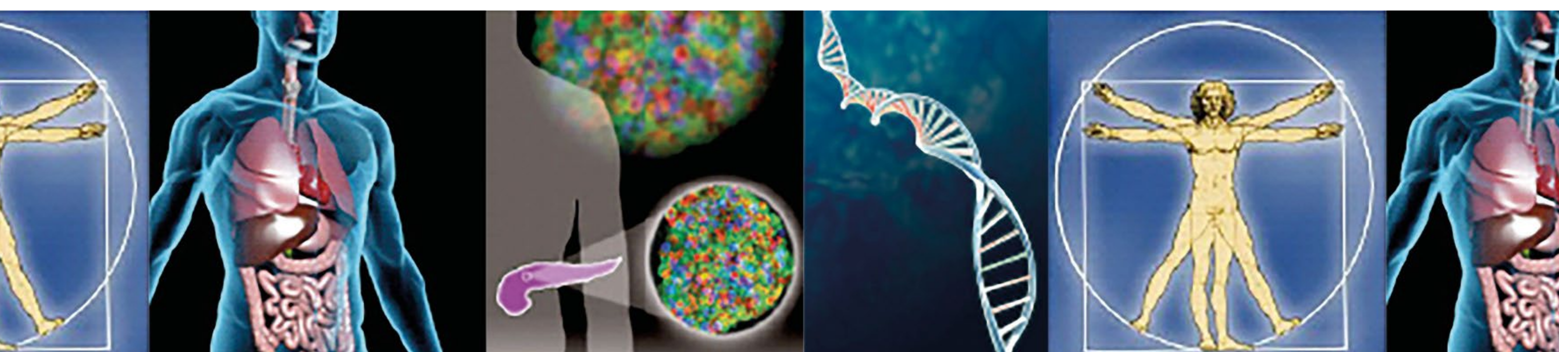
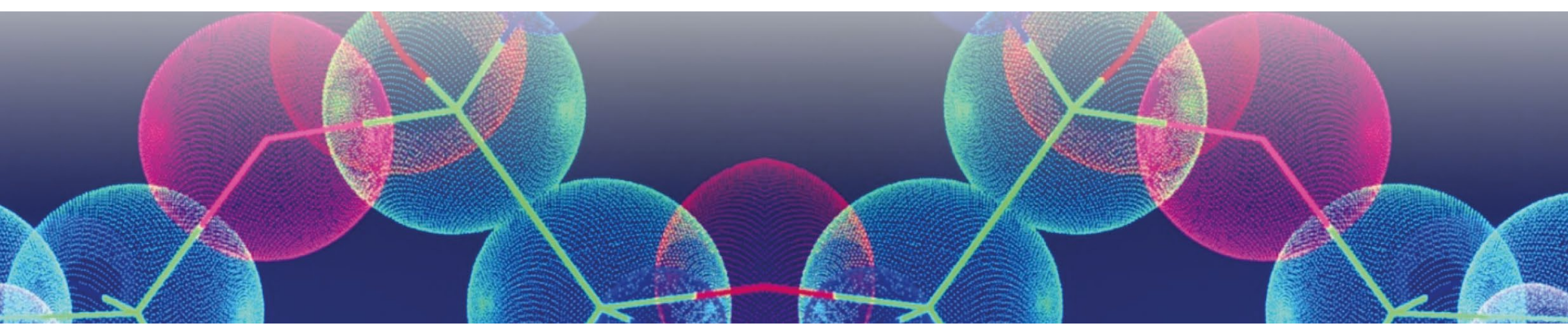
SRP DIABETES | ENDOMET | METENDO

12th SRP Diabetes-EndoMet-MetEndo Retreat



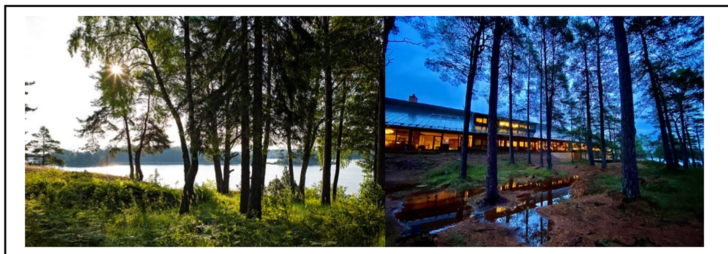
Program and Abstracts

May 15-16, 2023
Djurönäset Hotel & Conference



Venue information

Djurönäset Hotel & Conference
Seregårdsvägen 1
139 73 Djurhamn
(+46)08-571 490 00
info@djuronaset.com
https://www.djuronaset.com/en/the-hotel/



Sauna landscape & Pool

Bring your swim suit! PLEASE NOTE: *The Sauna landscape & 25-meter indoor pool and heated outdoor pool is open for our hotel guests at no further cost. Saunas, indoor pool and outdoor pool open after you checked in 13.30-23.00 May 15 and 06.00-11.00 May 16.*

Gym

There is a gym with cross-trainers, exercise bikes, rowing machines, and free weights. Open 06.00-23.00 daily at no further cost. Located one floor above the pool area.

Walking paths

Ask in the reception on the available paths.

Running track with outdoor gym

A lighted track of 1,3 km with training stations spaced out along the track. Ask in the reception for direction.

Wifi

In public areas at the venue the wifi available is **djuronaset_guest** and is an open network without password. In the seminar hall the wifi is **Kongressen** with password **Kongressen**.

Travel Information

Organized bus transport to and from the venue

Hired buses for the participants will **depart 08.30 sharp on May 15** from the central bus terminal "Cityterminalen" (next to Stockholm Central Train Station) heading for the venue. Look for *"SRP Diabetes Retreat"* on the digital screens to find the right gate in the bus terminal. Please, be there in good time at the gate not to miss the bus departure! The return is 16.20 on May 16 from the venue back to "Cityterminalen". Note: Check your registration details if you have signed up for the bus transfer as the number of seats are limited.

