulation. Our findings demonstrate that hyperglycemia deteriorates human β cell exocytosis by exaggerating CaVchannel-calcineurin-HSFsignaling that at least in part mechanistically accounts for reciprocal aggravation of hyperglycemia and impaired GSIS.

P72. Maturation of insulin-expressing cells within human embryonic stem cell-derived islets in the anterior chamber of the eye of immunodeficient mice

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Background and aims: In-vitro generated human embryonic stem cell-derived islets (ESC-islets) offer a renewable source for diabetes therapy. However, they remain functionally immature and require an in vivo milieu to achieve functional maturation. This study aims to explore the maturation of insulin-expressing cells within ESC-islets in the anterior chamber of the eye (ACE) of immunodeficient mice.
Methods: We transplanted ESC-islets into ACE of NSG mice and used immunostaining, confocal microscopy, patch-clamp, blood glucose monitoring, C-peptide assay and IPGTT in this study.
Results: ESC-islets grafted into the ACE gradually underwent rich vascularization and gave intense insulin-granule-derived backscatter signals during post-transplantation. Intracameral ESC-islet grafts displayed islet hormone profiles similar to native human islets and expressed the β cell maturation marker UCN^3. These grafts released human insulin to maintain normoglycemia and glucose tolerance in recipient mice treated with STZ. Their insulin-expressing cells expressed functional KATP, CaV, Na+, and K+ channels. Importantly, insulin-expressing cells within ESC-islet grafts retrieved from the ACE exhibited action potential firing in response to glucose stimulation, but insulin-expressing cells within pre-transplanted ESC-islets did not.
Conclusion: The immunodeficient mouse ACE serves as a unique, profitable niche for ESC-islet maturation. In this niche, intracameral ESC-islet grafts become well able to maintain glucose homeostasis.

P73. Proteome profiling of plasma and extracellular vesicles to identify biomarkers for enterovirus induced type diabetes

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While the mechanisms underlying the destruction of pancreatic beta cells in type diabetes (T1D) remain incompletely understood, the triggering of islet autoimmunity by enteroviral infections has been suggested to be involved. Biomarkers of early infection thus hold great potential for personalised disease interventions. Extracellular vesicles (EVs), secreted by all cells – including beta cells – and detectable in various body fluids such as plasma, may provide powerful, minimal invasive means to detect biomarkers of beta cell damage and the earliest stages of T1D.

Here, we performed proteome profiling of plasma and EV-enriched plasma fractions from an experimental model of enterovirus-induced T1D, using HiRIEF or data-independent acquisition LC-MS/MS. Mock-infected animals served as controls.

Profiling both plasma and plasma-derived EV fractions expanded the detectable protein repertoire, thereby enhancing our ability to uncover early disease-related markers. More than and unique proteins were detected in plasma and plasma-derived EVs, respectively, with around 50% overlap. Of the proteins found in EVs, over were detected in a separate analysis on a new cohort of samples using a complementary LC-MS/MS methodology. A comparison between samples from infected and uninfected animals showed widespread changes in protein expression upon virus infection, with many differentially expressed proteins enriched in immune- and defence-related pathways, demonstrating the method’s ability to detect infection-specific alterations in the proteome.

P74. Spatially Resolved Transcriptomic Profiling Identifies Fibrin-Driven Trophoblast Arrest in Placentas of Mothers with Type Diabetes

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In utero exposure to maternal type diabetes (T^1D) significantly increases offspring cardiometabolic risk. As the maternal-fetal interface, the placenta is central to this developmental programming, yet its molecular and structural alterations under T^1D remain poorly defined. While single-nucleus RNA sequencing (snRNA-seq) profiles molecular landscapes, it fails to capture acellular pathological features like perivillous fibrin deposition. To overcome this, we integrated snRNA-seq with HAPPY, a deep-learning algorithm that infers cell types and tissue architecture from H&E-stained images. HAPPY's predictions showed high concordance with snRNA-seq, retrieving critical spatial context lost in transcriptomics.

The physiological differentiation from cytotrophoblasts (CTB) to syncytiotrophoblasts (STB) is frequently arrested in pathological microenvironments, manifesting as delayed villous maturation. This arrest is strongly associated with ectopic fibrin deposition, though the underlying mechanisms remain unclear. By leveraging the in situ multi-modal mapping enabled by snRNA-seq and HAPPY, we aim to investigate whether the T^1D-induced adverse microenvironment drives spatial differentiation arrest in trophoblasts and how this relates to fibrin deposition. Furthermore, we seek to elucidate the specific transcriptomic alterations within these arrested CTB populations, with the ultimate goal of identifying novel molecular biomarkers for perivillous fibrin deposition and T^1D-associated placental dysfunction.

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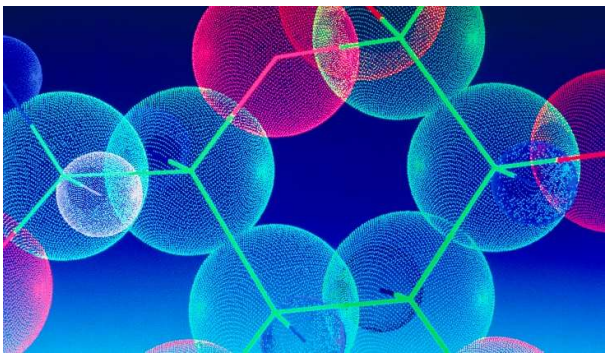

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