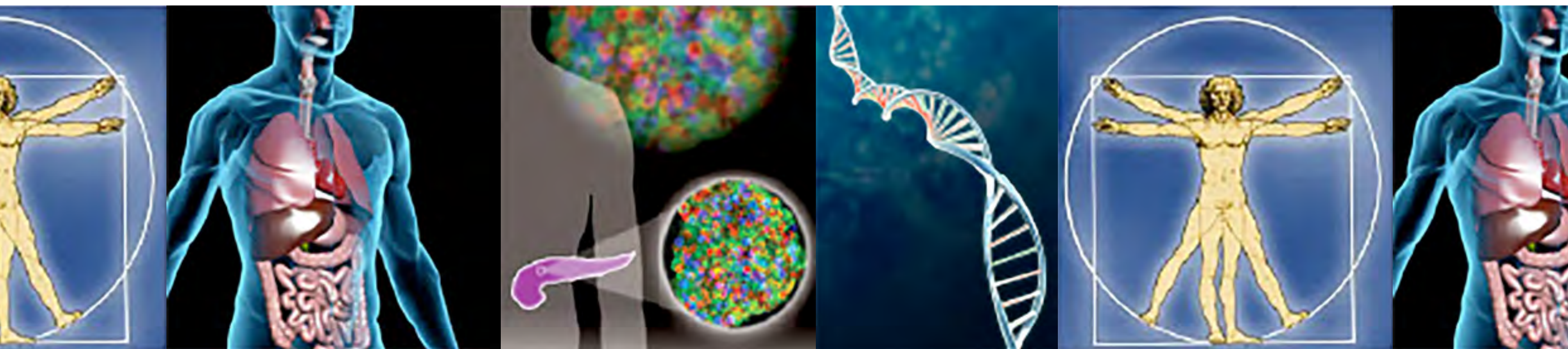
SRP DIABETES | ENDOMET | METENDO

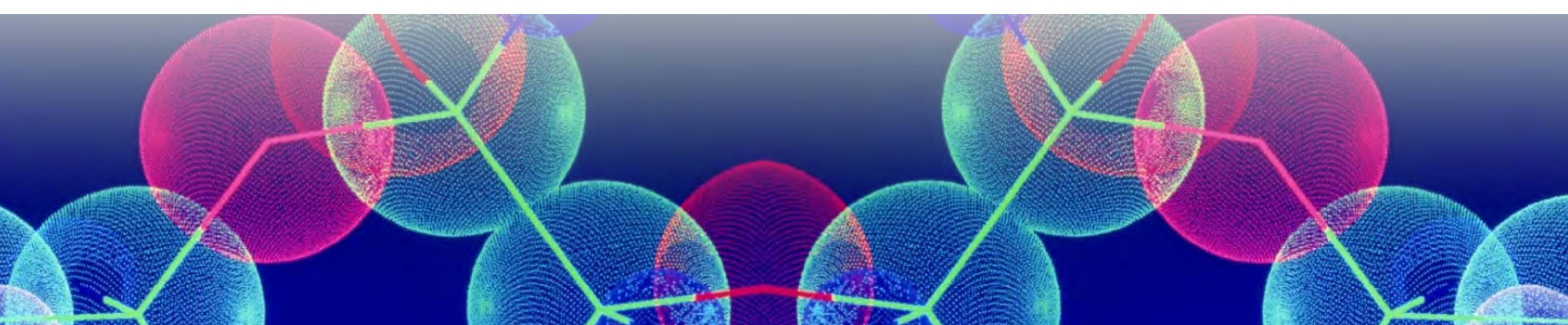
10 YEARS Joint Retreat ANNIVERSARY



Program and Abstracts

November 15–16, 2021

Vår Gård Conference Center



Venue information

Venue:

Vår Gård
Ringvägen 6
SE-133 80 SALTSJÖBADEN
TEL: +46(0)8-748 77 00
www.vargard.se

Travel Information

With Saltsjöbanan

From Stockholm central/T-centralen take the metro south to Slussen. Then follow the signs to "Ersättningsbuss" Saltsjöbanan. You take the bus 25 or 25B two stops from Slussen to Henriksdal and from there continue with the train to Saltsjöbaden (end station). From the station you can see a red brick building on the other side of the tracks. The walk to the reception takes just a couple of minutes.You can find the time-table for Saltsjöbanan at: www.sl.se

By car

Take highway 222 east (Värmdöleden) and make a right on the exit to Saltsjöbaden (highway 228). Once you are in Saltsjöbaden follow the signs to Ångbåtsbryggan and Vår Gård Saltsjöbaden.

GPS coordinates

Lat: N 59° 16.825
Long: E 018° 18.653

By bicycle

For those of you who want to take your bikes to the venue please contact the Reception at Vår Gård upon arrival for information about where to take a shower.

Poster session - Best Poster Awards

Posters will be on display just outside the seminar hall on screens that are **1.85 m high and 0.88 m wide**. Materials to attach with will be available. Posters should be mounted in the morning or lunchtime on the first day, November 15. The poster numbers will be given in the abstract book (printed copies handed out at the meeting plus sent electronically in advance). The number will designate the location of the poster board. Posters can be left on the poster screens during the whole meeting, where after they need to be removed.

November 15, 17.00 – 19.00 Poster presentations
Posters will be shown in two consecutive sessions; in the first session from 17.00 – 18.00 posters with odd numbers will be presented and in the second session from 18.00 – 19.00 posters with even numbers will be presented. This will allow the presenters to see the other posters.
Please Note: Poster presenters are expected to prepare a brief 3 min oral presentation.

Poster presenters eligible for the **poster awards** are PhD students and postdocs. A committee will evaluate the eligible abstracts/posters for best posters in two different categories:

Best poster for completed work
Best poster for most promising project

Awards will be announced during the dinner on November 15.

See page 26 and onwards for poster numbers and abstracts

Wifi

There is a free wifi available at Vår Gård: VAR-GARD-GUEST

No password is needed, just accept when prompted and you should have access to internet.

Best short talk award

As always, it's the **short talks by postdocs and PhD students that are eligible** for the best short talk award who are listed below as well as highlighted with *[VOTE BSTA]* in the program.

We will use the same online voting system as we used last year (voxvote), meaning that **you need a smartphone/computer connected to the internet to vote** (wifi at the venue: VAR-GARD-GUEST).
Info on link and pincode for the voting system will be given at the meeting. The voting takes place at the end of the meeting.

The best short talk award will be handed out at the concluding remarks on Tuesday November 16.

Eligible speakers for short talk awards (make notes for your voting at the end)

Monday November 15

Session 1: Skeletal muscle, exercise, metabolism

11.00 - 11.15 Jonathon Smith "*HES1 is an insulin- and exercise- induced transcription factor that regulates skeletal muscle glucose metabolism"*

11.20 - 11.35 Zhengye Liu "*Altered mitochondrial function in skeletal muscle afflicted by peripheral artery disease"*

Tuesday November 16

Session 6: Immune system, metabolic disease, adipose tissue

09.35 - 09.50 Angelo Ascani "*The role of B cells in immune cell activation in Polycystic Ovary Syndrome"*

Session 7: Liver, lipids

11.25 - 11.40 Patricia Recio-López *"Improvement of the metabolic syndrome by lowering apolipoprotein CIII with siRNAs"*

13.00 - 13.15 Oihane Garcia-Irigoyen "*Liver-specific deletion of the corepressor GPS2 alleviates atherosclerosis and dyslipidemia"*

Session 8: Diabetes complications

13.40 - 13.55 Julia Sánchez-Ceinos "*Targeting histone methyltransferase EZH2 attenuates hyperglycaemia-induced oxidative stress and inflammation in human endothelium"*

14.00 - 14.15 Sofie Eliasson Angelstig *"Repression of Hypoxia-Inducible Factor-1 Contributes to Increased Mitochondrial Reactive Oxygen Species Production in Diabetes"*

Program

Monday November 15

09.15 Arrival on your own to the venue Vår Gård.

Coffee + sandwich, mounting posters

[Note: time given for all talks below include 5 min for questions.]

10.00 - 10.20 Welcome note: Anna Krook (SRP Diabetes), Håkan Karlsson (SRP Diabetes), Marie Björnholm (MetEndo)

Session 1: Skeletal muscle, exercise, metabolism Chair: Juleen Zierath

10.25 - 10.55 John Kirwan (Pennington BRC) *"Metabolic Diseases – Solutions from Cells to Society"*

11.00 - 11.15 Jonathon Smith *"HES1 is an insulin- and exercise- induced transcription factor that regulates skeletal muscle glucose metabolism" [VOTE BSTA]*

11.20 - 11.35 Zhengye Liu *"Altered mitochondrial function in skeletal muscle afflicted by peripheral artery disease" [VOTE BSTA]*

11.40-12.00 Coffee Break

12.00 - 12.15 Jorge Correia *"Zfp697 is a novel regulator of skeletal muscle inflammation and regeneration"*

Session 2: Rolf Luft Grants for Instrumentation Chair: Anna Krook

12.20 - 12.40
Myriam Aouadi lab *"Digital droplet QPCR"*
Mattias Carlström lab, *"INALYZER: High Resolution DXA Body Composition Analyzer"*
Carolina Hagberg lab *"ProteinSimple capillary Jess western blot system"*
Kirsty Spalding lab *"Biorep Perifusion integrated system"*

13.00-14.20 Lunch + mounting posters + check-in

Session 3: Targeting beta cells, type 1 diabetes Chair: Malin Flodström Tullberg

14.20 - 14.50 Mikael Knip (U Helsinki) *"Primary prevention of type 1 diabetes: challenges and hopes"*

14.55 - 15.10 Dimitri Van Simaeys *"β-cell-specific Aptamer, Vegf-A targeting small activating RNA conjugates improves vascularisation speed in the anterior chamber of the eye"*

15.15 - 15.45 Lena Eliasson (Lund U) *"Human islet microRNAs and their role in the pathogenesis of type 2 diabetes."*

15.45-16.00 Break

Session 4: Targeting brain in type 2 diabetes (digital) Chair: Kirsty Spalding

16.00 - 16.30 Lora Heisler (U Aberdeen) ***"****Targeting brain circuits to improve obesity and type 2 diabetes"*

Session 5: Scientific Publishing Chair: Johanna Lanner

16.35 - 16.50 Isabella Samuelson (Nature Metabolism) *"Careers in scientific publishing"*

17.00 - 19.00 Poster Session with refreshments served

Posters are on display in the area just outside the seminar hall and poster presenters are expected to stand by their posters in two consecutive sessions; in the first session from 17.00 - 18.00 posters with odd numbers will be presented and in the second session from 18.00-19.00 posters with even numbers will be presented. This to allow also the presenters to see all posters. *[Note: Poster presenters are expected to prepare a brief 3 min oral presentation].*

19.30 Dinner and activities – Best Poster Awards

Tuesday November 16

07.00-09.00 Breakfast + check out from your rooms

Session 6: Immune system, metabolic disease, adipose tissue Chair: Peter Arner

09.00 - 09.30 Anthony Ferrante (Columbia U) *"An Immune-Metabolic Interface"*

09.35 - 09.50 Angelo Ascani "*The role of B cells in immune cell activation in Polycystic Ovary Syndrome" [VOTE BSTA]*

09.55 - 10.10 Kirsty Spalding "*Fat cell contribution to metabolic disease in humans"*

10.15-10.45 Coffee Break

10.45 - 11.00 Anna Benrick (U Gothenburg) *"Adiponectin drives hyperplastic expansion and beiging of brown and white adipose tissue in mice"*

Session 7: Liver, lipids Chair: Sara Straniero

11.05 - 11.20 Valerio Azzimato "*Hepatic miR-144 drives fumarase activity preventing NRF2 activation during obesity"*

11.25 - 11.40 Patricia Recio-López *"Improvement of the metabolic syndrome by lowering apolipoprotein CIII with siRNAs" [VOTE BSTA]*

11.40-13.00 Lunch + networking

13.00 - 13.15 Oihane Garcia-Irigoyen "*Liver-specific deletion of the corepressor GPS2 alleviates atherosclerosis and dyslipidemia" [VOTE BSTA]*

Session 8: Diabetes complications Chair: Mikael Rydén

13.20 - 13.35 Vladimer Darsalia "*Normalisation of glucose metabolism by Exendin-4 in the chronic phase after stroke promotes functional recovery in male diabetic mice"*

13.40 - 13.55 Julia Sánchez-Ceinos "*Targeting histone methyltransferase EZH2 attenuates hyperglycaemia-induced oxidative stress and inflammation in human endothelium" [VOTE BSTA]*

14.00 - 14.15 Sofie Eliasson Angelstig *"Repression of Hypoxia-Inducible Factor-1 Contributes to Increased Mitochondrial Reactive Oxygen Species Production in Diabetes" [VOTE BSTA]*

14.20-14.40: Concluding remarks and short talk award (by voting online)
Anna Krook (SRP Diabetes), Mats Rudling (EndoMet), Sara Straniero (EndoMet) and Stefan Nobel (SRP Diabetes)

Departure

Speaker Abstracts

Session 1: Skeletal muscle, exercise, metabolism

Metabolic Diseases – Solutions from Cells to Society

John Kirwan
Pennington Biomedical Research Center, Louisiana State University

Type 2 diabetes is a disease that affects over 450 million people worldwide. Furthermore, it is a leading cause of death driven primarily by increased risk of cardiovascular, liver, and renal disease, as well as numerous forms of cancer. The dramatic rise in prevalence and length of duration of diabetes is associated with an economic burden that exceeds trillions of dollars. Despite the scale of the problem our fundamental understanding of the cellular and molecular mechanisms of the disease itself remains incomplete. Consequently, treatment strategies are limited and those that are available, including medications, lifestyle, and bariatric surgery, have either modest efficacy, are not sustainable, or are viewed as too radical.

Our research is addressing fundamental questions related to the underlying basis for insulin resistance with a focus on skeletal muscle mitochondria in obesity and type 2 diabetes. Mitochondria are essential regulators of energy in all organs and play a critical role in controlling the rate of nutrient utilization. The traditional view of mitochondria as isolated, spherical, energy producing organelles, is evolving based on emerging data that show mitochondria form a dynamic reticulum of networked tubules that are regulated by cycles of fission and fusion. The discovery of a number of proteins that regulate these activities has led to significant advances in understanding human disease and our data support a role for mitochondrial fission in obesity-related insulin resistance. Since much of the diabetes epidemic is linked to obesity and can be attributed to an imbalance in nutrient supply and demand, mitochondrial dynamics may hold an important key to discovering novel treatment targets for managing patients with the disease.

Based on recent advances in small molecule screening and biochemical engineering we have begun to investigate mitochondrially targeted agents including mitochondrial uncouplers for the treatment of metabolic disease. Today, we will discuss the potential role of BAM15, a mitochondrial-targeted weak lipophilic acid with protonophore activity, as a pharmacologic strategy for the treatment of obesity and hyperglycemia. Our data suggests that BAM15 is safe, tolerable, and efficacious and has therapeutic potential for the treatment of obesity and associated comorbidities.

We will also discuss clinical data from the ongoing Alliance of Randomized Trials of Medicine versus Metabolic Surgery in Type 2 Diabetes (ARMMS-T2D) consortium which assessed the durability and longer-term effectiveness of metabolic surgery compared to medical/lifestyle management in patients who were overweight and had obesity and type 2 diabetes. Three-year follow-up of this largest cohort of randomized patients followed to date, demonstrates that metabolic/bariatric surgery is more effective and durable than medical/lifestyle intervention in remission of type 2 diabetes, including among individuals with class 1 obesity, for whom surgery is not widely used.

Finally, how do we convince society and the public at large to do something about the enormous health burden of obesity and diabetes? To address this problem, Pennington Biomedical recently launched the “Obecity, USA” awareness and advocacy campaign which reframes the obesity narrative and aims to effectively curb the obesity epidemic by 2040. We will discuss what is “Obecity, USA” and what do the early awareness metrics look like.