Public transport – bus

There are scheduled local buses (SL, www.sl.se) from Slussen station in Stockholm: buses 433 and 434. Travel time is approximately 50 minutes and the bus stop is named 'Djurönäset'.

By car

Take the road 222 and follow signs towards 'NACKA' and 'GUSTAVSBERG'. Carry on driving on Värmdöleden (road 222). Do not turn off when you see signs for Gustavsberg but follow the road and signs towards 'STAVSNÄS' and 'DJURÖ'. Drive along the 222 and drive past the exit to Stavsnäs but continue on the same road and you will then drive across the Djuröbron (The Djurö bridge). You will find Djurönäset on the right-hand side about 100 m after the bridge**.**

GPS coordinates lat. 59, 2985; long. 18,6740

Poster sessions - Best Poster Awards

May 15, 17.00 - 19.10 Poster Sessions A-B with refreshments served.

Posters will be on display in two areas, in the lobby just opposite the seminar hall and in a bar area down the stairs outside the seminar hall.
The **poster screens** are **1.80 m high and 0.72 m wide**. Materials to attach with will be available.
The **poster numbers** are given at page 30, the first page of the poster section in this book. The number will designate the location of the poster board.
Posters should be **mounted** in the morning or lunchtime **before 14.00, May 15**.
Posters should be removed after the last poster session or latest in the morning the day after, before 09.00 May 16.

Best Poster Awards
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

Scoring posters by participants
All participants will also have the chance to score the posters by PhD students and postdocs to give feedback to the presenters. Please avoid scoring posters where you have conflict of interest. Scoring will be done using the **Mentimeter** online system and a poster specific QR code will be given at each eligible poster screen. **You will need a smartphone/computer connected to the internet to score**.

Please score each criterion with 1-5 (5 best)
1. Oral presentation skills
2. Poster organisation of text and figures
3. Quality of discussion, knowledge around topic
4. Novelty of science presented

Poster Awards will be announced at the dinner on May 15.

Poster session A: 17.10 - 18.10
Presenters for posters with **odd numbers** are expected to stand by their posters prepared to present.
[Note: Poster presenters are expected to prepare a brief 3 min oral presentation].

Poster session B: 18.10 - 19.10
Presenters for posters with **even numbers** are expected to stand by their posters prepared to present.
[Note: Poster presenters are expected to prepare a brief 3 min oral presentation].

Best short talk award

Only **short talks by postdocs and (PhD) students are eligible** for the best short talk award, and are highlighted with *[SCORE BSTA]* in the program. Scoring by the participants will be done directly after each eligible short talk using the **Mentimeter** online system. **You will need a smartphone/computer connected to the internet to score**.

Short talks are scored both for content and for presentation. Please provide a score between 1-5 (5 is best) to each of the four questions directly after each eligible talk:

1) Was the science novel and interesting?
2) Was the quality of and analysis of data good?
3) Was the speaker engaged and enthusiastic about the topic/study?
4) Were the slides easy to follow to convey the aim and the conclusion?

Info on link/QR code for the scoring system will be given at the meeting.

The best short talk award will be handed out at the end of the meeting.

Program

Monday May 15

08.30 Bus departure Cityterminalen (bus terminal next to Stockholm Central train station)

09.30 Bus arrival to the venue Djurönäset

Coffee + sandwich, mounting posters

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

10.10 - 10.30 Welcome note Anna Krook (SRP Diabetes), EndoMet+MetEndo repr

Session 1: Diabetes complications and cardiovascular disease Chairs: Zhichao Zhou and Anna Witasp

10.35 - 11.05 Ann Marie Schmidt (New York U) *"RAGE-DIAPH1 - New Insights on Cardiometabolic Disease from the Inside - Out!"*

11.10 - 11.25 Julia Sánchez-Ceinos *"Epigenetically-driven transmission of cardiovascular risk in offspring exposed to gestational diabetes: role of histone methyltransferase MLL1" [SCORE BSTA]*

11.30-12.00 Coffee Break

12.00 - 12.15 Allan Zhao *"Male but not female offspring of dams with well-controlled type 1 diabetes develop endothelial dysfunction without obvious metabolic derangement" [SCORE BSTA]*

12.20 – 12.35 Eduard Iustian Ghemeș "*Repression of hypoxia miR-210 contributes to the development of diabetes kidney disease" [SCORE BSTA]*

12.45-13.45 Lunch + mounting posters
13.45- 14.40 Networking

Session 2: Non-alcoholic fatty liver disease Chair: Shane Wright

14.40 - 14.55 Annelie Falkevall *"Inhibition of VEGF-B signaling prevents non-alcoholic fatty liver disease development by targeting lipolysis in the white adipose tissue"*

15.00 – 15.15 Peter Saliba Gustafsson *"A functional genomic framework to elucidating novel causal nonalcoholic fatty liver disease genes"*

15.20-15.45 Coffee break + check-in to your rooms

Session 3: Islet physiology and pathology Chair: Noah Moruzzi

15.45 - 16.15 Jakob Grunnet Knudsen (U Copenhagen) *"Alpha cell metabolism - A new framework for the regulation of glucagon secretion"*

16.20 – 16.35 Joakim Lehrstrand *"Optical 3D characterization of the complete human β-cell mass in health and diabetes - evidence for local pockets of resistance?"*

16.40 – 16.55 Patricia Recio-López "*The fate of the pancreatic islet is decided by its environment***"** *[SCORE BSTA]*

17.00 - 19.10 Poster Sessions A-B with refreshments served

Posters are on display in two areas, in the lobby just opposite the seminar hall and in a bar area down the stairs outside the seminar hall.

Poster session A: 17.10 - 18.10
Presenters for posters with **odd numbers** are expected to stand by their posters prepared to present.
[Note: Poster presenters are expected to prepare a brief 3 min oral presentation].

Poster session B: 18.10 - 19.10
Presenters for posters with **even numbers** are expected to stand by their posters prepared to present.
[Note: Poster presenters are expected to prepare a brief 3 min oral presentation].

19.30 Dinner and activities – Best Poster Awards

Tuesday May 16

07.00-09.00 Breakfast + check out from your rooms + take down posters

Session 4: Clinical diabetology Chair: Thomas Ebert

09.00 - 09.30 Chantal Mathieu (UZ Leuven) *"The new face of clinical diabetes"*

09.35 - 09.50 Youssef Chninou "*eHealth – A new approach to achieve diabetes remission" [SCORE BSTA]*

09.55-10.15 Coffee Break

Session 5: Adipose, skeletal muscle metabolism Chair: Scott Frendo-Cumbo

10.15 - 10.45 Tore Bengtsson (Stockholm U) *"Beta-adrenergic stimulation and glucose homeostasis"*

10.50 - 11.05 Jutta Jalkanen *"White adipose tissue cytoarchitecture in obesity across depots" [SCORE BSTA]*

11.10 - 11.25 Kaisa Hofwimmer *"Interleukin 1β regulates white adipose tissue remodeling by targeting adipocyte precursors" [SCORE BSTA]*

11.30-12.30 Lunch + networking

12.30 - 12.45 Tova Eurén "*Diacylglycerols in the interplay between peripheral insulin sensitivity, myofiber composition and ethnicity" [SCORE BSTA]*

12.50 - 13.05 Baptiste Jude "*Can PGC-1α1 counteract skeletal muscle capillary rarefaction and reduced glucose uptake induced by chronic inflammation?" [SCORE BSTA]*

13.10-13.20 Break

Session 6: Inflammation and metabolism Chair: Nicolas Pillon

13.20 - 13.35 Achilleas Fardellas "*ADAR1 suppresses meta-inflammation in myeloid cells" [SCORE BSTA]*

13.40 - 13.55 Sara Torstensson "*The immune profile in adipose tissue of a mouse model of polycystic ovary syndrome" [SCORE BSTA]*

14.00 - 14.15 Ahmed Abdelmoez *"Thromboxane is elevated in men after exercise and improves skeletal muscle glucose uptake and whole-body glucose homeostasis" [SCORE BSTA]*

14.20-14.50 Coffee Break

Session 7: Circadian Rhythms and metabolism Chair: Elin Chorell

14.50 – 15.20 Kenneth Dyar (Helmholtz Munich) "*Circadian Catecholamine Sensitivity: Does Time Matter?*"

15.25 – 15.40 Kirstin MacGregor "*The transcriptional response to acute exercise is HIF1α dependent and time-of-day specific" [SCORE BSTA]*

15.45-16.00 Concluding remarks and Short Talk Award
Anna Krook (SRP Diabetes), EndoMet+MetEndo repr

16.20 Bus Departure to Stockholm cityterminal

Speaker Abstracts

Session 1: Diabetes complications and cardiovascular disease

RAGE-DIAPH1 1 - New Insights on Cardiometabolic Disease from the Inside – Out!

Ann Marie Schmidt, M.D.
New York University Grossman School of Medicine, NY NY

The Receptor for Advanced Glycation End Products (RAGE) transduces the effects of Damage Associated Molecular Pattern (DAMP) molecules, which accumulate in metabolic and ischemic stresses. RAGE and its ligands are upregulated in diabetes and its complications, obesity, atherosclerosis and ischemic tissues. RAGE is expressed on cell types such as immune cells, vascular cells, cardiomyocytes, hepatocytes and adipocytes. Our research suggests that RAGE's natural function is the dampening of energy expenditure, at least in part through ligand-dependent suppression of Protein Kinase A phosphorylation of hormone sensitive lipase and p38 mitogen activated protein kinase. *In vivo*, global or adipocyte-specific deletion of *Ager* (gene encoding RAGE) protects mice from high fat diet-induced obesity, glucose and insulin insensitivity and adipose inflammation.

Pivotal to understanding RAGE mechanisms in cardiometabolic disease was the discovery that the binding of the cytoplasmic domain of RAGE to the formin, Diaphanous 1 (DIAPH1), regulates RAGE signal transduction. Numerous studies have demonstrated key roles for RAGE in the progression and regression of atherosclerosis; recent work showed that deletion of *Diaph1* in mice devoid of the *Ldlr* resulted in significant reduction in atherosclerosis in mice, which was accompanied by reduced plasma and hepatic concentrations of cholesterol and triglycerides. RNA-sequencing of hepatic tissues unveiled unexpected roles for RAGE/DIAPH1 in hepatic lipid metabolism, at least in part through the regulation of nuclear translocation of SREBP1, without differences in its mRNA or total protein expression. Regulation of nuclear translocation of SREBP1 via DIAPH1 was dependent, at least in part, on modulation of actin cytoskeleton organization, through phosphorylation of Cofilin 1 in hepatocytes.

The discovery of small molecules that block RAGE-DIAPH1, such as RAGE229, hold promise for treatment of cardiometabolic diseases. Recent work illustrated that administration of RAGE229 to mice with obesity undergoing weight loss further accelerated loss of body mass and adiposity and the reversal of insulin resistance and glucose intolerance. Even in lean mice fed a low fat diet, treatment with RAGE229 resulted in assumption of a more healthful body mass and composition, and glucose and lipid metabolism.

In summary, through "inside-out" regulation of intracellular signal transduction and actin cytoskeleton organization, RAGE/DIAPH1suppresses energy expenditure. Although of benefit in nutrient deprivation, in contrast, in nutrient excess, this pathway contributes to pathological body mass and adiposity and the accumulation of lipid substrates – consequences of which include maladaptive inflammation and insulin resistance. Unleashing this brake is a novel treatment to break the cycle of obesity and cardiometabolic dysfunction.