HES1 is an insulin- and exercise- induced transcription factor that regulates skeletal muscle glucose metabolism

Jonathon A.B. Smith[1], Nicolas J. Pillon[1], Julie Massart[2], David Rizo-Roca[2], Michael S. Kuefner[2], Thais de Castro Barbosa[1], Ahmed M. Abdelmoez[2], Brendan M. Gabriel[1], Erik Näslund[3], Anna Krook[1], and Juleen R. Zierath[1,2]
[1]*Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*
[2]*Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden*
[3]*Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden*

Introduction
In human skeletal muscle, insulin and exercise reportedly stimulate transcription factor, Hairy and Enhancer of Split 1 (*HES1*). Yet, the metabolic role of HES1 remains largely unexplored. We hypothesised that altering HES1 expression would affect glucose metabolism in differentiated skeletal muscle.
Methods and results
Meta-analysis of the skeletal muscle transcriptomic response to hyperinsulinaemic-euglycaemic clamp confirmed *HES1* upregulation by insulin (1.5-fold), in human *vastus lateralis* muscle. Accordingly, HES1 increased during a 2-h oral glucose tolerance test (OGTT) in the skeletal muscle of men with normal glucose tolerance or type 2 diabetes. The magnitude of change in *HES1* mRNA was attenuated in individuals with type 2 diabetes (2.3 *vs.* 3.7-fold); however, no difference in HES1 protein abundance was observed between groups. A 56-fold overexpression of *Hes1* in mouse *tibialis anterior* enlarged muscle weight (+9%), without altering glycogen content, and improved glucose uptake (+16%) during a modified OGTT, compared to contralateral control. In primary human skeletal muscle myotubes, an 11-fold lentiviral overexpression of *HES1* increased glucose oxidation (+14%) and lactate release (+11%). Conversely, also in primary human myotubes, small interfering RNA silencing of *HES1*, by 46%, decreased glucose uptake (-17%), oxidation (-10%), and incorporation into glycogen (-17%).
Thirty minutes of cycling exercise, at 85% of maximum heart rate, transiently induced skeletal muscle *HES1* to similar extents in men with normal glucose tolerance or type 2 diabetes; which may be a consequence of AMP-activated protein kinase or calcium signalling, as suggested by increased *HES1* mRNA after electrical pulse stimulation (1.5-fold), and AICAR (3.3-fold) or ionomycin exposure (11.9-fold), in primary human myotubes.
Conclusion
Upregulation of HES1 may mediate downstream effects of insulin and exercise on glucose metabolism in differentiated skeletal muscle. As such, interventions that increase HES1 could represent viable strategies for the improvement of skeletal muscle metabolic health.

Altered mitochondrial function in skeletal muscle afflicted by peripheral artery disease

Liu Zhengye, Baptiste Jude, Dinah Mous, Johanna T Lanner
Department of Physiology & Pharmacology, Karolinska Institutet, Stockholm, Sweden

Aims: Subjects with obesity and type II diabetes (T2D) have a higher risk to develop skeletal muscle ischemic disease, e.g. peripheral artery disease (PAD). PAD lowers blood flow to the lower limbs, causing debilitating skeletal muscle myopathy. Interventions that improve distal arterial pressures (ie. bypass surgery) generally fail to normalize the functional performance of muscle indicating pathophysiological mechanisms inside the skeletal myofibers that reduce overall muscle function. Here we aim to elucidate the role of the mitochondrial function in PAD-induced muscle dysfunction.
Methods: Unilateral femoral artery ligation (FAL), a mouse model of PAD, was induced in C57BL6 mice on a normal chow diet (ND) or a high-fat diet (HFD) for eight weeks before FAL.
Results: In normal mice, FAL resulted in skeletal muscle weakness and the weakness was exacerbated further in muscle from obese mice. Besides, FAL-induced fibrosis and ectopic fat accumulation were also worsened in muscle from obese mice. Our RNA-sequencing results showed that mitochondrial gene expressions were reduced in muscle from ND-FAL legs, while the reduction was attenuated in HFD-FAL legs. Mitochondrial assembly and cellular respiration were identified as the top suppressed pathway in ND-FAL legs, but not in HFD mice. Fibrosis, fat metabolism, and myosin heavy chain isoforms were amongst the top variable genes in control and FAL muscle from normal and obese mice. Applying deconvolution using CIBERSORTx on our global RNA-seq data identified that HFD alone induced an inflammatory response in control muscles with an altered proportion of CD4+ and CD8+ T cells. Moreover, FAL induced an increased expression of Cytl1+ and Fabp4+ endothelial cell populations, as well as fibroblast and tenocytes, but no difference was observed between diets (i.e ND-FAL and HFD-FAL).
Conclusion: FAL-induced muscle weakness was exacerbated by eight weeks of HFD. Genes involved in mitochondrial assembly and cellular respiration are highly affected by FAL while the response is attenuated in obese mice. Therapeutical interventions to enhance mitochondrial health appear as a promising target to improve muscle function in PAD associated with obesity and T2D.

Zfp697 is a novel regulator of skeletal muscle inflammation and regeneration

Jorge C. Correia[1], Paulo R. Jannig[1], Vicente Martínez-Redondo[1], Liu Zhengye[2], Johanna T. Lanner[2], Paul Gregorevic[3], and Jorge L. Ruas[1]

[1] *Molecular & Cellular Exercise Physiology, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.*
[2] *Molecular Muscle Physiology and Pathophysiology, Department of Physiology and Pharmacology, Biomedicum, Karolinska Institutet, 17165 Stockholm, Sweden.*

[3] *Baker Heart and Diabetes Institute, Melbourne, Australia.*

Loss of skeletal muscle mass and function constitutes the major clinical feature of several neuromuscular diseases and is commonly observed in many other clinical settings, such as long-term bed rest, cancer, chronic inflammatory disease, and even aging. The interplay between muscle and the immune system is critical for muscle function and repair, and poor control over immune cell recruitment and activity greatly contributes for muscle dysfunction in a plethora of pathophysiological settings. However, the mechanisms that orchestrate immune cell recruitment to muscle remain poorly understood. In this study, we identify Zfp697, an uncharacterized protein of unknown function, as a damage-induced regulator of muscle inflammation and regeneration. Our results show that Zfp697 is rapidly induced in mouse and human skeletal muscle by damage-inducing stimuli and activates a broad inflammatory gene program, associated with immune cell recruitment and function. To assess the role of Zfp697 in muscle regeneration, we subjected skeletal muscle-specific Zfp697 knock-out mice to a hindlimb unloading/reloading protocol, designed to mimic a scenario of disuse-induced muscle atrophy and subsequent recovery upon reloading. Zfp697 ablation had a profound effect on the regenerative transcriptional response to reloading, with impaired activation of gene programs associated with inflammation, extracellular matrix remodeling, angiogenesis and cell proliferation. This was accompanied but impaired recovery of muscle mass upon reloading. Likewise, $Zfp697^{mKO}$ mice showed impaired recovery of muscle strength following a single bout of damage-inducing downhill running. Lastly, we used an adeno-associated viral vector to achieve body-wide and sustained Zfp697 gene delivery to muscle. Strikingly, mice that received a single injection of AAV6-Zfp697 showed improved muscle strength and running performance. Together, our data indicate that Zfp697 is an important regulator of muscle regeneration that can be targeted to improve muscle function.

Session 3: Targeting beta cells, type 1 diabetes

Primary Prevention of Type 1 Diabetes: Challenges and Hopes

Mikael Knip
Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital; Research Program for Clinical and Molecular Medicine, Faculty of Medicine, University of Helsinki

Primary prevention of type 1 diabetes (T1D) aimed at preventing the initiation of the disease process leading to clinical T1D would be the most effective way to reduce the incidence and burden of T1D, if successful. The appearance of diabetes-associated autoantibodies is the first detectable sign of the initiation of the T1D disease process. From birth cohort studies we have learned that the first autoantibodies may appear already during the first year of life and a there is a clear peak in the autoantibody seroconversion rate during the first 3 years. These observations underline that preventive measures have to be started very early in life. Recent studies have also shown that T1D is a heterogenous disease with different endotypes. One of the endotype classifications is based on which autoantibody emerges first. Insulin autoantibodies (IAA) are the first autoantibodies in 30-40% of the cases and a similar proportion develops GAD antibodies (GADA) first. Coxsackie B viruses (CBV) are a strong candidate for being the exogenous trigger of the disease process in the "IAA first" endotype. A pentavalent vaccine including five CBV serotypes is currently tested in a phase 1 trial in healthy adults in Finland. In the best scenario such a vaccine could become available for prevention of T1D within the next 6 years. Reduced levels of phosphatidylcholines and sphingomyelins in cord blood have been observed to be associated with rapid progression of T1D in the offspring. Milk fat globule membrane is rich in such lipids and could be given to pregnant women and/or offspring in infancy. Progression to clinical T1D is associated with a reduced diversity of the intestinal microbiota and a decreased relative abundance of beneficial bacteria, Bifidobacterium longum subsp. infantis, in particular. An international multicenter trial (SINT1A) with early supplemention with this species has been started in April this year in infants with a 10% risk of progressing to clinical T1D. There is hope that T1D will become a preventable disease, but there is also a series of challenges in the field. Who should be included in the optimal target group for primary prevention, when should the treatment be started and what degree of potential harmful effects is acceptable?

β-cell-specific Aptamer, Vegf-A targeting small activating RNA conjugates improves vascularisation speed in the anterior chamber of the eye

Dimitri Van Simaeys[1], Ingo Leibiger[1], Per-Olof Berggren[1,2]
1: The Rolf Luft Research Center for Diabetes and Endocrinology, Karolinska Institutet, Karolinska University Hospital L1, SE-171 76 Stockholm, Sweden
2: Division of Integrative Bioscience and Biotechnology, Pohang University of Science and Technology, Pohang, Gyeongbuk 37673, Republic of Korea;
Diabetes Research Institute, University of Miami Miller School of Medicine, Miami, FL 33136, USA;
Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 637553, Singapore;
Center for Diabetes and Metabolism Research, Department of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, PR China.

Aims: Tissue-specific delivery of therapeutic RNA is an unmet medical need with a wide potential. By pre-treating pancreatic islets with beta cell-specific aptamer chimera, we hypothesize that vascularization of islets will improve, potentially leading to a reduced need of donor material to re-establish glycemic control in diabetic mice.
Methods: Mouse and human pancreatic islet are pre-treated with a beta-cell specific aptamer mix conjugated with VEGF-A-targeting saRNA. The engraftment is monitored using *in vivo* confocal microscopy on islets transplanted in the anterior chamber of the eye of mice. The vascularization is visualized by the use of a Dextran alexafluor 647 conjugate.
Results and discussion: The upregulation of Vegf-A can be done in both human and mouse islets, where we can observe a ten-fold and a five-fold increase of Vegf-A upregulation in mouse and human islets, respectively. In vitro results indicate that the upregulation of Vegf-A in the islet is of a transient nature that last about a week. Chimera-treated islets showed an increase in engraftment speed in mice. Human islet studies in immune-deficient mice are ongoing. These results show an exciting new avenue towards increasing the transplantation efficiency in primary islets. SaRNA, conjugated with β-cell-specific aptamer delivery shows promise to treat primary tissue as its effects are transient.

Human islet microRNAs and their role in the pathogenesis of type 2 diabetes.

Prof. Lena Eliasson
Islet Cell Exocytosis, Lund University Diabetes Centre, Dept of Clinical Sciences Malmö, Lund University, Sweden

Insulin released from β-cells within the pancreatic islet of Langerhans is central in the control of blood glucose homeostasis. A combination of environmental and genetic factors can lead to defects in this regulation and lead to type 2 diabetes (T2D). Hyperglycaemia due to reduced glucose uptake in target tissues needs to be compensated by the β-cell through increased insulin secretion. Failure of the β-cells to secrete enough insulin results in T2D. MicroRNAs are small non-coding RNAs post-transcriptionally regulating gene expression. Their ability for rapid regulation of alterations in target gene expression make microRNAs ideal in the β-cell adaptations needed during development of T2D. However, whereas changes in the expression of some microRNAs occur as a compensatory mechanism for insulin resistance others are part of the etiology of T2D. MicroRNAs are also involved in the maintenance of β-cell phenotypic identities via cell-specific, or cell-enriched expression, and some microRNAs, such as e.g., miR-29, reduce the expression of beta-cell disallowed genes. One of the most highly abundant microRNAs in the β-cell is miR-375. MiR-375 is highly involved in several cellular processes essential for maintaining the β-cell phenotypic identity. Despite the importance of miR-375, it has not been shown to be differentially expressed in T2D islets. On the contrary, other microRNAs such as miR-200c, miR-130a/b and miR-152 are deregulated in T2D islets. I will discuss human islet miRNA-mRNA networks, the involvement of microRNAs in β-cell dysfunction underlying T2D pathogenesis, and introduce how microRNAs can be involved in future treatment.

Session 4: Targeting brain in type 2 diabetes

Targeting brain circuits to improve obesity and type 2 diabetes

Lora Heisler
Chair in Human Nutrition Lead, Obesity and Food Choice Lead, Obesity and Food Choice Theme
Rowett Institute, University of Aberdeen, Foresterhill Aberdeen

The increasing prevalence of type 2 diabetes (T2D) and associated morbidity and mortality emphasizes the need for a more complete understanding of the mechanisms mediating glucose homeostasis to accelerate the identification of new medications. Recent reports indicate that obesity medication, 5-hydroxytryptamine (5-HT, serotonin)2C receptor (5-HT_{2C}R) agonist lorcaserin improves glycemic control in association with weight loss in obese patients with T2D. We examined whether lorcaserin has a direct effect on insulin sensitivity and how this effect is achieved. We clarify that lorcaserin dose-dependently improves glycemic control in a mouse model of T2D without altering body weight. Examining the mechanism of this effect, we reveal a necessary and sufficient neurochemical mediator of lorcaserin's glucoregulatory effects, via activation of brain pro-opiomelanocortin (POMC) peptides. We observed that lorcaserin reduces hepatic glucose production and improves insulin sensitivity. These data suggest that lorcaserin's action within the brain represents a mechanistically novel treatment for T2D: findings of significance to a prevalent global disease.

Session 5: Scientific Publishing

Careers in scientific publishing

Isabella Samuelson
Associate Editor, Nature Metabolism, Nature Research 2 Crinan St, London N1 9XW, UK

A scientific editor gets exposed to cutting-edge research every day. It is our job to filter the many submissions we get, enhance the research quality through review and revision, and give the published work high visibility. As scientific editors, we are at the forefront of scientific research, constantly learning, and have the opportunity to meet and engage with researchers. Simultaneously, a scientific editor is often the bearer of bad news, and our negative decisions can have serious consequences for researchers. I will give an introduction to the scientific editor role at Nature Metabolism, our responsibilities, required skills, and the perks as well as downsides to the job.

Session 6: Immune system, metabolic disease, adipose tissue

An Immune-Metabolic Interface

Anthony W. Ferrante Jr., Stephen Flaherty III, Ambar Grijlava, Xiaoyuan Xu, Kunheng Cai, Joshua Goodman

Naomi Berrie Diabetes Center
Division of Preventive Medicine & Nutrition
Vagelos College of Physicians & Surgeons, Columbia University

The immune system senses perturbations to tissues and organs and responds in ways that attempt to restore homeostasis. Classical, stereotypical immune responses that occur in nearly all tissues include the responses to infection and injury. However, the immune system also helps maintain homeostasis through tissue specific function of specialized immune cells. For example, in bone multinucleated osteoclasts (bone macrophages) degrade bone as part of a calcium-phosphate cycle without which bone become either fragile or brittle. The function of the immune system in adipose tissue has been less clear. It has been known for almost twenty years that in adipose tissue changes in metabolic state lead to large changes in the size and character of immune cells in fat. For example, while in the fat of lean individuals and animals ~ 5% of cells are adipose tissue macrophages (ATMs), in the severely obese more than 50% of cells are ATMs. How the immune system senses changes in metabolic state has remained poorly defined. In addition, the adaptive tissue-specific functions of adipose tissue immune cells are just emerging. Recently, our group found that adipocytes release lipid filled extracellular vesicles (AdExos) in a manner that is determined by metabolic state and that these AdExos regulate the migration and phenotype of immune cells in a manner that increases their ability to metabolize neutral lipids. Furthermore, the trafficking of lipid between adipocytes and macrophages appears to establish a local lipid cycle with important implications for normal function of adipose tissue in a manner that is analogous to the mineralize bone cycle. We are continuing to characterize the functions of AdExos in adipose tissue biology and their systemic effects.