Epigenetically-driven transmission of cardiovascular risk in offspring exposed to gestational diabetes: role of histone methyltransferase MLL1

Julia Sánchez-Ceinos[1], Nadia Di Pietrantonio[1, 2], Mariana Shumliakivska[1], Alexander Rakow[3], Domitilla Mandatori[2], Pamela Di Tomo[2], Gloria Formoso[4], Tiziana Bonfini[5], Maria Pompea Antonia Baldassarre[4], Maria Sennström[6], Wael Almahmeed[7], Assunta Pandolfi[2], Francesco Cosentino[1]

[1] *Cardiology Unit, Dept. of Medicine, Solna, Karolinska Institute; Karolinska University Hospital; Stockholm, Sweden.*

[2] *Dept. of Medical, Oral, and Biotechnological Sciences, Center for Advanced Studies and Technology-CAST, University G. D'Annunzio of Chieti-Pescara, Chieti, Italy.*

[3] *Dept. of Neonatology, Karolinska University Hospital, Stockholm, Sweden.*

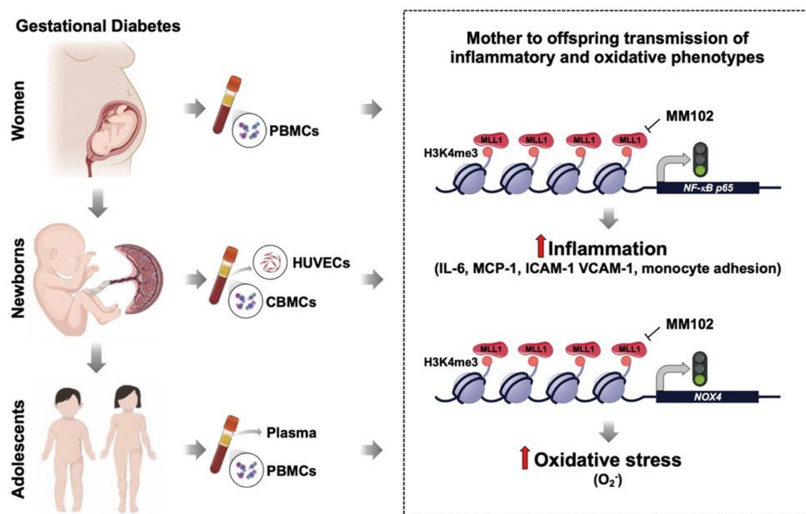
[4] *Dept. of Medicine and Aging Sciences, Center for Advanced Studies and Technology-CAST, University G. D'Annunzio of Chieti-Pescara, Chieti, Italy.*

[5] *Dept. of Oncology Hematology, Spirito Santo Hospital, Pescara, Italy.*

[6] *Dept. of Women's and Children's Health, Karolinska University Hospital, Stockholm, Sweden.*

[7] *Heart and Vascular Institute, Cleveland Clinic Abu Dhabi, Abu Dhabi, UAE.*

Abstract text: Environmental stimuli *in-utero* can permanently affect health and vulnerability to diseases later in life. Indeed, the offspring of women with gestational diabetes (GD) exhibit a high risk for obesity, diabetes, and cardiovascular complications in adulthood. Epigenetics has emerged as a crucial determinant for this transmission. Histone methyltransferase MLL1 activates inflammation and oxidative stress through the trimethylation of H3 at lysine 4 (H3K4me3). However, whether this regulation also occurs in GD-women or if it can be transmitted to the progeny remains unknown. Here, we aimed to investigate the role of MLL1-induced H3K4me3 in the mother-to-offspring transmission of inflammatory and oxidative stress processes in GD. Thus, we isolated peripheral blood mononuclear cells (PBMCs), cord blood mononuclear cells (CBMCs), and umbilical cord vein endothelial cells (HUVECs) from GD and control pregnant women, and PBMCs and plasma samples from a retrospective cohort of adolescents born to control- or GD-women. Gene/protein expression was investigated by RT-PCR, immunoblotting, and/or confocal microscopy. H3K4me3 levels were determined by chromatin immunoprecipitation (ChIP). Mechanistic analyses were performed in HUVECs using MLL1 inhibitor MM-102. Our data show increased levels of MLL1 and H3K4me3 in PBMCs, HUVECs, and CBMCs isolated from GD- *vs.* control-women. ChIP analysis revealed H3K4me3 enrichment at the promoter region of NF-kB p65 and NOX4 in these cells. Notably, H3K4me3 was also increased in NF-kB p65 promoter in PBMCs from adolescents born to GD- *vs.* control-women. All cells exhibited enhanced content of NF-kB p65, its target genes (IL-6, MCP-1, ICAM-1, VCAM-1), and reactive oxygen species. Finally, inhibition of MLL1 reduced H3K4me3 levels and restored the pro-inflammatory and -oxidant phenotype in GD-HUVECs. Taken together, our findings suggest that GD induces inflammation and oxidative stress through MLL1-mediated H3K4me3. Our results also unravel the potential transmission of this epigenetic modification to the GD-offspring, which may have further consequences for their cardiovascular health.



Male but not female offspring of dams with well-controlled type 1 diabetes develop endothelial dysfunction without obvious metabolic derangement

Allan Zhao[1], Alice Larsson[1], Eftychia Kontidou[2], Sanjiv Risal[1], Aida Collado[2], Jacob Grünler[3], Haojiang Lu[1], Sara Torstensson[1], Eva Lindgren[1], Xiaowei Zheng[3], Zhichao Zhou[2], Sergiu-Bogdan Catrina[3], Qiaolin Deng[1]

[1] *Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden*
[2] *Division of Cardiology, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden*
[3] *Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden*

Abstract text: Background: Cardiovascular complications associated with diabetes constitute major clinical problems which contribute to increasing mortality and morbidity among these patients. Epidemiological studies have suggested that the offspring of mothers with type 1 diabetes (T1D) are at a higher risk of developing cardiovascular complications. However, it remains unexplored whether this elevated risk is confounded by the increased rate of metabolic disease in offspring to mothers with T1D, or an independent occurrence.

Methods: T1D was induced in female mice using streptozotocin (STZ) in repeated low-dose intraperitoneal injections (50mg/kg, 5 days). Controls received PBS. Mice were kept for 10 weeks after induction to mimic human conditions, where early onset of T1D leads to long-term disease exposure before reproduction. Afterwards, mice were mated with healthy male mice. Offspring were weighed biweekly until 16 weeks of age. Then, oral glucose tolerance test (OGTT), metabolic cage analysis, and body composition analysis were performed. Moreover, endothelium-dependent relaxation (EDR), a well-established readout for endothelial function, of isolated aortas was assessed using a wire myograph.

Results: STZ-induced dams had normal fasting blood glucose levels, but clear glucose intolerance once challenged. Maternal HbA1c levels were only slightly elevated. Both male and female STZ offspring had similar weight gain compared to controls until 16 weeks of age. Moreover, both male and female STZ offspring displayed normal OGTT response, insulin sensitivity, respiratory exchange rate, and energy expenditure, implying absence of metabolic derangement. Interestingly, EDR was markedly impaired in isolated aortas from male but not female offspring. This suggests that endothelial dysfunction in male offspring of T1D mothers occurs independently of detectable metabolic alterations.

Conclusions: Male offspring of T1D mothers with acceptable metabolic control develop endothelial dysfunction without obvious metabolic derangement. These observations highlight the potential importance of identifying and monitoring male offspring of diabetic mothers as a high-risk population for cardiovascular disease.

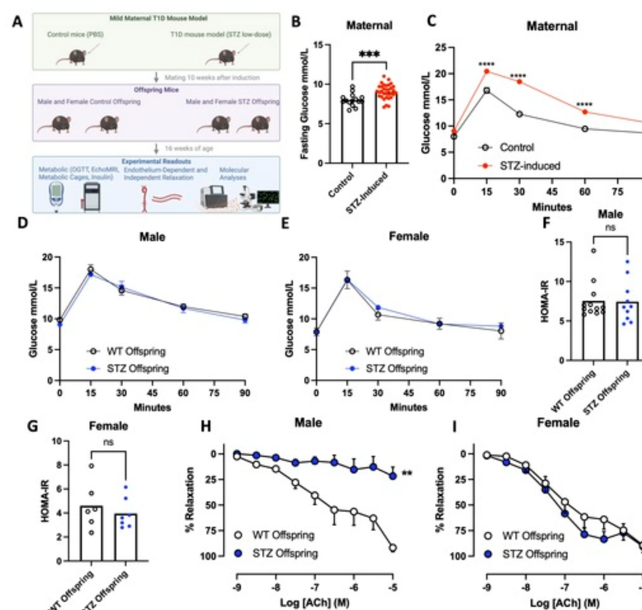


Figure 1. Maternal T1D leads to endothelial dysfunction in male offspring without obvious metabolic derangement. (A) Schematic overview over study. **(B)** Fasting blood glucose for maternal mice. **(C)** OGTT curve for maternal mice. **(D-E)** OGTT curve for male **(D)** and female **(E)** offspring. **(F-G)** HOMA-IR for male **(F)** and female **(G)** offspring. **(H-I)** Endothelium-dependent relaxation for male **(H)** and female **(I)** offspring. STZ, Streptozotocin; WT, Wild Type; OGTT, Oral Glucose Tolerance Test; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance. **, *P<0.01*; ***, *P<0.001*; ****, *P<0.0001.*

Repression of hypoxa miR-210 contributes to the development of diabetes kidney disease

Graciela Teran Vasquez[1], Eduard Iustian Ghemeş[1], Li Jiang[1], Jacob Grünler[1], Cheng Xu[1], Sampath Narayanan[1], Ran Luo[1], Stelios Karayiannides[1], Henrik Falhammar[2], Mircea Ivan[3], Xiaowei Zheng[1], Sergiu Catrina[1, 2, 4]

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[3] *Departments of Medicine, Microbiology and Immunology, Indiana University, Indianapolis, IN, USA.*

[4] *Center for Diabetes, Academic Specialist Centrum, Stockholm, Sweden*

Abstract text: Diabetic kidney disease (DKD) is a major cause of mortality in patients with diabetes. Recently, hypoxia has been identified as an important pathogenic factor for DKD. However, the adaptive responses to hypoxia are impaired in diabetic kidney due to the inhibition of Hypoxia Inducible Factor-1 (HIF-1) signaling.

MicroRNA-210 (MiR-210) is a unique microRNA that is exclusively regulated by HIF-1 signalling. It influences the expression of genes that are involved in the adaptive responses to hypoxia. We have shown that the decreased miR-210 expression contributes to the impaired wound healing in diabetes. In this study, we want to investigate the role of miR-210 in the development of DKD.

We first analyzed plasma miR-210 expression in patients with type 1 diabetes (T1D) and its correlation with DKD. We found significant decrease in miR-210 levels in plasma from patients with DKD. The plasma miR-210 correlate negatively with blood HbA1c levels and positively correlate with the estimated Glomerular Filtration Rate (eGFR), indicating a potential role of plasma miR-210 as biomarker for the development of DKD. Moreover, the correlation between plasma miR-210 levels and DKD is maintained in the multiple regression analysis even when disease duration and HbA1c were included, suggesting its independent prognostic value for DKD. In order to further investigate the role of miR-210 in the development of DKD, we generated miR-210 KO mice, and induced diabetes in these mice. MiR-210 gene knockout increased ROS levels and aggravated albuminuria in diabetic mice.