The role of B cells in immune cell activation in Polycystic Ovary Syndrome

Angelo Ascani[1,3], Sara Torstensson[2,3], Sanjiv Risal[2], Haojiang Lu[2], Gustaw Eriksson[2], Congru Li[2], Sabrina Teshl[1], Joana Menezes[2], Katalin Sandor[2], Camilla I Svensson[2], Martin Helmut Stradner[1], Barbara Obermayer-Pietsch[1,4], Elisabet Stener-Victorin[2,4]

[1] *Department of Internal Medicine Medical University of Graz, Austria*

[2] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*

[3] *These authors contributed equally.*

[4] *These authors jointly supervised this work.*

Objective: Age-associated double negative (DN) B memory cells lacking surface expression of CD27 and immunoglobulin D (IgD) are associated with proinflammatory characteristics and higher disease activity in autoimmune diseases[1]. Serum levels of B-cell activating factor (BAFF), a known factor to promote survival of autoreactive B cells, are higher in women with polycystic ovary syndrome (PCOS)[2]. We here first characterized B cells phenotypes in women with and without PCOS. Thereafter we took an *in vivo* approach and transferred purified IgG extracted from serum of hyperandrogenic women with PCOS to mice to establish whether self-reactive B cells have a causal effect on the development of a PCOS-like immune profile and a reproductive and metabolic phenotype in mice.

Methods: First, we characterized the B cell development by analyzing major lineages and subpopulations based on pan B cell surface marker CD19 in serum of hyperandrogenic women with PCOS and of women without PCOS (controls). Second, we purified IgG from serum of women with PCOS or controls. The IgG was injected intraperitoneally (i.p.) on days 1, 3 and 10 into wild type (WT) female mice. Phenotypic testing started one week after last injection. Reproductive function was tested by measuring anogenital distance and estrous cyclicity. Body weight was recorded weekly and EchoMRI for analyses of body composition, followed by assessment of metabolic functions using metabolic cages and oral glucose tolerance test. 3 weeks after last injection, serum was collected for analysis of sex steroids by liquid chromatography mass spectrometry. Moreover, flow cytometric analyses of cell-surface markers were performed on whole blood, spleen, lymph node, ovary, endometrium, visceral adipose tissue, omentum for main expanded populations of lymphocytes and myeloid cells.

Results: Immunophenotypic analyses showed a significant remodeling of B cell repertoire in women with PCOS compared with controls. The frequency of age-associated double DN B memory cells lacking surface expression of CD27 and IgD was significantly higher in PCOS patients (P=0.002), with declined IgD+ B memory cells (P=0.011). There was a strong inverse correlation between IgM prevalence and androgen levels in both women with and without PCOS. Total testosterone was an independent predicting variable for IgM variability (P=0.01). Preliminary results from immune profiling of the transfer of human IgG into female WT mice showed an overall increase of DN B cells, particularly DN2 subsets with a $CD21^-$ phenotype, along with increased frequencies of active naïve cells and neutrophils, particularly in the ovaries in mice receiving the IgG from women with PCOS. Unswitched memory cells were reduced both in blood and lymph nodes. The mice injected with PCOS-IgG weigh more than control mice ($P<0.05$). No effect was seen on circulating sex steroids, anogenital distance or estrous cyclicity in PCOS-IgG mice.

Conclusions: Women with PCOS display an increased peripheral expansion of DN B cells. Exposing mice with IgG from women with PCOS rapidly induced an altered immune cell profile with an immediate increased body weight. Thus, PCOS appears to be associated with specific antigenic stimuli. The follow-up experiment with transfer of purified B cells from prepubertal hyperandrogenic mouse model into muMt- mice (B cell deficient mice) will define the overall impact of androgen exposure on B cell phenotypes. Altogether, these results indicate that PCOS may represent a state of inflammatory-cell hypersensitivity, leading to persistent antigen stimulation and chronic inflammation, which affect the adaptive immune cells resulting in remodeling of the lymphocytes.

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Fat cell contribution to metabolic disease in humans

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Many metabolic diseases, including type 2 diabetes, strongly associate with obesity, making obesity one of the major health challenges facing the world today. Obesity is usually accompanied by an increase in fat cell size. As fat cells become enlarged they begin to secrete factors which promote adipose tissue inflammation and dysfunction. The underlying mechanisms connecting increases in fat cell size (hypertrophy) and the secretion of pro-inflammatory factors are not well understood. We show that despite long being considered post-mitotic, mature human fat cells can activate a cell cycle program in association with obesity and hyperinsulinemia, with a concomitant increase in adipocyte cell size, nuclear size and DNA content. Chronic hyperinsulinemia in vitro or in humans, however, is associated with subsequent cell cycle exit, leading to a premature senescent transcriptomic and secretory profile in adipocytes. Premature senescence is rapidly becoming recognized as an important mediator of stress-induced tissue dysfunction. We demonstrate that senescent fat cells secrete factors known to be pro-inflammatory and propose that these factors drive inflammation and pathology in human adipose tissue, impacting whole body health. Using drugs currently on the market for other purposes, we show that manipulating adipocyte cell cycle entry and progression enables one to influence the formation of senescent cells. These studies identify an unappreciated aspect of human adipocyte biology, the activation of a cell cycle program in obesity and hyperinsulinemia, which could pave the way for novel treatment strategies for obesity and associated co-morbidities, such as type 2 diabetes.

Adiponectin drives hyperplastic expansion and beiging of brown and white adipose tissue in mice

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#jointly supervised this work

Adiponectin stimulates hyperplastic expansion and browning of white adipose tissue and is associated with reduced type-2 diabetes risk. In contrast, elevated adiponectin levels are associated with whiter brown adipose tissue (BAT). Here, we aimed to determine the effect of adiponectin on BAT functionality and adipose tissue hyperplasia.

Brown and white adipose tissues from adiponectin overexpressing (APNtg) mice and littermate wildtype controls, housed at room and cold temperature, were studied by histological, gene/protein expression and flow cytometry analyses. Metabolic function was studied by radiotracers and Seahorse-based technology.

APNtg BAT displayed increased proliferation prenatally leading to enlarged BAT. Postnatally, APNtg BAT turned whiter than control BAT. Furthermore, elevated adiponectin augmented the sympathetic innervation/activation within adipose tissue. APNtg BAT displayed reduced metabolic activity and reduced mitochondrial oxygen consumption. In contrast, APNtg IWAT displayed enhanced metabolic activity. These metabolic differences between genotypes were apparent also in cultured adipocytes differentiated from BAT and IWAT stroma vascular fraction. Furthermore, mesenchymal stem cell-related genes were upregulated along with an increased number of Lineage$^-$Sca1$^+$CD34$^-$ “beige-like” adipocyte precursor cells in both APNtg BAT and IWAT. We therefore propose that the seemingly opposite effect of adiponectin on BAT and WAT is mediated by a common mechanism; adiponectin stimulates expansion of adipocyte precursor cells that produce adipocytes with intrinsically higher metabolic rate than classical white but lower metabolic rate than classical brown adipocytes. Moreover, adiponectin can modify the adipocytes’ metabolic activity by enhancing the sympathetic innervation within a fat depot.

Session 7: Liver, lipids

Hepatic miR-144 drives fumarase activity preventing NRF2 activation during obesity.

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BACKGROUND AND AIMS: Oxidative stress is central in the development of obesity-associated metabolic complications, including insulin resistance and non-alcoholic fatty liver disease. We recently discovered that microRNA miR-144 regulates the master mediator of the antioxidant response, NRF2. Upon miR-144 silencing, NRF2 target genes were significantly upregulated, suggesting that miR-144 controls NRF2 protein expression *and* activity. Here we explored a mechanism whereby upon obesity miR-144 inhibited NRF2 activity regulating a tricarboxylic acid (TCA) metabolite, fumarate. METHODS: Transcriptomic analysis in liver macrophages (LMs) of obese mice identified the immuno-responsive gene 1 (*Irg1*) as a target of miR-144. IRG1 catalyzes the production of a TCA derivative, itaconate, which inhibits succinate dehydrogenase (SDH). TCA enzyme activities and kinetics were analyzed after miR-144 silencing in obese mice and human liver organoids using single cell activity assays *in situ* and molecular dynamic simulations. RESULTS: miR-144 upregulation in obesity was associated with reduced expression of *Irg1,* which was restored upon miR-144 silencing *in vitro* and *in vivo.* Furthermore, miR-144 overexpression reduces *Irg1* expression and the production of itaconate *in vitro*. In alignment with the reduction in *IRG1* and itaconate levels, SDH activity was upregulated during obesity. Surprisingly, also fumarate hydratase (FH) activity was upregulated in obese livers, leading to fumarate depletion. miR-144 silencing selectively reduced the activities of both SDH and FH resulting in the accumulation of their related substrates succinate and fumarate. Moreover, molecular dynamics analyses revealed the potential role of itaconate as a competitive inhibitor of not only SDH but also FH. Combined, these results demonstrate that miR-144 inhibits the *activity* of NRF2 through decreased fumarate production in obesity. CONCLUSIONS: miR-144 triggers hyperactivation of FH in the TCA cycle and consumption of its substrate fumarate, eventually inhibiting NRF2 activity. Therefore, herein we unraveled miR-144 immunometabolic role in obesity in the regulation of endogenous antioxidant response.

Improvement of the metabolic syndrome by lowering apolipoprotein CIII with siRNAs

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Background and Aims: Apolipoprotein CIII (apoCIII) is increased in obesity-induced insulin resistance and type-2 diabetes. Emerging evidence support the advantages of small interfering RNAs (siRNAs) to target disease-causing genes. We aimed to develop a new RNA-based platform for *in vivo* silencing of apoCIII and investigate its protective effects against the metabolic syndrome.
Material and Methods: We used 12-week-old male and female *ob/ob* mice. Before start of treatment body weight (BW) and blood glucose (BG) were determined to randomize and assign the mice to two different groups: i) apoCIII-siRNA; and ii) control-siRNA treatment. The siRNAS were given intravenously (i.v.), at a dose of 0.008 mg/kg, for three consecutive days every 15 days for eight weeks. Plasma apoCIII, BW and BG were monitored during the study. After the last treatment plasma lipoprotein lipase (LPL) activity was determined and intraperitoneal glucose tolerance test (IPGTT) and glucose-stimulated insulin secretion (GSIS) were performed. At the end of the study samples for lipid profiles, apoCIII gene expression and protein levels and off-target effects were taken.
Results: Our results show that circulating apoCIII was progressively lowered upon administration of apoCIII-siRNA. Plasma LPL activity was higher in mice with reduced apoCIII levels and this resulted in lower levels of triglycerides. Decreasing apoCIII induced a progressive reduction in weight gain and non-fasting BG levels, as well as improved IPGTT and GSIS. At the end of the study, it was confirmed that apoCIII gene and protein levels were reduced in liver from apoCIII-siRNA treated mice. To test the specificity of the siRNA the expression of apoCIII was analyzed in duodenum, the second largest source of the apolipoprotein, and the levels were unaffected. Furthermore, since the apoCIII gene is located within a gene cluster with apoAI, apoAIV and apoAV we confirmed that there were no off-target effects of the siRNA on these apolipoproteins.
Conclusion: Our data demonstrate that obese, insulin resistant and hyperglycemic mice, treated with siRNA targeting the apoCIII gene, cease in gaining weight, improve their insulin sensitivity, lipid- and glucose homeostasis without any observed side effects. Therefore, our siRNA strategy to decrease apoCIII might become a new tool in the fight against the metabolic syndrome.

Liver-specific deletion of the corepressor GPS2 alleviates atherosclerosis and dyslipidemia

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Atherosclerosis is a chronic inflammatory disease and a major cause of cardiovascular disease (CVD). The chronic inflammation resulting from the interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial wall lead to the development of atherosclerotic lesions that protrude into the arterial lumen. Atherosclerosis is a dynamic disorder and it is not possible to consider the disease as an independent process, unaffected by other systems in the body. Several tasks performed by the liver, including lipid metabolism, and inflammatory liver disorders have been implicated in the pathogenesis of atherosclerosis. Non-alcoholic fatty liver disease (NAFLD) has been linked with insulin resistance, obesity, and metabolic syndrome, conditions known to be associated with CVD and subclinical atherosclerosis. Our previous work showed that hepatocyte-specific GPS2 knock out in mice alleviates the development of diet-induced steatosis and fibrosis and causes activation of lipid catabolic genes by the derepression of PPARα. Taking all this into account, we generated and characterized liver-specific GPS2 KO mice in the ApoE KO background (ApoE-LKO) to analyse how liver GPS2 pathways affect atherogenesis. After 12 weeks on an atherogenic diet, ApoE-LKO mice showed smaller atherosclerotic lesion areas in the aortic root. Their body and epididymal fat pad weights were lower and exhibited better serum lipid profile (lower cholesterol and triglycerides levels) compare to their ApoEKO littermates. Liver total cholesterol content was significantly reduced in ApoE-LKO mice and the expression of sterol regulatory element-binding protein 2 (Srebp2), a key mediator of cholesterol biosynthesis, was also lower. In addition, the expression of inflammatory genes was reduced in the liver, while the expression of fibroblast growth factor 21 (FGF21), a target of PPARα, was significantly increased, along with the circulating FGF21 levels. In animal studies, FGF21 has been shown to prevent atherosclerosis by suppression of Srebp2 and induction of adiponectin. Furthermore, recent clinical trials demonstrated that the treatment with an FGF21 analog improved the cardiometabolic profile in obese patients with type 2 diabetes. Collectively, our study reveals that the absence in the liver of the corepressor GPS2 improves atherosclerosis development and alleviates dyslipidemia, probably in part by the induction of FGF21.

Session 8: Diabetes complications

Normalisation of glucose metabolism by Exendin-4 in the chronic phase after stroke promotes functional recovery in male diabetic mice

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Background and Purpose
Glucagon-like peptide-1 receptor (GLP-1R) activation decreases stroke risk in people with type 2 diabetes (T2D) while animal studies have shown the efficacy of this strategy to counteract stroke-induced acute brain damage. However, whether GLP-1R activation also improves stroke recovery in the chronic phase after stroke is unknown. We investigated whether post-acute, chronic administration of the GLP-1R agonist Exendin-4 improves post-stroke recovery and examined possible underlying mechanisms in T2D and non-T2D mice.
Experimental Approach
We induced stroke via transient middle cerebral artery occlusion (tMCAO) in T2D/obese mice (8 months of high-fat diet) and age-matched controls. Exendin-4 was administered for 8 weeks from day 3 post-tMCAO. We assessed functional recovery by weekly upper-limb grip strength tests. Insulin sensitivity and glycaemia were evaluated at 4 and 8 weeks post-tMCAO. Neuronal survival, stroke-induced neurogenesis, neuroinflammation, atrophy of GABAergic parvalbumin+ interneurons, poststroke vascular remodeling and fibrotic scar formation were investigated by immunohistochemistry.
Key Results
Exendin-4 entirely normalised T2D-induced impairment of forepaw grip strength recovery in correlation with normalised glycaemia and insulin sensitivity. Moreover Exendin-4 counteracted T2D-induced atrophy of parvalbumin+ interneurons and decreased microglia activation. Finally, Exendin-4 normalised density and pericyte coverage of micro-vessels and restored fibrotic scar formation in T2D mice. In non-T2D mice, the Exendin-4-mediated recovery was minor.
Conclusion and Implications
Chronic GLP-1R activation mediates post-stroke functional recovery in T2D mice by normalising glucose metabolism and improving neuroplasticity and vascular remodeling in the recovery phase. The results promote launching clinical trials investigating whether GLP-1R agonists improve rehabilitation after stroke in T2D.

Targeting histone methyltransferase EZH2 attenuates hyperglycaemia-induced oxidative stress and inflammation in human endothelium

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Histone post-translational modifications play a critical role in diabetes-associated endothelial dysfunction. Particularly, histone methyltransferase EZH2 catalyses trimethylation of lysine 27 on histone 3 (H3K27me3), a modification linked to gene repression. However, the role of EZH2-H3K27me3 on hyperglycaemia-induced oxidative stress and inflammation in endothelial cells remains unexplored. Likewise, whether targeting EZH2 may represents a valuable strategy to improve endothelial function in this setting has not been proven yet.