These results suggest that the repression of miR-210 contributes to the development of DKD. We will further assess the changes in histology and transcriptomics in the mouse kidneys, and study the role of miR-210 in regulating the metabolism, ROS and apoptosis of tubular epithelial cells. We will also test the therapeutic effects of miR-210 mimic on DKD using db/db diabetic mice.

Session 2: Non-alcoholic fatty liver disease

Inhibition of VEGF-B signaling prevents non-alcoholic fatty liver disease development by targeting lipolysis in the white adipose tissue

Annelie Falkevall[1], Annika Mehlem[1], Erika Folestad[1], Frank Chenfei Ning1[1], Óscar Osorio-Conles[2], Rosa Radmann[1], Ana de Hollanda[3, 4], Samuel D Wright[5], Pierre Scotney[6], Andrew Nash[6], Ulf Eriksson[1]
[1] *Division of Vascular Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden*
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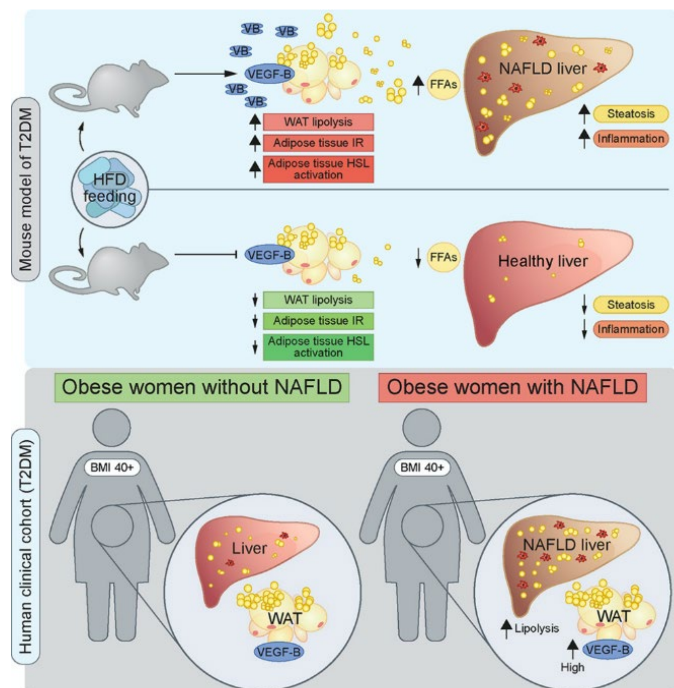
[3] *Obesity Unit. Clinical Hospital of Barcelona, Barcelona, Spain*
[4] *Centro de Investigación Biomédica en Red Fisiopatologia de la Obesidad y Nutrición (CIBEROBN), Madrid, Spain*
[5] *CSL Behring, King of Prussia, PA, USA*
[6] *CSL Innovation Pty Ltd, Parkville, Victoria, Australia*

Background & Aims: Hepatosteatosis is a hallmark of Non-alcoholic fatty liver disease (NAFLD), a common co-morbidity in Type 2 diabetes (T2DM). The pathogenesis of NAFLD is complex and involves the crosstalk between the liver and the white adipose tissues (WAT). Vascular Endothelial Growth Factor B (VEGF-B) has been described to control tissue lipid accumulation by regulating the transport properties of the vasculature. The role of VEGF-B-signaling and the contribution to hepatosteatosis and NAFLD in T2DM is currently not understood.

Methods: C57BL/6J mice treated with a neutralizing antibody against VEGF-B, or mice with adipocyte-specific overexpression- or under-expression of VEGF-B (Adipoq^{Cre+}/VEGF-B$^{TG/+}$ mice and Adipoq^{Cre+}/*Vegfb*$^{fl/+}$mice) were subjected to a 6-month high-fat diet (HFD), or chow-diet, whereafter NAFLD development was assessed. WAT biopsies from patients with obesity and NAFLD in a pre-existing clinical cohort (n=24 patients with NAFLD and n=24 without NAFLD) were analysed for VEGF-B expression and correlated to clinicopathological features.

Results: Pharmacological inhibition of VEGF-B signaling in diabetic mice reduced hepatosteatosis and NAFLD by blocking WAT lipolysis. Mechanistically we show, by using HFD-fed Adipoq^{Cre+}/VEGF-B$^{TG/+}$ mice and HFD-fed Adipoq^{Cre+}/*Vegfb*$^{fl/+}$mice, that inhibition of VEGF-B signaling targets lipolysis in adipocytes. Reducing VEGF-B signaling ameliorated NAFLD by decreasing WAT inflammation, resolving WAT insulin resistance, and lower the activity of the hormone sensitive lipase. Analyses of human WAT biopsies from NAFLD subjects supported that the VEGF-B signaling pathway contributes to NAFLD development. VEGF-B expression levels in adipocytes from two WAT depots correlated with development of dysfunctional WAT and NAFLD in human subjects.

Conclusions: Taken together, our data from mouse models and human subjects suggest that VEGF-B antagonism may represent an approach to combat NAFLD by targeting hepatosteatosis through suppression of lipolysis.



A functional genomic framework to elucidating novel causal nonalcoholic fatty liver disease genes

Peter Saliba Gustafsson[1, 2, 3, 4], Johanne M. Justesen[1, 5], Jiehan Li[1, 3, 4], Disha Sharma[1, 4], Amanda Ranta[1, 3, 4], Ping Chen[6], Laeya A. Najmi[1, 3, 4], Mike Gloudemans[7], Themistocles L. Assimes[1, 8], Ivan Carcamo-Orive[1, 3, 4], Chong Park[1, 4], Thomas Quertermous[1, 4], Joshua W. Knowles[1, 3, 4, 9]