Therefore, we analysed EZH2-H3K27me3 in human aortic endothelial cells (HAECs) exposed to high glucose and in endothelial cells from diabetic individuals (D-HAECs). Moreover, we investigated the effect of EZH2 modulation on hyperglycaemia-mediated activation of pro-oxidative and inflammatory genes in these cells.

Increased EZH2-H3K27me3 levels were found in HAECs exposed to high glucose and D-HAECs. ChIP assays revealed increased H3K27me3 levels on the promoters of important antioxidant genes (SOD1 and SOD2) and the transcriptional factor JunD. Further experiments confirmed the repression of these genes, whereas NOX4, a major source of oxidative stress, was upregulated. Along with an increase in reactive oxygen species (ROS), HAECs exposed to high glucose, as well as D-HAECs, exhibited molecular features indicative of inflammation and endothelial impairment. Interestingly, treatment with EZH2 selective inhibitor GSK126 or EZH2 silencing effectively promote a favourable transcriptional program and rescue endothelial function.

Our data provide experimental evidence suggesting that hyperglycaemia/diabetes enhance adverse epigenetic signatures, namely EZH2-H3K27me3, contributing to oxidative stress and inflammation in endothelial cells. Targeting EZH2 blunts H3K27me3 histone modification and preserves endothelial homeostasis, representing a promising pharmacological option to ensure cardiovascular health in diabetes.

Repression of Hypoxia-Inducible Factor-1 Contributes to Increased Mitochondrial Reactive Oxygen Species Production in Diabetes

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Excessive production of mitochondrial reactive oxygen species (ROS) is a central mechanism for the development of diabetes complications. Recently, hypoxia has been identified to play an additional pathogenic role in diabetes. In this study, we hypothesized that the ROS overproduction in diabetes was secondary to the impaired responses to hypoxia due to the inhibition of hypoxia-inducible factor-1 (HIF-1) by hyperglycemia. We found that exposure to hypoxia increased circulating ROS in subjects with diabetes, but not in subjects without diabetes. High glucose concentrations repressed HIF-1 both in hypoxic cells and in kidneys of mouse models of diabetes, through HIF prolyl-hydroxylase (PHD)-dependent mechanism. Impaired HIF-1 signaling contributed to excess production of mitochondrial ROS through increased mitochondrial respiration that was mediated by downregulated Pyruvate dehydrogenase kinase 1 (PDK1) and was followed by cellular injury with functional consequences. The restoration of HIF-1 function attenuated ROS overproduction despite persistent hyperglycemia, and conferred protection against apoptosis and renal injury in diabetes. We conclude that the repression of HIF-1 plays a critical role in mitochondrial ROS overproduction in diabetes and is a potential therapeutic target for diabetic complications. These findings are highly relevant since the first PHD inhibitor has been newly approved for clinical use.

Posters

There are 46 posters, where some posters have several presenters. The posters are ordered somewhat according to research theme. See also page 3 about the poster session.

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Poster Abstracts

P1

Longitudinal monitoring of glucose consumption dynamics in organotypic primary human tissue cultures using a novel glucose sensor with nanoliter input volumes

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Disturbance of glucose homeostasis in metabolically active tissues constitutes a key feature of metabolic syndrome. However, glucose consumption dynamics of human tissues are difficult to study *in vivo*. To overcome these limitations, we developed a highly sensitive and selective glucose sensor and integrated it with 3D primary human liver and adipose tissue culture platforms to monitor glucose consumption over multiple days. First, the bi-enzymatic colorimetric sensor based on Saifer-Gerstenfeld method was benchmarked to perform accurately within the physiological glucose concentration range even with nanoliter input volume. Using this system, we then demonstrated that glucose uptake of 3D human liver cultures closely resembles human hepatic glucose uptake *in vivo* as measured with positron emission tomography during a euglycemic-hyperinsulinemic clamp. By comparing isogenic insulin-resistant and insulin-sensitive liver cultures, we showed that insulin and extracellular glucose levels account for 55% and 45% of hepatic glucose consumption, respectively. Similarly, we showed that adipocyte hypertrophy reduced glucose consumption in 3D human adipose microtissues. By utilizing click-chemistry based on thiol-Michael additions, we covalently conjugated the sensor to custom-build culture devices made from a novel polymer with low drug absorption, enabling its integration into microphysiological system. In summary, combination of human organotypic *in vitro* models with benchmarked glucose sensor will facilitate studies into the biology and pathobiology of glycemic control, as well as antidiabetic drug screening.

P2

Identification of insulin receptor isoform-selective interaction partners

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The insulin receptor (IR) signalling pathway regulates diverse cell functions, from glucose and lipid metabolism to mitogenic pathways. Disturbance in insulin signalling plays a key role in the development of obesity, insulin resistance and type 2 diabetes mellitus. In mammals, insulin acts through two IR isoforms, IR-A and IR-B, which differ structurally and functionally. The activation of these receptor isoforms may involve the recruitment of specific binding proteins and activation of different pathways depending on the cell type. To uncover the IR isoform-specific interactomes in different cell types and in conditions mimicking type 2 diabetes, such as hyperglycemia, hyperinsulinemia or hyperlipidemia, we will use the BioID proximity-dependent labelling technique. This method uses expression of IR isoform fusion proteins that have the capacity to permanently biotinylate proteins that come close enough to interact with the receptor. The biotinylated proteins can be isolated and may subsequently be identified by mass spectrometry. The candidate binding partners identified via this approach will be further validated by co-immunoprecipitation techniques and their role in insulin signalling and cell function will be investigated via gene knockdown and overexpression approaches. The IR-A-BioID and IR-B-BioID fusion proteins we have generated maintain their membrane localization and native signalling activity and show biotinylation capacity. We have identified potential novel IR interactors and have performed preliminary validations. Understanding the molecular basis of IR signal transduction will aid the development of specific protein-targeting therapies to rescue or modulate insulin signalling.

P3

GPS2 antagonism of KDM1A governs M2 macrophage activation

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Macrophage-derived inflammation plays a major role in obesity-induced metabolic dysregulation. Our previous studies have revealed that G protein pathway suppressor 2 (GPS2), a subunit of the NCOR/SMRT/HDAC3 corepressor complex, acts as an anti-inflammatory chromatin modifying coregulator in classically activated (M1) macrophages (*Fan et al. Nat Med 2016, Huang et al Mol Cell 2021*). Here we investigate the role of GPS2 in alternatively activated M2 macrophages through transcriptomic, cistromic, epigenomic, and DNA topology analysis. We find that GPS2 depletion sensitizes the activation of IL4 target genes including *Ptgs1*, *Mrc1*, *Mgl2, Ccl24, Flt1, Clec7a*. Depletion of GPS2 or NCOR triggers comparable changes of the H3K27ac epigenome (enhancer activity), suggesting GPS2 to act via the corepressor complex. Detailed analysis at the *Ptgs1* locus reveals that this regulation requires the IL4-regulated transcription factor STAT6. Further, we demonstrate that GPS2 depletion alters DNA accessibility at the *Ptgs1* locus and promotes enhancer-promoter interaction to trigger gene transcription. Intriguingly, GPS2 depletion triggers chromatin recruitment of the histone demethylase KDM1A (LSD1), which specifically demethylates the H3K9me2/3 at IL4 target loci without disturbing H3K4me1/2. Overall, our findings suggest that functional antagonisms between GPS2 and KDM1A is critical for the epigenetic regulation of M2 macrophage activation and indicate a role of these coregulators in tissue homeostasis and wound healing during Th2-mediated immune responses.

P4

CD200R signaling regulates the response of adipose tissue macrophages in obesity-induced metabolic disease

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Obesity is characterized by an accumulation of macrophages in the adipose tissue. These macrophages contribute to the development of metabolic disease including insulin resistance and type 2 diabetes. However, while multiple macrophage populations have been described in the adipose tissue, little is known about their functional role in response to metabolic changes associated with obesity. Herein we characterized adipose tissue macrophages during the development of insulin resistance and metabolic disease. Adipose tissue biopsies from lean and obese mice and humans undergoing bariatric surgery were characterized by single cell RNA sequencing and flow cytometry. Here, we identified a distinct population of adipose tissue macrophages in mice and humans that expressed high levels of the inhibitory receptor CD200R in response to obesity. Moreover, signalling via CD200R in primary macrophages treated with high levels of lipids and insulin to mimic the condition of obesity, shaped their response in obesity and prevented their immune activation. Together, these results provide evidence for a protective role of CD200R signalling in triggering the reprogramming of inflammatory adipose tissue macrophages in obesity. Targeting specific adipose tissue macrophage populations could thus represent a promising therapeutic strategy for obesity-induced metabolic disease.

P5

Loss of Immune Cell Gpr35 Induces Insulin Resistance

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Immune cells are increasingly recognized as modulators of metabolic tissue homeostasis. Gpr35 is a receptor with high levels of expression in metabolically active tissues as well as in a variety of immune cells. We have previously shown that Gpr35 and kynurenine metabolites are involved in regulating systemic energy balance[1,2]. Activation of Gpr35 by kynurenic acid increases energy expenditure by inducing "browning" of adipocytes, while at the same time promoting an anti-inflammatory immune cell microenvironment in adipose tissue. Analysis of RNA expression data from adipose tissue indicates that the majority of Gpr35 expression in adipose tissue is found in certain resident immune cell populations. To elucidate the role of immune cell Gpr35 in the context of obesity and metabolic disease, we generated an immune cell Gpr35 knockout mouse model (VavGpr35KO). Under control conditions, we didn't observe any difference between VavGpr35KO mice and littermate controls in terms of weight, fasting glucose, glucose tolerance, or adipose tissue immune cell numbers. In addition, VavGpr35KO mice fed a high fat diet (HFD) showed no significant difference in weight gain or fasting glucose compared to controls. However, VavGpr35KO mice show significantly higher plasma insulin levels upon a bolus of glucose. At 8 weeks on HFD, VavGpr35KO mice were more glucose intolerant than controls despite having higher levels of insulin. These mice also exhibited altered macrophage profiles in their visceral adipose tissue. Preliminary RNA-sequencing data analysis suggests a disruption in genes involved in energy metabolism in both skeletal muscle and inguinal white adipose tissue of these mice. Collectively, our data suggests that immune cell Gpr35 is a regulator of systemic energy metabolism. The absence of Gpr35 in immune cells induces insulin resistance through a yet unknown mechanism of action.

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P6

Elucidating the Molecular Mechanisms Driving Senescence in Adipocytes

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Excessive intake of calories results in weight gain through increased fat accumulation. If the calorie surplus is sustained, this can lead to obesity. The prevalence of obesity is increasing globally, and projections are estimating that roughly 4 billion adults will be overweight or obese by year 2025. Many complications arise in conjunction with excessive fat accumulation, such as osteoarthritis, cancer and cardiovascular disease. Many of these obesity-associated complications have been linked to the inadequate lipid storage by the adipose tissue, leading to elevated serum-lipids and lipid storage in non-adipose tissue.

We recently showed that individuals with hyperinsulinemia display a higher proportion of senescent adipocytes. Senescence is characterized by cell cycle arrest, although unlike the name suggests, cells remain metabolically active. We show that senescent adipocytes have an increased pro-inflammatory profile with elevated cytokine production and secretion. Interestingly, we could only detect senescent adipocytes in subcutaneous adipose tissue, with little to no senescence observed in visceral adipocytes. Moreover, we were able to identify positive correlations between clinical parameters and senescent cell number. These correlations were further confirmed by mimicking a disease-state *in vitro* with prolonged insulin treatments. The exact mechanism driving this phenotype, however, remains elusive.

Given the lack of senescence observed in visceral adipocytes, and our limited understanding of the molecular pathways driving this state, we are interested in understanding the mechanisms whereby senescence is induced in adipocytes. DNA damage is frequently described as a strong promoter of senescence, however DNA damage in human adipocytes is not well documented. We will investigate whether adipocyte DNA damage correlates with adipocyte senescence, and if manipulating DNA integrity influences the proportion of senescent adipocytes. Understanding whether intrinsic differences in nuclear DNA damage exists between visceral and subcutaneous adipocytes may help decipher why some adipocytes are protected from senescence, whilst others are not.

P7

Interleukin 1 β promotes adipogenesis in human adipose tissue-derived stem cells

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Obesity-associated metabolic disorders are linked to a decreased capacity of the white adipose tissue (WAT) to safely store excess energy. WAT expansion through adipogenesis (differentiation of progenitors into mature adipocytes) maintains storage capacity and preserves metabolic health. Interestingly, although the obesity-related dysfunctional WAT is characterized by chronic inflammation, healthy WAT expansion requires inflammatory signals. The proinflammatory cytokine interleukin 1β (IL-1β) has been attributed both adverse and beneficial metabolic effects. In the WAT, the expression of its receptor is highest in the progenitor cells. We therefore aimed to investigate the role of IL-1β in adipogenesis.

IL-1β treatment of differentiating human WAT-derived stem cells during the early, but not late adipogenic stage increased lipid droplet accumulation, quantified by high-content fluorescent screening, as well as adipogenic gene expression (RNAseq). The expression of adipogenic regulators CEBPδ and CEBPβ were upregulated after 2 hours of treatment. In addition, IL-1β induced EdU-incorporation during the first 24 hours of differentiation, and inhibiting S-phase progression with hydroxyurea blocked its adipogenic effects, as did pharmacological inhibition of the JNK, but not Erk or p38, pathways. Pre-treatment with IL-1β two days before adipogenic induction prevented the pro-adipogenic effects, while exacerbating induction of inflammation, and similar trends were seen for continuous exposure compared to shorter pulses. The adipogenic effects of IL-1β were also observed in murine subcutaneous, but not visceral stroma-vascular fraction.

In conclusion, we have shown that IL-1β induces adipogenesis via pathways dependent on S-phase progression and JNK pathway activation, and that prolonged exposure causes resistance to these effects, while aggravating inflammation. We propose that IL-1β might regulate adipose tissue remodeling by redistributing fat storage from mature fat cells, where it induces insulin resistance, to newly differentiated progenitors, and that this process can be attenuated by chronic inflammation caused by obesity. The studies are continued in murine *in vivo* models.

P8

Fat cell contribution to metabolic disease in humans

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Many metabolic diseases, including type 2 diabetes, strongly associate with obesity, making obesity one of the major health challenges facing the world today. Obesity is usually accompanied by an increase in fat cell size. As fat cells become enlarged they begin to secrete factors which promote adipose tissue inflammation and dysfunction. The underlying mechanisms connecting increases in fat cell size (hypertrophy) and the secretion of pro-inflammatory factors are not well understood. We show that despite long being considered post-mitotic, mature human fat cells can activate a cell cycle program in association with obesity and hyperinsulinemia, with a concomitant increase in adipocyte cell size, nuclear size and DNA content. Chronic hyperinsulinemia in vitro or in humans, however, is associated with subsequent cell cycle exit, leading to a premature senescent transcriptomic and secretory profile in adipocytes. Premature senescence is rapidly becoming recognized as an important mediator of stress-induced tissue dysfunction. We demonstrate that senescent fat cells secrete factors known to be pro-inflammatory and propose that these factors drive inflammation and pathology in human adipose tissue, impacting whole body health. Using drugs currently on the market for other purposes, we show that manipulating adipocyte cell cycle entry and progression enables one to influence the formation of senescent cells. These studies identify an unappreciated aspect of human adipocyte biology, the activation of a cell cycle program in obesity and hyperinsulinemia, which could pave the way for novel treatment strategies for obesity and associated co-morbidities, such as type 2 diabetes.