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[5] *Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Denmark*
[6] *Department of Laboratory Medicine, Karolinska Institutet*
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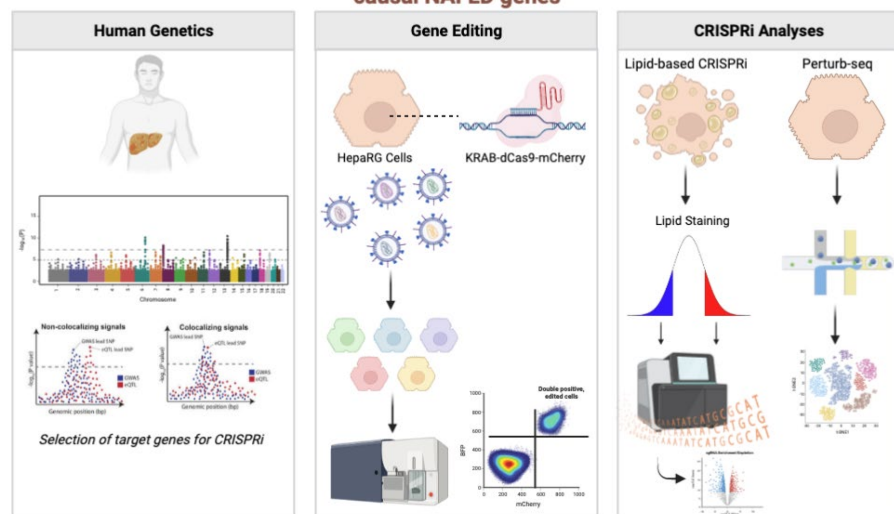
[8] *VA Palo Alto Health Care System, Palo Alto CA, USA*
[9] *Stanford Prevention Research Center, Stanford, CA, USA*

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver conditions, with serious public health consequences. Globally, about 25% of adults are estimated to suffer from NAFLD, and the most common cause of death amongst these patients is cardiovascular disease. Definitive diagnosis of NAFLD relies on imaging, where the gold standard being abdominal MRI. However, abdominal MR is not typically conducted on asymptomatic individuals, meaning NAFLD may go undiagnosed for years. Genome-wide association studies have been used to identify associations between common genetic variants and NAFLD, however, due to scarcity of imaging data, identification of risk loci for NAFLD has been much slower than for anthropometric (e.g., body mass index) or biochemical measures, and other complex cardiometabolic diseases such as obesity, and diabetes.
Aim: To increase our knowledge on the genetic basis of NAFLD, and test the genes' influence on hepatocyte biology *in vitro* and NAFLD *in vivo*.
Results/conclusion: We create a NAFLD score (NAFLDS), which is a composite surrogate for NAFLD consisting of anthropometric and biochemical variables to predict liver fat using multivariate modelling. We run a genome-wide association study in the UK Biobank using our NAFLD surrogate, and confirm known, and elucidate new genetic basis of NAFLD. We explore associations using genetic colocalization studies to prioritize genes for large-scale CRISPRi and Perturb-seq experiments. We identify novel putative NAFLD-associated loci and characterize a subset of these in *in vitro* using large-scale CRISPRi screens and Perturb-seq, and *in vivo* models of NAFLD/NASH. Our results suggest a role for the *VKORC1* gene in NAFLD, which has also been suggested by several independent research groups. Above all we present a functional genomic framework to study putative NAFLD genes.



Session 3: Islet physiology and pathology

Alpha cell metabolism - A new framework for the regulation of glucagon secretion

Jakob G Knudsen, *University of Copenhagen*

Alpha cells secrete glucagon when circulating glucose levels are low to increase glucose production from the liver, which is an essential part of the counterregulatory response. Although glucagon secretion is important during hypoglycaemia, alpha cells also seem to play an important role in the development of diabetes, where dysregulated glucagon secretion leads to impaired regulation of hepatic metabolism and is thought to contribute to the elevated circulating glucose levels. Despite this we still do not understand how glucose regulates glucagon secretion.

The current hypothesis suggests that glucose regulates glucagon secretion through paracrine signalling or intrinsic mechanisms in which elevations in glucose leads to increased intracellular ATP and closure of the ATP sensitive potassium channels, depolarizing the membrane and reducing glucagon secretion. However, alpha cells oxidise very little glucose and use fatty acids as substrate for ATP production. This could suggest that alpha cell metabolism is different than previously thought. We have therefore explored alpha cell metabolism and electrical activity under conditions with physiological levels of glucose and fatty acids.

Optical 3D characterization of the complete human β-cell mass in health and diabetes - evidence for local pockets of resistance?