P9

Metabolic Consequences of Adipocyte-specific Deletion of IL-1 Receptor 1 in Mice

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The pro-inflammatory master cytokine interleukin 1beta (IL-1b) is chronically elevated in obesity and type 2 diabetes. Recently, a physiological role for IL-1b in glucose metabolism was described. Feeding induces IL-1b which in turn contributes to insulin secretion. Strikingly, the IL-1 pathway in adipose tissue macrophages was identified as an early sensor of metabolic changes upon food intake. To study the biological role of the adipocyte IL-1 system, we generated a mouse line with genetic ablation of IL-1 receptor 1 (IL-1R1), specifically in adipocytes. With chow diet, Adipo-Cre IL-1R KO (ARILKO) mice showed similar body weight development and glucose tolerance compared to their littermate controls. When challenged with an acute dose of IL-1b prior to the glucose bolus, ARILKO mice and littermate controls showed similar glucose tolerance, indicating that whole body glucose disposal is not dependent on IL-1 signaling in adipocytes. Interestingly, upon acute IL-1b treatment, ARILKO mice showed a trend towards reduced glucose uptake in the epididymal white adipose tissue (eWAT), suggesting that IL-1 signaling plays role in glucose uptake particularly in this fat pad. When fed a high-fat diet (HFD), ARILKO mice initially showed improved insulin sensitivity at 21 weeks of age, transitioning to an impaired metabolic phenotype after 35 weeks of in aging. Western blot analysis of adipose tissue isolated from aged HFD-fed mice suggested decreased insulin signaling in eWAT of ARILKO mice compared to their littermate controls, with reduced AKT phosphorylation upon insulin treatment. Aged HFD-fed ARILKO mice showed similar body weight and glucose excursion to their littermate controls, besides a trend towards elevated insulin secretion. Collectively, these results indicate that activation of the IL-1 pathway in adipocytes results in metabolic effects beyond the adipose tissue, affecting whole-body glucose homeostasis.

P10

Time-of-day influences post-exercise metabolism in mouse adipose tissue

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AIMS: This study aims to determine if time-of-day modulates the exercise response in adipose tissue metabolism and function.
METHODS: We exposed mice to a 60-minute exercise bout (or sham) during the early rest ("rest"; day, ZT3) or early active ("active", night, ZT15) phase. Tissue and serum samples were collected at 0, 4, 8, 12, 16, and 20h post-exercise. An additional subset of mice was exercised (or sham) at ZT3 in either an *ad libitum* or 10h-fasted state. We measured post-exercise serum parameters and performed RNA-sequencing on inguinal white adipose tissue (iWAT).
RESULTS: Exercise had no effect on core clock gene expression in iWAT. Time of day did not significantly affect exercise-mediated serum adrenaline or corticosterone release; nevertheless, serum NEFA and serum TG significantly increased only with active phase exercise. RNAseq data identified increased expression of 179 exercise-responsive genes in the active phase, with no genes altered by exercise in the rest phase samples. To decipher the influence of nutritional status on these time-of-day specific effects, we compared the response to exercise in the rest phase in fed or 10h-fasted mice. Fasted rest phase exercise led to an increase in serum NEFA and a depletion of liver glycogen relative to *ad libitum* fed mice; however, fasted rest phase exercise did not recapitulate expression of genes identified in the active phase response to exercise.
CONCLUSIONS: Active phase exercise in mice models modulates adipose tissue gene expression and is associated with a shift in serum lipids, indicating increased lipolysis. While nutritional state may explain the lipolytic response, fasting does not sufficiently recapture the time-of-day effect of exercise on gene expression. Our results provide evidence to suggest that exercise and the circadian clock interact to fine-tune the beneficial effects of exercise.

P11

Maternal obesity alters the brown adipose tissue in murine offspring in a sex-dependent manner

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Introduction: Obesity among women of reproductive age and during pregnancy predisposes fetus to metabolic complications later in life. It has been demonstrated that the susceptibility to metabolic diseases is sex-related. Brown adipose tissue (BAT) is considered as an important target against obesity due to its ability to dissipate energy as heat. Maternal obesity has recently been demonstrated to damage BAT in offspring, but the underlying mechanism is still unidentified.
Aim: The aim of our study is to explore the impact of exposure to maternal obesity on offspring metabolism and investigate the sex differences with focus on brown adipose tissue in order to improve our understanding in metabolic disease mechanism and develop better therapeutic methods.
Methods: Female C57BL/6J mice were fed a control diet or a high-fat diet before mating and maintained on their respective diet during pregnancy and lactation. Offspring were on control diet after weaning and BAT was collected from both female and male offspring at 6 months of age. To determine the effect of maternal obesity on BAT biology and metabolism we used magnetic resonance imaging and spectroscopy, lipidomic analysis and RNA-seq.
Results: Males had larger BAT compared to females but both sexes had increased lipid infusion in BAT (whitening) when coming from obese mothers. Lipidomic analysis showed that male BAT contained more long-chain fatty acids compared to females from obese mothers. We observed both sex- and mother-diet effects on the transcriptional regulation in BAT ´s offspring.
Conclusion: Exposing mothers to obesogenic diet prior mating, during pregnancy and lactation has a strong sex-dependent impact on the programming of BAT in offspring which may alter metabolic balance in a sex specific manner later in life.

P12

Sex-specific regulation of IL-10 expression in human adipose tissue in obesity

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Aim:
Sexual dimorphism in obesity and diabetes and the cytokine alteration during these metabolic abnormalities are becoming interestingly striking. We have previously shown that the interleukin-10 (IL-10) from human white adipose tissue (hWAT) is upregulated during obesity and type 2 diabetes (T2D) in women. Clinical studies addressing regulation of hWAT IL-10 secretions in both genders were lacking.
Methods:
IL-10 hWAT secretion, mRNA expression and percentage of IL-10-secreting macrophages were analysed in three different groups of individuals: non-obese healthy, non-obese T2D and obese T2D men and women. IL-10 secretion was also determined in a cohort of individuals undergoing sex conversion. To investigate the role of sex hormones, hWAT hormone levels were correlated with IL-10 expression. In addition, in vitro hormone treatments of THP1 monocytes and human stroma vascular fraction (SVF) were performed.
Results:
We found increased hWAT IL-10 secretion, mRNA expression and higher numbers of IL-10 secreting macrophages in obese T2D women compared to non-obese healthy and T2D individuals. This difference was not observed in men indicating a sex specific regulation of IL-10 in hWAT. In-addition, IL-10 correlated significantly with estrone (E1) in obese women. However, in vitro treatment of SVF by recombinant E1 did not upregulate IL-10 significantly.
Conclusions:
In hWAT, IL-10 expression is upregulated during obesity and T2D in women, but not in men, which could indicate a protective anti-inflammatory mechanism in women that is lacking in men. However, we do not provide evidence that IL-10 might be directly regulated by sex hormones.

P13

LONG-TERM IMPROVEMENT OF ADIPOCYTE INSULIN ACTION DURING BODY WEIGHT RELAPSE AFTER BARIATRIC SURGERY: A LONGITUDINAL COHORT STUDY

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Background: There are few long-term mechanistic studies in adipose tissue that investigate the metabolic effects of bariatric surgery. Changes in lipogenesis may be involved in long-term weight development.
Aim: To investigate the long-term effect of bariatric surgery on lipogenesis in abdominal fat cells and whether surgical treatment could induce an epigenetic memory that would maintain improved lipogenesis despite body weight relapse.
Methods: 22 obese women living in the Stockholm area were examined before, 2, 5 and 10 years after bariatric surgery. Abdominal adipose tissue biopsies were obtained. Fat cells were isolated and spontaneous and insulin stimulated glucose incorporation into lipids were assayed. GpG-methylation profiling was performed on adipocytes using the Infinium EPIC BeadChips**.**
Results: Bariatric surgery was associated with improvement in adipocyte spontaneous and insulin stimulated lipogenesis, which was maintained despite late weight regain. There were 7,729 differentially methylated CpG sites (DMS) at 2 y's that showed no sign of return to baseline at either 5 or 10 years. Merging results with expression profiles identified 1,259 genes with DMS which showed early response or continual change in expression in one direction after surgery. Up-regulated genes with DMS were enriched in gene sets linked to cellular response to insulin stimulus (e.g. *IRS1, IRS2, PDE3B*, and *AKT2)* and regulation of lipid metabolic processes.
Conclusions: Bariatric surgery leads to long-term improvement of lipogenesis and insulin responsiveness in subcutaneous adipocytes in women. This may to some extent explain the long-term relapse in fat mass and epigenetic modifications could be involved.

P14

Endometrial cell-type-specific disease signatures and endometrial epithelial organoids in polycystic ovary syndrome

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Introduction
Polycystic ovary syndrome (PCOS) is the leading cause of female infertility and is associated with type 2 diabetes and endometrial cancer. Hyperandrogenemia is a hallmark of PCOS and contributes to endometrial-related dysfunctions, including implantation failure and miscarriage. Whether cellular heterogeneity contributes to the functioning of the endometrium is not previously studied. Therefore, the aim is to reveal cell-type-specific disease signatures in the endometrium in women with PCOS at the single-cell level and to validate the role of molecular targets in endometrial epithelial organoids (EEOs).
Method
Single nuclei are extracted from frozen endometrial biopsies collected from women with PCOS (n=11) and healthy controls (n=5). The nuclei RNA libraries are prepared following the 10x genomics protocol allowing us to sequence ~10,000 cells per sample. Sequencing data is analyzed using the Seurat package and integrated with public snRNA-seq data for further QC and cell labeling. In parallel, 3D EEOs are established from fresh endometrial biopsies. Following several passages, EEOs are cryopreserved to create a biobank to be used for functional analyses of identified molecular targets.
Results
In a pilot study, single-nuclei were extracted from two endometrial samples from women with PCOS for 10x snRNA-sequencing. The endometrial tissue of women with PCOS has a distinct single-cell transcriptomic profile, with PCOS-specific cell clusters that differ from the controls. The EEO protocol has been established and two have been cryopreserved. In both cases, the EEOs have been reestablished after thawing. Validation with immunofluorescent staining shows that the 3D EEOs consist of an intact proliferative basolateral epithelial membrane.
Conclusion
This rigorous mapping of endometrial tissue samples will increase the understanding of the cellular complexity and dysfunction and will be linked to phenotypic features in women with PCOS. By successive formation of PCOS-EEOs, we can further study cellular and molecular mechanisms causing PCOS-specific endometrial dysfunction.

P15

Developmental programming by maternal androgen excess is mediated by androgen receptor pathways

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Introduction
The hyperandrogenic *in utero* environment in pregnant women with polycystic ovary syndrome (PCOS) can affect embryo development and impair offspring health at adult age. Moreover, PCOS jeopardizes the pregnancy to miscarriage, preterm delivery, and perinatal mortality. The underlying mechanism(s) of pregnancy complications associated with PCOS and the consequence of hyperandrogenic intrauterine environment on the offspring is not well known.
Method
We used a PCOS-like mouse model induced by continuous exposure of dihydrotestosterone from prepuberty that develops obesity, anovulation and dysfunctional ovarian morphology, to study the effects of maternal hyperandrogenism during pregnancy. To explore molecular mechanisms that might contribute to the developmental defects, whole genome bisulfite and RNA sequencing of primordial germ cells and placenta were performed.
Results
We found a lower pregnancy rate and impaired placenta and embryonic development, which was partially prevented when co-treated with the androgen receptor blocker, flutamide. Moreover, germ cell specification was greatly compromised at embryonic day 10.5 and 13.5. The results of whole genome bisulfite sequencing and RNA sequencing of the primordial germ cells and placentas are currently under analysis.
Conclusion
Our results so far suggest that hyperandrogenism greatly compromise the PCOS-pregnancy and embryo development due to placenta dysfunction. Such effects are mainly mediated by the androgen receptor pathway as administration of flutamide partially prevented the compromised placenta and fetal development.

P16

Thromboxane is elevated in men after exercise and improves skeletal muscle glucose uptake and whole-body glucose homeostasis

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Introduction: Prostanoids (thromboxane and prostaglandins) are lipid mediators that signal through receptors which are expressed at the surface of multiple cell types, including skeletal muscle. Type 2 diabetes and exercise are associated with increased levels of prostanoids in the circulation and in skeletal muscle tissue in males. We therefore hypothesized that prostanoids play a role in skeletal muscle remodelling and metabolism.
Methods: The exercise response of genes involved in prostanoids synthesis was assessed using the MetaMEx database. Blood samples were obtained from healthy men and women before and immediately after an acute (30 minutes) aerobic exercise bout. Levels of thromboxane B2 in plasma were measured using ELISA. Primary human myotubes were incubated with the thromboxane receptor agonist I-BOP and levels of glucose uptake and oxidation, and glycogen synthesis were measured using radiolabelled substrates. Western blot was performed to track signalling events. EDL and soleus from mice were incubated ex-vivo with I-BOP, and glucose oxidation was measured using [^{14}C]-glucose. Glucose tolerance test in mice was performed after an acute injection with I-BOP.
Results: Levels of the thromboxane synthase (*TBXAS1*) mRNA were elevated after exercise in the skeletal muscle of men but not women. Concomitantly, preliminary results indicate that levels of thromboxane B2 in plasma were higher after exercise only in men. Activating the receptor of thromboxane (TP receptor) in skeletal muscle cells resulted in increased glucose uptake (+53%, p=0.0019) and oxidation (+28%, p=0.017), and glycogen synthesis (+398%, p<0.001). This was found to coalesce with signalling indicative of active actin cytoskeleton remodelling and GLUT4 translocation to the plasma membrane. Skeletal muscles from male mice that were incubated with the TP receptor agonist increased glucose oxidation (+118%, p<0.001), and male mice acutely injected with the TP receptor agonist exhibited improved glucose tolerance.
Conclusions: Plasma thromboxane is distinctly regulated between men and women in response to exercise. Activating the thromboxane receptor leads to improved glucose uptake and oxidation, and thus may be a target for improving whole body glucose homeostasis.

P17

A nuclear structural node of one-carbon and oxygen metabolism to regulate cellular activity.

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A variety of pathological and non-pathological conditions can reduce oxygen supply, either to the whole organism or to specific tissues. To cite some, these could be cancer, intense physical exercise, high altitude breathing, strokes, and lung failure. The cellular responses to low oxygen conditions (hypoxia) are mostly driven by the hypoxia-inducible factor-1alpha (HIF-1a). When activated, HIF-1a drives a coordinated program of gene transcription that promotes glycolysis and cell proliferation.
This project aims to identify and characterize new cofactors that participate in the regulation of HIF-1a activity. Using a biochemical approach, we found that HIF-1a forms a nuclear complex with the one-carbon metabolism enzyme tetrahydrofolate synthase (THFs, encoded by the gene MTHFD1). In a luciferase reporter assay, we have seen that THFs promotes HIF-1a transcriptional activity in a dose-dependent manner which suggests that THFs is recruited by HIF-1a at the DNA level. To verify this hypothesis, we have performed ChIP-seq experiment. Our results show that THFs localizes on the promoter region of HIF-1a target genes only in hypoxic conditions. Unexpectedly, we also found that THFs localizes in chromosomal areas that are enriched in consensus sequences for the RE1-Silencing Transcription factor (REST), which has been shown to repress gene expression during hypoxia.
To further understand the nuclear function of THFs, we are now performing metabolomics and proteomics experiments to a) isolate which one-carbon metabolites are affected by hypoxia, and b) identify the other components of the HIF-1α:THFs nuclear complex.
Concluding, we have identified a new component of the HIF-1a transcriptional machinery. Understanding the physiological regulation of the HIF-1a:THFs complex could open new lines of intervention for situations in which the activity of HIF-1a is crucial, such as cancer and/or immune system metabolism.

P18

Exploring the role of sensory neurons in resolving noxious stimuli in skeletal muscle and adipose tissue

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Detection of noxious stimuli in tissue microenvironments is a fundamental property of sensory neurons and is often altered in diseases. Thus, understanding how tissue microenvironments influence communication to their sensory neurons may reveal an underappreciated mode of inter-organ crosstalk. We hypothesize that this could involve the classical afferent function of sensory neurons, but also alterations in the exchange of molecular messengers between neuronal termini and local tissues. This project aims to study how communication between sensory neurons and two metabolically important tissues is affected by the status of the innervated tissue. We will use a microfluidic device that allows for compartmentalized culture of different cell types with micro-channels that are only large enough for axonal projections or secreted molecules. C57BL/6-derived primary myoblasts or pre-adipocytes will be plated on one side of the microfluidic device and hESC-derived sensory neurons on the other. The skeletal muscle and adipose compartments will be pre-treated with compounds to mimic a healthy (or less healthy) tissue status (e.g., PGC-1α1 overexpression for skeletal muscle and kynurenine metabolites for adipocytes). We will then evaluate how communication between the tissue is affected by noxious stimuli such as cytokines and/or neurotoxic components of the kynurenine pathway—both common in metabolic diseases. The sensory neuron response will be assessed by immunocytochemistry and/or qRT-PCR to detect changes in morphology and axon recruitment, and expression of sensory-specific ion channels, proteins, neuropeptides, and cell survival genes. Preliminary results suggest an altered cytokine profile in PGC-1α1-overexpressing myotubes, and that sensory neuron-derived peptides influence metabolism. Following the in vitro component, we will generate tissue-specific metabolic alterations in mice and evaluate how the associated sensory neurons respond to stimuli such as muscle injury or high-fat diet. We will use methods from the in vitro component to assess neural health along with CLARITY-based tissue imaging and protein analysis.

P19

Exercise-responsive non-coding RNAs in the regulation of skeletal muscle metabolism

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Exercise triggers profound structural and metabolic adaptations in numerous tissues, including skeletal muscle. While this accounts for the benefits of exercise in type 2 diabetes (T2D), a detailed understanding of the molecular mechanisms involved remains unclear. Non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are important regulators of skeletal muscle physiology, and their expression in skeletal muscle is modulated by different exercise training programs. miRNAs are also incorporated into exosomes and released into the circulation to facilitate tissue crosstalk, and their circulating levels are also affected by exercise. In this study, we investigated differentially expressed miRNAs derived from serum exosomes and skeletal muscle derived lncRNAs from normal glucose tolerant (NGT) and T2D subjects at rest and following an acute exercise bout or upon endurance exercise training.
In **Study I**, seventeen individuals with normal glucose tolerance (NGT) and nineteen individuals with T2D underwent an acute bout of cycling exercise and RNA sequencing was performed on vastus lateralis biopsies collected before (basal), immediately after, and 3 hours after (recovery). In **Study II**, a separate cohort of 12 healthy individuals underwent 3 weeks of supervised endurance training exercise and serum was collected before and after. Differentially expressed miRNAs were identified using a miRNA focus PCR panel.
Our preliminary analysis reveals that a single bout of exercise altered the skeletal muscle lncRNA profile more profoundly in the T2D group with more than two hundred lncRNAs being differentially expressed compared to the basal state. As a training effect, we found that the levels of exosomal miR-139-5p and miR-136-3p were significantly increased in the serum of healthy individuals after the 3-week training period.
This study will provide mechanistic insights into the regulation of skeletal muscle metabolism by exercise, which may lead to the identification of new targets for the treatment of T2D.

P20

Role of muscle-secreted Mustn1 in peripheral perfusion and energy homeostasis

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Skeletal muscle is a highly adaptive tissue that changes with many physiological stimuli such as exercise/training, disuse, weightlessness and age. However, deregulation of skeletal muscle physiology can lead to pathologies affecting the whole organism. Using transcriptional analysis of muscle in a mouse model of hindlimb unloading/reloading we aimed to identify genes with novel roles in the regulation of these adaptive processes. One of the most prominent candidates is a gene called *mustn1*, encoding a conserved micropeptide of 82 amino acids. Although *mustn1* expression in muscle is known to be heavily regulated under a myriad of (patho)physiological conditions, the function of Mustn1 is still largely unknown.

Single-cell transcriptomic data places the origin of *mustn1* expression in smooth muscle cells (SMC) and myonuclei of skeletal muscle fibers, and we confirmed Mustn1 protein expression in blood vessels of the muscle. Although Mustn1 has previously been thought of as a nuclear protein, predictions indicate it might be non-classically secreted. Indeed, we found that muscle cells could secrete ectopically expressed Mustn1 into the extracellular milieu. Furthermore, using the LigandTracer system, we found that recombinant Mustn1 (rMustn1) could interact with a (still unknown) receptor expressed on specific target cells. Treating muscle cells with rMustn1 resulted in activation of signaling pathways regulating cellular energy homeostasis, including robust activation of AMP-activated protein kinase. Using a genetic mouse model, we found that whole-body Mustn1 deficiency resulted in a mild phenotype that included reduction in fat mass, increased exercise performance, and reduced weight gain on a high-fat diet.

The next steps in this project include investigating the role of SMC in secreting Mustn1 under conditions such as exercise and muscle injury, and the effects this has on peripheral perfusion and energy homeostasis. The cellular effects of Mustn1 on target cells, including finding its receptor, are further aims of this study.

P21

Exploring muscle-derived extracellular vesicles (EVs) as mediators of inter-organ communication

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Physical activity is crucial for the prevention or alleviation of type 2 Diabetes mellitus and metabolic diseases. Skeletal muscle secretes contraction-induced factors, with numerous beneficial effects on different tissues and the metabolic state in general. However, the communication between muscle and other organs is still not well understood. Muscle-derived factors comprise several peptides (myokines), nucleic acids and metabolites that can act in autocrine, paracrine or endocrine manner. In addition to their direct secretion into the circulation, these factors can be transported between donor and recipient cells by small extracellular vesicles (EVs).
With this project we aimed to determine if it was possible to identify and characterize skeletal muscle-derived EVs. We established a protocol for the isolation of EVs from primary muscle cells (PMCs) using ultracentrifugation or size-exclusion chromatography. PMC-derived EVs exhibit a homogenous size distribution and a typical nanovesicular shape. Furthermore, we analyzed the muscle-derived EVs proteome and identified potential specific surface proteins for targeted purification or tracking of EVs. One of these proteins was Cacng1 (Calcium channel gamma subunit 1), which showed to be skeletal muscle-specific. Next, we transiently expressed a Cacng1-GFP fusion protein in mouse *gastrocnemius* muscle using a recombinant adenoviral vector. By collecting liver samples, we could determine a muscle-liver crosstalk mediated by muscle-derived EVs. In humans, muscle-derived EVs with were increased after endurance exercise but the amount of muscle-derived EVs with Cacng1 did not change. We are further exploring the potential of Cacng1 as a skeletal muscle-specific EV marker that could allow us to isolate or track that specific EV population in different situations.

P22

Gene regulatory elements controlling PGC-1α expression

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The peroxisome proliferator-activated receptor γ (PPARγ) co-activator 1α (PGC-1α) has a central role in skeletal muscle homeostasis, connecting external stimuli to the co-activation of gene networks related to oxidative phosphorylation, angiogenesis, fatty acid metabolism, fibre-type switching and muscle hypertrophy. This wide range of action comes from the fact that the PGC-1α gene can express multiple protein variants by alternative promoter usage and alternative splicing. Although it is known that the proximal/canonical PGC-1α promoter is constitutively expressed at baseline, while its alternative promoter is highly inducible, the physiological consequences of differential promoter usage is not very well understood. Moreover, distal genomic regulatory elements controlling the activity of each PGC-1α promoter were never described. Thus, our aim is to characterize mice lacking the PGC-1α isoforms expressed from its alternative promoter and to identify novel genomic regulatory elements controlling the expression of the PGC-1α gene. For that, we generated whole-body and muscle-specific the PGC-1α alternative promoter knockout mice, and challenged these animals with cold exposure, beta adrenergic stimulation, and acute and chronic exercise interventions. Our preliminary results indicate that some of the gene networks controlled by PGC-1α are disturbed by the absence of the alternative promoter, however these animals show normal physiological response to the challenges applied. This seems be explained by a compensatory activation of the PGC-1α proximal promoter. In our attempt to understand how each PGC-1α promoter is controlled, we identified three novel genomic regulatory regions upstream of the PGC-1α locus. Luciferase reporter assays performed in C2C12 myotubes indicate that these regions work as network of regulatory elements, enhancing and/or silencing each of the PGC-1α promoters. Our next steps involve characterizing the functional role of these novel genomic regulatory regions in mice by using epigenome editing tools.

P23

Abnormal muscle glucose metabolism in chronic inflammation

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Skeletal muscle is essential for our ability to move and the major site for the post-prandial glucose uptake. The polyol pathway is a two-steps process converting glucose to sorbitol by aldose-reductase (AR) and fructose by sorbitol-dehydrogenase (SORD). This pathway, mostly explored in diabetes, is associated with oxidative stress. Rheumatoid arthritis (RA) is a chronic inflammatory disorder with oxidative stress being associated with the pathological processes. We have shown that oxidative stress contributes to muscle weakness in patients with RA. In addition, patients with RA have a two-fold increased risk of developing diabetes. Here we investigated the polyol pathway in chronic inflammation using our mice model of RA.

Arthritis was evoked by an ankle injection with complete Freund's adjuvant (CFA) and muscles were collected 14 days post-injection. Glucose tolerance test and insulin secretion were assessed after an intra-peritoneal glucose injection. Protein expression was assessed by immunoblotting, glycogen content and glutathione by enzymatic assays. Basal blood glucose was lower in mice with CFA, but no difference in the glucose clearance rate or insulin secretion were observed. Protein expression of glucose transporter 4 (GLUT4), responsible for insulin/contraction-mediated glucose uptake, was increased by ~160% in muscle from the leg with arthritis. However, the expression of enzymes involved in the glycolysis were decreased, including hexokinase (HK2) (-55%), phosphofructokinase (PFKm) (-38%) and phosphofructokinase-2/fructose-2,6-bisphosphatase (PFKFB3) (-35%). Muscle glycogen synthase (Gys1) was decreased by ~23%. Nevertheless, AR and SORD protein expression were elevated by ~50% and ~20% respectively in muscle with CFA. The glycogen content was unchanged. Decreased level of total glutathione, reduced form and the ratio reduced/oxidized was observed in muscle afflicted with CFA, which imply a lower antioxidant capacity. Thus, arthritis is associated with a shift in muscle glucose metabolism, in favor of the polyol pathway, which can contribute to oxidative stress and muscle dysfunction.

P24

The role of Diacylglycerol Kinase δ on glucose energy homeostasis in skeletal muscle

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Insulin resistance in type 2 diabetes (T2D) involves the complex interplay of multiple metabolic pathways over different organs and tissues. Although many hypotheses have been proposed to explain the occurrence the disruption of glucose homeostasis, the molecular mechanisms involved are not entirely understood. Diacylglycerol kinase δ (DGKδ) belongs to a family of enzymes that catalyze the ATP-dependent phosphorylation of diacylglycerol (DAG) to phosphatidic acid. The accumulation of DAG leads to insulin resistance through attenuation of the insulin signaling cascade. Reduced DGKδ protein abundance, which results in increased DAG accumulation, directly contributes to the development of lipid-induced insulin resistance and obesity, leading to dysglycemia and T2D. However, the direct role of DGKδ in the development of insulin resistance in human skeletal muscle cells (HSMCs) is still unknown.
The objective of this project is to determine the mechanism by which DGKδ contributes to peripheral insulin resistance. The hypothesis is that alterations in DGKδ expression lead to perturbances of glucose homeostasis in T2D, ultimately resulting in insulin resistance. To test this hypothesis, we will study insulin signaling and glucose metabolism in response to changes in DGKδ expression in differentiated primary HSMCs from T2D and healthy patients. Western blot, qPCR analysis, lactate measurements and glycogen synthesis assays will be performed to assess glucose homeostasis *in vitro*. Achieving a further understanding of the involvement of DGKδ in skeletal muscle physiology and how this enzyme controls insulin sensitivity may influence future development of pharmacological intervention strategies aimed to improve glucose homeostasis.

P25

The role of pancreatic islet phospholipid metabolism in type 2 diabetes

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Type 2 diabetes (T2D) has been considered a permanent disease that deteriorates over time. There is now ample evidence to support that T2D remission is possible and that the success rate is closely linked with the individual's ability to reduce pancreatic lipids. Although the role of lipids is accepted, both in terms of disease progression and remission, molecular mechanisms by which islet lipids mediate T2D development are widely debated. This project aims to investigate specific phospholipid pathways that regulate pancreatic diacylglycerol (DAGs) and ceramides, which are putative mediators of altered pancreatic insulin secretion. To achieve this, a comprehensive approach is outlined where state-of-the-art mass spectrometry analyses will be applied to cover the chemical diversity of bioactive lipids together with measures of organelle-specific proteins that are involved in phospholipid metabolism in human and mice islets of Langerhans during prediabetic and T2D conditions. Preliminary data from our lab show that diminished insulin secretion capacity during T2D, in both mice and humans, was associated with increases in specific ceramides, e.g., C16-ceramides, and lower estimate of sphingomyelin synthase, which converts ceramide to DAG via phospholipids. We hypothesize that sphingomyelin synthase activity in the ER/Golgi network is reduced during T2D development and linked with the accumulation of ER-stress markers, i.e., C16-ceramides, and altered mitochondrial biogenesis, all of which have implications on the insulin secretion machinery. Ongoing work include detailed measures of phospholipid metabolism in human donor islets and mice models with altered insulin secretion: **i)** obesity-induced prediabetic hypersecretion of insulin; and **ii)** islet amyloid plaque induced T2D with diminished insulin secretion. We will also investigate the effects of disease remission via supplementation of an AMPK-activator, which have shown to increase insulin secretion capacity. Results from this research have the possibility to provide novel therapeutic targets that can be of use to control and maintain insulin secretion against β-cell dysfunction in T2D.

P26

Phenotyping streptozotocin treated pancreatic islets using monolayer adherent whole islets in vitro; A novel method for detailed characterization of damaged pancreatic islets.

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Introduction: The cells within the pancreatic islets are difficult to characterize during injury, due its three-dimensional structure and variation of islets size. When isolated pancreatic islets are cultured on laminin-521 coated plastic, they adhere strongly and spread flat. The aim of our study was to establish cell quantification methods using a flat islet model, and to characterize the effect of Streptozotocin (STZ) induced diabetes in flat islets in vitro.
Methods: Pancreatic islets were isolated from Balb/c mice by gradient centrifugation, sorted by size by hand-picking and cultured on laminin-521 for 7 days to form flat islets in 96 well micro culture plates. Some of flat islets were treated with STZ for 24 hours. Islet function was examined by glucose-stimulated insulin secretion (GSIS) assay using ELISA, immunohistochemistry staining to detect insulin and glucagon secreting cells, 3D spinning-disc imaging and quantitation of the 3D images.
Results: Small whole islets, about 90 micrometers in diameter, on laminin-521 can form monolayer flat islets. Monolayer flat islets maintain normal insulin secretion capacity in response to glucose, the amount of insulin released per β cell was higher than 6 pg/β cell/hour during high glucose stimulation. In the STZ treated group, the number total islet cells was reduced, especially, β cell population and α-to-β ratio was increased. The glucose stimulation index was lower in the streptozotocin-treated group compared to the control group. However, amount of insulin produced per β cell was maintained.
Conclusions: This novel approach allows equal access of STZ suspended in the culture medium to all the cells within an islet. Our method is user-friendly, affordable, and semi-automated, which could be useful as drug screening method for cells within islets. The method is not limited to STZ-induced diabetes model. It is a versatile platform for a variety of studies aimed to explore islet biology.

P27

A small molecule that enhances β-cell maturation and reduces basal insulin secretion

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Recovering β-cell function or generating transplantable mature β-cells from stem cells is a promising approach for future diabetes therapies. Here, we identified a small molecule as a stimulator of functional maturation of zebrafish and mouse β-cells. It enhanced *insulin* promoter activity, increased *insulin* expression, and reduced glucose levels in zebrafish, without altering the number of β-cells. Treatment of isolated neonatal mouse islets with the small molecule stimulated *Mafa* expression and reduced their basal insulin secretion. Moreover, its effect on β-cell maturation and the accompanying reduction in basal insulin secretion was independent of thyroid hormone, which is a previously known factor stimulating β-cell maturation. Together, our results suggest that the small molecule increases the glucose threshold for insulin secretion and directs functional maturation of pancreatic β-cells through increasing the expression of *Mafa*, an effect that should be further studied in islets and diabetes.

P28

Transduction of islet organoids using adeno-associated virus vectors

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Genetically modified islet organoids assembled *in vitro* prior to transplantation are an emerging alternative to direct *in vivo* genetic manipulations for a number of clinical and research applications. Pseudoislets maintain the physiological functions of native islets while the dispersion step — where single islet cells remain in suspension for hours — allows for efficient transduction with viral vectors.
Adeno-associated viruses (AAVs) are the most widely used vectors in gene therapy development due to their advantageous safety profile characterised by low cytotoxicity and immunogenicity. However, AAV-mediated transduction of islet cells during organoid formation was never examined.

Here we have characterised in detail AAV serotype 8 performance in transduction of dispersed islet cells during pseudoislet formation and compared it to the most commonly used *in vitro* adenoviral vector AdV5. Both AAV8 and AdV5 carried a CMV-TurboRFP construct which allowed us to monitor expression kinetics and cell tropism of both vectors. To follow expression kinetics, we have measured fluorescence intensity development over time and assessed TurboRFP mRNA levels by qRT-PCR at several time-points. To evaluate cell tropism of AAV8 we have stained transduced pseudoislets for major endocrine cell types and determined the proportion of each endocrine cell type among TurboRFP positive cells using flow cytometry and confocal microscopy.

Based on fluorescence intensity, transduction with AAV8 during pseudoislet formation was 2.5 times more efficient than transduction of intact islets. AAV8-driven expression was characterised by slow but sustained kinetics while AdV5-introduced fluorescence was short-lived and started to decline already after 2 weeks post-transduction. Both vectors preferentially targeted insulin-producing cells. We have detected very few TurboRFP positive alpha- and delta-cells with over 90% of TurboRFP-expressing cells being beta-cells.
Our data demonstrate that AAV8 can be successfully used to modify insulin-producing cells during pseudoislet formation and will serve well for longitudinal studies and applications potentially including *in vivo* transplantation.

P29

Developing data-driven strategies to decipher the roles of monocytes in non-alcoholic fatty liver disease progression

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease particularly in obese individuals and can progress from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, cirrhosis, liver failure and hepatocellular carcinoma. The severity of liver fibrosis can predict the mortality and time of developing severe and irreversible disease stages in NAFLD patients, but the current fibrosis scoring methods based on histological assessment of biopsies are invasive. Non-invasive tools, such as ultrasound systems or algorithms using blood test results, are usually used to justify the collection of biopsies. Both invasive and non-invasive approaches do not allow the accurate scoring of disease stages and cannot rigorously predict disease progression at an early stage. Non-invasive strategies for dynamic scoring of liver fibrosis using blood tests are therefore in urgent need. While such strategies may reveal novel longitudinal patient subgroups, to devise personalized therapeutic strategies requires in-depth understanding of the molecular mechanisms that drive disease progression. In NAFLD, monocyte-derived liver macrophages were found to be detrimental in NASH and liver cirrhosis. However, the contribution of circulating monocytes, a vital immune cell population in the bloodstream that can differentiate into tissue macrophages, to longitudinal NAFLD progression is still unclear. Therefore, in this research, my objective is to develop a data-driven translational research platform that combines biobanks, longitudinal clinical data and population registries, cutting-edge omics technologies and novel computational methods/tools. I will build an innovative prediction model to predict patient current and future disease states from blood test values and stratify patient subgroups based on longitudinal disease states. With comprehensive multi-omics data analysis in combination with functional validation using established preclinical models developed by my collaborators, my goal is to decipher the cellular and molecular mechanisms that drive disease progression and reveal new diagnostic and therapeutic strategies for severe disease prevention.

P30

Dissecting A-to-I RNA editome of liver macrophages in metabolic diseases

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RNA editing is an epitranscriptomic process referring to modifications in the chemical structure of RNA molecules. Adenosine-to-Inosine (A-to-I) editing is the most common RNA editing event catalyzed by a family of enzymes called ADARs (adenosine deaminases acting on RNA). While aberrant RNA editing profiles have been associated with many diseases including neurological disorders and cancers, it has not been previously studied in the setting of non-alcoholic fatty liver disease (NAFLD). We have recently discovered that IGFBP7 transcripts in liver macrophage (LMs) of insulin resistant patients underwent A-to-I RNA editing at a high frequency leading to the production of a variant isoform with a higher capacity to regulate insulin signaling. Consistently, preliminary results from RNAseq analysis of 266 liver biopsy samples from two independent studies indicate that the RNA editing rate of NAFLD patients is considerably higher compared to lean and "healthy" obese. However, analyses of the overall RNA editome of LMs revealed that the RNA editing rate is unexpectedly decreased in obesity. In addition, treatment of LMs with a media mimicking the NAFLD environment increased the expression of ADARB2, a negative regulator of RNA editing. These data suggest that the RNA editome of the liver changes dynamically in the setting of metabolic diseases and interestingly, in a cell-specific manner. Understanding the epitranscriptomic landscape of NAFLD and its role in the disease development may lay the foundation for the development of novel therapeutic strategies and new disease biomarkers.

P31

An experimental system for studying metabolism in intact human liver *ex vivo*

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The human liver is central for metabolic homeostasis, and derangements in liver metabolism plays a key role in the development of diabetes and other metabolic disorders. While liver metabolism has been extensively studied in animal models, obtaining data on human liver remains difficult. In particular, metabolic tracing is essential to understand flux through metabolic pathways, but is prohibitively expensive in humans *in vivo*. To address this challenge, we are developing an experimental system for stable isotope tracing in intact human liver tissue cultured *ex vivo*. In this system, freshly resected human liver is sliced into 150–250um sections and cultured in a custom-made medium containing selected ^{13}C nutrients. The resulting ^{13}C incorporation into tissue metabolites and metabolic products released into the medium is measured by high-resolution mass spectrometry. Metabolic fluxes can then be quantified based on an atom-level network model of liver metabolism.
In preliminary experiments, liver tissue structure was well maintained in culture, and nutrient diffusion through the tissue was satisfactory. Transcriptomics data from liver slices showed relatively stable expression of liver-specific enzymes, but also revealed activation of resident immune cells, presumably due to tissue wounding. We found ^{13}C incorporation into hundreds of metabolites, and activity in central liver pathways such as the TCA and urea cycles was readily observable. We also noted clear metabolic differences between human and rat liver cultures, suggesting possible species differences.
Due to the small scale of these experiments, the isotope cost is low (about 50 SEK per culture), which makes larger population studies affordable. In addition, a typical liver sample yields dozens of slices, which allows modulating hormone or nutrient levels, or treatments with metabolic inhibitors or other drugs. We therefore hope that this system will prove useful to better understand the spectrum of liver metabolism in human populations, in health and disease.

P32

Dynamics of the cellular liver landscape across different stages of non-alcoholic steatohepatitis

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The prevalence of metabolic dysfunction-associated fatty liver disease (MAFLD) is increasing at an alarming rate worldwide. Early stage MAFLD is characterised by fat deposition (Steatosis) and progresses into a non-alcoholic steatohepatitis (NASH) and fibrosis. This condition, if untreated, can progress to end-stage liver diseases, cirrhosis or hepatocellular carcinoma (HCC), the main cause for liver transplantation.
The increasing number of patients with MAFLD and the lack of appropriate treatments highlight the pressing need for a better understanding of the underlying cellular and molecular processes driving for the progression of MAFLD. We have demonstrated that liver macrophages (LMs) play an important role in the development of metabolic and oxidative stress associated with MAFLD independent of their inflammatory status. Preliminary data indicate an origin-dependent functional diversity of LMs subpopulations in health and MAFLD. Here we aim to characterise the dynamics and function of these LM subpopulations and their interaction with their neighbouring cells in the progression of MAFLD towards NASH and fibrosis.
In this ongoing study, nuclei of 10 cryopreserved unfixed liver biopsies from patients with confirmed mild or advanced NASH together with 5 cryopreserved control samples without NASH were isolated. To investigate the gene expression and epigenomic profile of parenchymal and non-parenchymal cells by single nuclei RNA sequencing (snRNA-seq) and single cell assay for transposase-accessible chromatin using sequencing (scATAC-seq), libraries were prepared by using the 10X Genomics Multiome platform.
The results of this study will be a valuable resource to get a better understanding of the functional diversity of LMs in NASH progression. This will serve as a starting point for subsequent targeted functional analyses and drug development using pre-clinical models, such as human liver spheroids that recapitulate NASH *in vitro*.

P33

Involvement of the CYP4F family as a culprit in NAFLD complications

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Non-alcoholic fatty liver (NAFLD) is among the leading causes for liver cirrhosis with a global prevalence of 25%. Many factors have been associated with the onset of NAFLD and its progression such as obesity, type 2 diabetes and hyperlipidemia. We recently found an association between NAFLD and elevated levels of the cytotoxic deoxy sphingolipids (SL) in subjects with liver steatosis. To elucidate the etiology of the accumulation of these toxic SL in NAFLD, we used a 3D primary human hepatocyte model of NAFLD. Notably, we found that steatosis in this model resulted in a significant increase in deoxy SL, which was paralleled by a reduction of CYP4F expression. Similar effects were observed in control spheroids treated with the CYP4F inhibitor HET0016, corroborating a central role of CYP4Fs in metabolizing deoxy SL. These results were moreover confirmed using supersome assays, which showed that specifically CYP4F2 and CYP4F12 metabolized deoxy SLs. In summary, our results reveal a novel route of deoxy SL metabolism by CYP4F enzymes and demonstrate that altered CYP4F expression contributes to the accumulation of cytotoxic deoxy SL in NAFLD.

P34

Renoprotective mechanisms of soluble Klotho in diabetic nephropathy

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Podocyte injury is considered a major culprit in the pathogenesis of diabetic nephropathy (DN). In recent years, multiple reports have suggested that soluble Klotho protects against DN by preventing podocyte hypertrophy and apoptosis. In addition to its demonstrated protective actions, Klotho overexpression has also been linked to hyperglycemia and increased resistance to insulin and IGF1, complicating the potential therapeutic use of soluble Klotho in patients with DN. Our hypothesis is that soluble Klotho protects podocyte health and ameliorates DN through systemic regulation of endoplasmic reticulum (ER) stress and unfolded protein response (UPR), despite causing hyperglycemia and reduced insulin sensitivity in non-diabetic subjects. We will pursue three lines of research: 1) To understand how soluble Klotho regulates insulin metabolism in the context of diabetes we will induce DN in transgenic mice overexpressing Klotho in the liver. This mouse line is characterized by high levels of serum Klotho without changes in endogenous renal Klotho expression or urinary Klotho excretion (unpublished data). The effects of Klotho overexpression on insulin sensitivity and glomerular health will be assessed. 2) To study the clinical relevance of soluble Klotho as a potential therapy for DN patients, a novel transgenic mouse line with Tamoxifen-inducible overexpression of Klotho in skeletal muscle will be generated. To mimic as closely as possible a potential future clinical situation, Klotho will be overexpressed in skeletal muscle cells after the induction of DN. The effects of Klotho overexpression on glomerular health, renal function and insulin sensitivity will be assessed. 3) To confirm that the effects of soluble Klotho in DN is conserved in humans, Precision Cut Kidney Slices (PCKS) from tumor free parts of diabetic renal cancer nephrectomies will be used. PCKS will be treated *ex vivo* with soluble Klotho or vehicle control, and effects on ER/UPR, podocyte integrity and fibrosis will be assessed.

P35

Downregulation of erythrocyte miR-210 in diabetes induces endothelial dysfunction by targeting vascular PTP1B

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miR-210 has been shown to play a protective role in atherosclerotic cardiovascular disease, and its levels in whole blood are reduced in mice with type 2 diabetes mellitus (T2DM). We recently demonstrated that red blood cells (RBCs) from patients with T2DM induce endothelial dysfunction. This study aimed to determine the functional role of RBC miR-210 for vascular injury associated with T2DM.

Expression levels of miR-210 and its target protein tyrosine phosphatase 1B (PTP1B) were measured in RBCs from patients with T2DM (T2DM-RBCs) and age-matched healthy controls (H-RBCs) as well as in human endothelial cells and mouse aortas following incubation with human and mouse RBCs using qPCR and immunohistochemistry. Endothelial function in isolated aortas was examined using wire myography following incubation with RBCs.

miR-210 levels were lower in T2DM-RBCs in comparison with H-RBCs. T2DM-RBCs induced endothelial dysfunction, which was reversed by miR-210 overexpression in RBCs. In addition, miR-210 inhibition in H-RBCs and RBCs from miR-210 knockout mice impaired endothelial function. T2DM-RBCs increased PTP1B expression in endothelial cells and aortas, while miR-210 overexpression in T2DM-RBCs normalized PTP1B expression in aortas. PTP1B inhibition in aortas attenuated endothelial dysfunction induced by RBCs from miR-210 knockout mice. Administration of miR-210 mimic in T2DM *db/db* mice *in vivo* not only attenuated endothelial dysfunction induced by RBCs but also normalized elevated PTP1B expression in aortas following incubation with RBCs.

These data shed light on a new pathological mechanism underlying T2DM-associated vascular dysfunction through downregulation of RBC miR-210, which may represent a future pharmacological target.

P36

Attenuated cardiac post-ischemic injury and endothelial dysfunction by stimulation of soluble guanylyl cyclase in red blood cells from patients with type 2 diabetes

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Background: Reduced bioavailability of nitric oxide (NO) contributes to ischemic heart disease in type 2 diabetes (T2D). Red blood cells (RBCs) are known to produce NO bioactivity and to contain a functional soluble guanylyl cyclase (sGC) that is activated by NO. Recent studies revealed that RBCs from patients with T2D exacerbates ischemia-reperfusion (I/R) injury via a mechanism that is dependent on reduced export of NO bioactivity from RBCs. It remains unknown whether stimulation of sGC in RBCs from patients with T2D protects against myocardial IR injury.
Purpose: To test the hypothesis that stimulation of sGC in RBCs from T2D patients protects against myocardial I/R injury.
Methods: RBCs collected from T2D patients and healthy subjects were incubated with vehicle or the sGC stimulator CYR715 before being administered to isolated Langendorff-perfused rat hearts subjected to 25 min global ischemia and 60 min reperfusion. Left ventricular developed pressure (LVDP) and infarct size were determined.
Results: Administration of RBCs from T2D patients impaired post-ischemic recovery of LVDP in comparison with RBCs from healthy subjects ($p<0.001$). Pre-incubation of RBCs from patients with T2D with CYR715 prior to administration to the isolated heart enhanced the recovery of LVDP and reduced infarct size. CYR715 did not induce cardioprotection in the absence of RBCs. The sGC inhibitor ODQ did not significantly affect cardiac recovery *per se* but totally abolished the protective effect of CYR715.
Conclusions: Stimulation of sGC in RBCs from patients with T2D protects against cardiac I/R injury. Stimulation of the NO-sGC pathway in RBCs appears to be an attractive therapeutic strategy to prevent cardiovascular injury in T2D.

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Lipid profile and uremic toxins in patients with end-stage kidney disease

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Background and Aims:
Patients with end-stage kidney disease (ESKD) have an extremely high incidence of cardiovascular (CV) disease, partly driven by insufficient clearance of uremic toxins. To determine if uremic toxins associate with lipid profile in ESKD potentially mediating adverse CV events, we studied a large, trinational cohort using a comprehensive panel of uremic toxins.

Method:
Total, high density lipoprotein (HDL), non-HDL, low density lipoprotein (LDL), and remnant cholesterol, as well as triglyceride, levels were associated with a panel of 11 uremic toxins in a combined cohort of 611 European, adult patients with ESKD from Leuven, Belgium, Stockholm, Sweden and Leipzig, Germany. In all subjects, the panel of uremic toxins was centrally quantified in a single lab by liquid chromatography – tandem mass spectrometry. Univariate correlations were assessed using non-parametric Spearman's rank correlation method, adjusted for multiple testing using Bonferroni correction. To identify independent associations, multivariate linear regression models were used with adjustment for age, sex, and several clinical covariates.

Results:
The median age of the entire cohort was 55 years and 69.1% were treated with dialysis therapy. Male patients showed higher levels of the uremic toxins indoxyl sulphate, kynurenine and kynurenic acid compared to female subjects, whereas all other uremic toxins did not depend on sex. Univariate analyses revealed negative correlations of all cholesterols with most of uremic toxins. Multivariate linear regression analyses confirmed independent, negative associations of indole-3 acetic acid and phenylacetylglutamine with total, non-HDL and LDL cholesterol. Furthermore, trimethylamine-N-Oxide (TMAO) was independently and negatively associated with non-HDL cholesterol. In addition, kynurenic acid was also negatively associated with total cholesterol.

Conclusion:
Significant inverse associations between lipid profile and uremic toxins in ESKD highlight the complexity of the uremic environment in CKD. Our data suggest that not all uremic toxin interactions with conventional CV risk markers may be pathogenic.

P38

Identification and characterization of new AMPK activators for the treatment of streptozotocin/high fat diet-induced diabetic nephropathy in mice

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Diabetic nephropathy (DN) is one of the main complications of diabetes mellitus (DM). DN affects 20-40% of diabetic patients. However, the efficacy of current DN treatment is largely insufficient. Therefore, there is an urgent need to identify novel therapeutic agents that target underlying mechanisms of DN pathogenesis in order to improve renal outcomes of DN patients. DN is associated with insulin resistance in podocyte which result in podocyte dysfunction or loss of podocyte number. Their dysfunction and loss lead to albuminuria. Interestingly, stimulation of AMP-activated protein kinase (AMPK) is known to ameliorate insulin resistance in podocyte and improve renal outcomes *in vivo* models. Therefore, AMPK activators hold promise in the treatment of DN. This study aimed to identify novel AMPK activators in podocytes by screening in-house synthetic compound library and to evaluate their mechanism of action. From the screening of 133 compounds, as analyzed by western blot analysis, $AMPK_{act}$-01 was identified as the most potent AMPK activator in podocyte. Western blot analysis and Fluo-8 calcium flux assay indicate that $AMPK_{act}$-01 activates AMPK via calcium/calmodulin-dependent protein kinase kinase β (CaMKKβ) dependent pathway without alteration in intracellular calcium. Furthermore, therapeutic potential of $AMPK_{act}$-01 for DN treatment was investigated in a DN mouse model, in which C57BL6 mice were exposed to multiple low dose injection of streptozotocin (STZ, 40 mg/kg, i.p.) for 5 consecutive days. Then, mice were fed with high fat diet (HFD) with or without IP004 (20 mg/kg/day/i.p.) for 12 weeks. Interestingly, $AMPK_{act}$-01 treatment significantly decreased fasting blood glucose level and improved glucose tolerance in the STZ/HFD-induced DN mice. Moreover, $AMPK_{act}$-01 treatment ameliorated renal impairment as indicated by reduction of albuminuria. The present study reveals that $AMPK_{act}$-01 may have a potential to be a therapeutic candidate for treatment of DN.
Keywords: Diabetic nephropathy, AMPK activator, AMPK, Streptozotocin, High fat diet

P39

Mendelian Randomization Study on the Causal Effects of Tumor Necrosis Factor Inhibition on Coronary Artery Disease and Ischemic Stroke

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Aim Tumor necrosis factor (TNF) is a potent inflammatory cytokine that has been causally associated with coronary artery disease (CAD) and any ischemic stroke (AIS). We aim to assess whether TNF inhibition may reduce the risk of developing CAD and/or AIS.
Methods Leveraging summary statistics of several genome-wide association studies (GWAS), we assessed the repurposing potential of TNF inhibitors for CAD and AIS using Mendelian randomization (MR) design. Pharmacologic blockade of the pro-inflammatory TNF signaling mediated by TNF receptor 1 (TNFR1) was instrumented by four validated variants. Causal effects of TNF/TNFR1 blockade on CAD ($N_{case/control}$ upto 122,733/424,528) and AIS ($N_{case/control}$ upto 60,341/454,450) were then estimated via various MR estimators using circulating C-reactive protein (CRP; N_{GWAS}=204,402) as downstream effector to reflect treatment effect. Associations of a functional variant, rs1800693, with CRP, CAD and AIS were also examined.
Results No protective effect of TNF/TNFR1 inhibition on CAD or AIS was observed. For every 10% decrease of circulating CRP achieved by TNF/TNFR1 blockade, odds ratio was 0.98 (95% confidence interval [CI]: 0.60-1.60) for CAD and 0.77 (95% CI: 0.36-1.63) for AIS. Findings remained null in all supplement analyses.
Conclusions Our findings do not support TNFR1 as a promising target for CAD or AIS prevention among the general population. Future research are warranted to investigate whether the detrimental effect of circulating TNF on CAD and AIS might be counteracted by modulating other relevant drug targets

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Diet-induced weight loss in obese/diabetic mice promotes functional recovery after stroke

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Post-stroke functional recovery is severely impaired by type 2 diabetes (T2D). This is an important clinical problem since no cure to enhance rehabilitation in T2D stroke patients is available. Because weight loss-based strategies have been shown to decrease stroke risk in people with T2D, we aimed to investigate whether diet-induced weight loss can also improve post-stroke functional recovery and identify some of the underlying mechanisms. T2D/obesity was induced by 6 months of high-fat diet (HFD) feeding in the mouse. Weight loss was achieved by a short- or long-term dietary change replacing HFD with standard diet for 2 or 4 months, respectively. Stroke was induced by middle cerebral artery occlusion and post-stroke recovery was assessed by sensorimotor tests. Both short- and long-term dietary change led to similar weight loss. However, only the latter enhanced functional recovery after stroke. This effect was associated with pre-stroke normalization of fasting glucose and insulin resistance. The global diabetes epidemic will dramatically increase the number of people in need of post-stroke treatment and care. Our unpublished results suggest that diet-induced weight loss leading to pre-stroke normalization of glucose metabolism may have great potential to improve post-stroke rehabilitation in the diabetic population.

P41

Identification of reno-protective molecular signatures in diabetic nephropathy using single cell transcriptomics

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Diabetic nephropathy (DN) affects 20-40% of patients with diabetes. Current therapeutical options for DN are unspecific and often ineffective. Angiotensin convertin enzyme inhibitors (ACEi) are a standard of care for DN today, whereas sodium-glucose cotransporter 2 inhibitors (SGLT2i) have recently proven to be reno-protective and are being introduced to the clinical practice. However, why SGLT2i preserve kidney function is unclear. In this project, investigate the molecular effects of ACEi and SGLT2i in DN. We analyze molecular changes induced by ACEi- and SGLT2i-therapy using single cell transcriptomics in a mouse model of DN, in renal biopsies collected from patients with DN and in a human kidney organoid model treated with ACEi/SGLT2i. Molecular fingerprints from mouse, human and kidney organoids will be integrated to one comprehensive database. We aim to identify common, species- and drug-specific molecular mechanisms activated by SGLT2i/ACEi. Key mechanisms identified will be studied further in transgenic mouse lines in which target genes are activated/inactivated in a cell-specific fashion. Our study can pinpoint biomarkers for drug responses as well as unravel novel therapeutical targets for DN.

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HEREDITARY HYPERCHOLESTEROLEMIA AND ITS IMPACT ON ATHEROSCLEROTIC CARDIOVASCULAR DISEASE IN THE SWEDISH TYPE 1 DIABETES COHORT ASSESSED BY INNOVATIVE DIGIPHYSICAL SCREENING

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Aim
The inherited dyslipidemias Familial Hypercholesterolemia (FH) and elevated Lipoprotein (a) [Lp(a)] cause premature cardiovascular disease (CVD). Similarly, patients with type 1 diabetes (T1D) have increased CVD risk. However, the knowledge about the impact of combined diabetes and dyslipidemia traits is limited.
We aim to:
- diagnose FH in patients with T1D with an innovative open-entry digiphysical screening program.
- investigate the impact of FH, high Lp(a) and, hypercholesterolemia on CVD in patients with T1D.

Methods
In this cross-sectional study, all Swedish patients with T1D (n=48000) will be invited to an open-entry digiphysical prescreening program which combines data from the National Diabetic register (NDR) and patient-reported information through a secure web-based platform. Individuals will be categorized into non-FH or probable-FH (≥6 points) according to a registry-applied version of the Dutch Lipid Clinic Network criteria. Genetical testing for FH will be performed in individuals with probable-FH (approx. N=300). In addition, Lp(a) will be measured in 2000 randomly selected individuals. The screening results will be communicated through the web-based platform. Patients diagnosed with FH will be invited to a digital meeting with a physician.
Based on screening results the impact of FH, high Lp(a), and hypercholesterolemia on CVD will be investigated.

Preliminary results:
We performed a registered based cohort study of patients with T1D enlisted in NDR 2002-2018 (N=42,429). Patients with probable-FH (N=137) had increased hazard ratios (95%CI) for all-cause mortality 3.9 (3.0-5.1) and CVD 4.2 (3.2-5.5), and higher mean (95%CI) LDL-cholesterol (5.59 (5.41-5.77)) compared to non-FH (2.65 (2.64-2.65)) despite higher statin usage.

Conclusions:
This innovative study will offer high-throughput screening and diagnosis of FH in the Swedish T1D cohort by combining patient self-reported data with national quality registry data. The study will increase knowledge about the impact of FH, high Lp(a), and hypercholesterolemia on CVD in T1D patients.

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Is adipose tissue dysfunction driving the development of pro-atherogenic dyslipidemia?

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Obesity and **dyslipidemia** are major risk factors for the development of cardiovascular diseases (CVD), but their interconnectivity remains poorly understood. While **white adipose tissue (WAT) is the largest reservoir of cholesterol in our body**, the consequences of increased cholesterol uptake and storage on human adipocyte function (and dysfunction) require further research.

The **purpose** of this projects is to elucidate: 1) cellular mechanisms regulating adipocyte uptake of cholesterol in obesity; 2) the effects of cholesterol loading on adipocyte function; and 3) how WAT impacts systemic levels of circulating lipoproteins, driving dyslipidemia and subsequent CVD.

Our **preliminary data** of mRNA sequenced isolated human adipocytes shows that obese patients with dysfunctional WAT are associated with higher expression of cholesterol-uptake genes (*LDLR* and *SR-B1*) and lower expression of triglyceride-uptake genes (*VLDL* and *LPL*), which could initiate the development of dyslipidemia. Currently, we are expanding our cohort and validating these findings on protein level.
By using our Human Unilocular Vascularized Adipocyte Spheroid model (HUVAS, Ioannidou et al, 2021), we are studying how cholesterol supplementation impacts adipocyte dysfunction in lean (control) and obese (fattened) adipocyte cultures. In fact, supplementation with micellar cholesterol leads to significantly enlarged lipid droplets and higher leptin secretion. We will now access adipocyte function by quantifying insulin signaling by capillary western blot, and proinflammatory adipokine secretion levels.
Moreover, we are developing a new tamoxifen-inducible mouse model for adipocyte-specific LDLR overexpression to tests to what extent adipose tissue cholesterol loading induces dyslipidemia *in vivo*.

Together these experiments will prove if adipose tissue cholesterol can drive the development of dyslipidemia during obesity and constitutes an interesting target in **CVD prevention**.

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Silencing of methyltransferase Set7 rescues obesity-induced endothelial dysfunction

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It is recently emerging that epigenetic changes play an important role in the development of cardiovascular disease. Metabolic diseases such as obesity and type 2 diabetes (T2D) can increase cardiovascular risk and are alarmingly increasing worldwide. We demonstrated that methyltransferase Set7 is upregulated in peripheral blood mononuclear cells isolated from T2D patients as compared to healthy controls. Set7-induced transcription of NF-kB p65 promoter leads to increased expression of inflammatory and oxidant genes triggering endothelial dysfunction and inflammation in this setting. The current study was designed to investigate whether endothelium-specific Set-7 knock-out mice are protected against obesity-induced endothelial dysfunction.

In this study, we used both endothelium-specific conditional knock-out of Set7 (E-$Set7^{-/-}$) and wild-type (WT) mice. Both groups were fed with normal (ND) or high fat diet (HFD;45 kcal% fat) for 12 weeks to induce obesity. The aortas from different experimental groups were harvested and cut into aortic rings to assess endothelium-dependent relaxations to acetylcholine as well as reactive oxygen species (ROS) generation by ESR spectroscopy. Metabolic parameters e.g. blood glucose, triglyceride and cholesterol levels, were also measured.

Our preliminary data shows that HFD-induced obesity alters endothelium-dependent relaxation to acetylcholine only in WT mice. Interestingly enough, E-$Set7^{-/-}$ mice are protected from obesity-induced endothelial dysfunction and increased ROS production. Our findings provide insights for the role of Set7-induced epigenetic changes in obesity-induced ROS-dependent endothelial dysfunction.

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Plasma purification treatment relieves the damage of hyperlipidemia to PBMCs

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Background
Hyperlipidaemia [hypercholesterolemia (cholesterol>5.18 mmol/L) or hypertriglyceridemia (triglycerides>2.3 mmol/L), mixed hyperlipidaemia (cholesterol>5.18 mmol/L and triglycerides>2.3 mmol/L), and high low-density lipoproteinemia (low-density lipoprotein>3.4 mmol/L)] is a strong risk factor for arteriosclerosis and cardiovascular disease (CVD). Therapy with lipid-lowering drugs often results in many side effects. Our study aimed to investigate the potential effects of non-drug therapy with double-filtration plasmapheresis (DFPP) on lipid metabolism-, endoplasmic reticulum (ER) stress- and apoptosis-related proteins in peripheral blood mononuclear cells (PBMCs) before and after lipid clearance in patients with hyperlipidaemia.
Methods
Thirty-five hyperlipidaemia patients were selected. Proteins related to lipid metabolism [CD36, proprotein convertase subtilisin/kexin type 9 (PCSK9) and LDL receptor], ER stress [glucose-regulated protein 78 (Grp78), C/EBP homoiogousprotein (CHOP), Activating Transcription Factor 4 (ATF4), eukaryotic initiation factor2α (EIF2α)], and apoptosis [B-cell lymphoma-2 (Bcl-2), BAX, and cysteinyl aspartate specific proteinase-3 (Caspase-3)]were assayed by western blot, reactive oxygen species (ROS) were measured by flow cytometry (FCM), and ELISA detected serum inflammatory (IL-1β IL-6, TNF-α) factors.
Results
Compared with their pre-DFPP values, the values of most lipid metabolic parameters, such as cholesterol, triglycerides, LDL, lipoprotein a [Lp(a)] and small dense LDL cholesterol (sdLDL), were reduced after DFPP. DFPP was associated with the downregulation of proteins related to lipid metabolism, ER stress and apoptosis, resulting in decreased ROS and serum inflammatory factor release.
Conclusion
DFPP has lipid-lowering activity and can also regulate lipid metabolism-, ER stress- and apoptosis-related proteins in PBMCs and reduce the levels of inflammatory factors in patients with hyperlipidaemia (ClinicalTrials.gov number: NCT03491956).

P46

Impact of inflammation in diabetic late complications

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This study aims to

I. demonstrate if diabetic complications, e.g. in eyes (retinopathy) and in nerves (neuropathy) are associated with signs of inflammation; and
II. evaluate if anti-inflammatory intervention is of therapeutic value in patients with neuropathy.

Methods: The inflammatory parameter high-sensitive C-reactive protein (CRP) was determined in patient serum. Anti-inflammatory intervention in patients with neuropathy was performed with cibenetide, a novel TNFalpha antagonist, with evaluation of neuropathic pain and HbA1c levels.

Results: CRP levels were 4 times higher in patients with known complications (retino- or neuropathy, n=24) as in those without known complications (n=11). Patient groups had similar age and diabetes duration. Anti-inflammatory treatment for 8 weeks with cibinetide significantly reduced the neuropathic pain and also improved glycemic control, i.e. decreased HbA1c levels.

Conclusions: Low-intensive inflammation may be a part of the mechanism behind diabetic late complications. Hence, anti-inflammatory therapy can be valuable to complement regular treatment or prevention of these complications.

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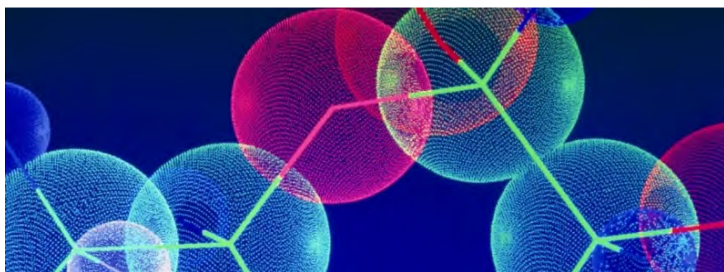

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