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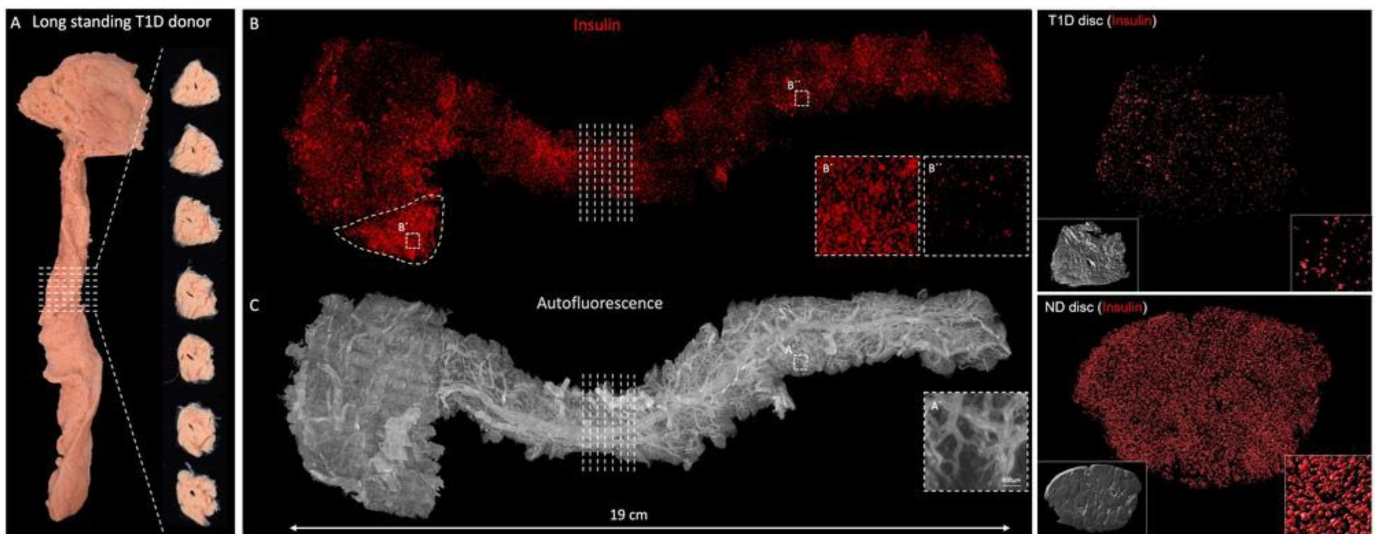
Although major advances have been made in the field of mesoscopic imaging and associated tissue clearing protocols, these still fall short in imaging of specifically labelled structures in larger specimens, primarily due to poor penetration of labelling agents. We have developed an approach by which the entire volume of the human pancreas can be labelled with antibody markers and visualized at micrometre resolution. In order to assess the possibility to determine the global impact on pancreatic ß-cell mass in pancreata from diabetic individuals, we imaged individual > cm3-sized tissue discs of entire pancreata with optical projection tomography and light sheet fluorescence microscopy. The resultant tomographic data files were subsequently reconstructed back into 3D space, providing the first whole organ account of the 3D volume distribution of insulin producing β-cells in non-diabetic (ND) and T1D (long standing) donors.

In contrast to previous reports, our findings show the ß-cell mass to be relatively homogenously distributed across the ND pancreas. By contrast, insulin positive objects were reduced 12-fold in the T1D pancreas, with a significant proportion remaining specifically in the pancreatic head and the uncinate process in particular. Interestingly, ß-cells in this region appear more resilient (8x more ß-cells) despite showing a high degree of immune infiltration. We hypothesize that this phenomenon could be attributed to either an exhaustion immunophenotype, alternatively an ability of remaining ß-cells to resist the autoimmunity. Further, we identified regions showcasing mature ß-cells grouped in clusters, completely lacking other endocrine cell types, appearing consistently across the pancreas. The identification of local factors influencing/promoting these ß-cell distribution patterns may contribute to the identification of targets for protective, preservative or even therapeutic regimens in T1D.



The fate of the pancreatic islet is decided by its environment

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Type-1 diabetes mellitus (T1D) is an autoimmune disease in which the pancreatic b-cells are destroyed. The mechanisms are still not clear, but a combination of environmental, immune and genetic factors are involved. **Aim:** By using the innovative approach to transplant islets into the anterior chamber of the eye (ACE), which enables noninvasive and longitudinal studies *in vivo* of the islets, we addressed whether β-cells in islets from a prediabetic donor transplanted into the ACE of a healthy recipient or *vice versa* will survive.
Methods: 25-day old diabetes-prone (DP), which develop a human-like T1D at about 60-days, and diabetes-resistant (DR) BioBreeding (BB) rats, were used. Pancreatic islets from DPBB rats were transplanted into the ACE of DRBB rats and *vice versa*. Three-weeks post-transplantation, islets were examined for islet integrity, vascularization and vascular leakage after intravenous injection of three different molecular weight dextrans. Infiltration of phagocytic cells was investigated 24h after the dextran injections. A second *in vivo* imaging session was performed five-weeks post-transplantation.
Results: After three weeks, there were no differences between the transplantation groups. All islets were vascularized, without signs of inflammation as assessed by vascular leakage and infiltration of phagocytic cells. However, two weeks later, there was a change in size of the healthy islets in DPBB rats compared to prediabetic islets in the healthy environment that remained unchanged. Vessels of the healthy islets in the prediabetic milieu showed more leakage as a sign of inflammation, while the prediabetic islets in the DR recipients remained normal. No differences were observed in the number of phagocytic cells between the groups.
Conclusions: Islets from prediabetic rats transferred to healthy animals are preserved and, healthy islets are affected by a prediabetic milieu. This shows that it is the *in vivo* environment that decides the fate of the islet, not the islet itself.

Session 4: Clinical diabetology

The new face of clinical diabetes

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Since the first clinical use of insulin, more than one hundred years ago, the face of diabetes has dramatically changed. Diabetes turns out to be a 'hydra' with many faces, with many pathophysiological routes, with many diagnostic paths and more importantly with many therapeutic opportunities. The last 20-30 years have seen an explosion in our knowledge and in our therapeutic approach of people living with diabetes, ranging from the introduction of novel insulins and novel technologies for measuring glucose and administering insulin, to the availability of direct organ protecting agents and disease modifying therapeutics, in particular in type 2 diabetes, but more recently also in type 1 diabetes. Research is moving on rapidly, with the promise of precision medicine for all just around the corner. In the whirlwind of progress, it will remain important to stay focused on what really matters: the quality of life of the person living with diabetes. For people to live longer and healthier lives, not only tools and techniques are important, but even more so education, motivation, accompaniment of the person living with diabetes. Making the person with diabetes a member of the multidisciplinary team will ultimately determine success. The way we communicate all the novelties and make them matter, really matter for those with diabetes, is crucial and we should never forget that there are as many faces to diabetes as there are people living with this disease. Importantly, we need to strive for an all-inclusive strategy in diabetes care: access to care should be there for all… independent on age, gender, where you are born in the world, your socio-economic status…. And probably that is the greatest challenge to be faced in the next years. A challenge however this community can and WILL overcome.

eHealth – A new approach to achieve diabetes remission

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Objective: In the recent DiRECT study, remission of type 2 diabetes was achievable through intense weight loss by using a low-calorie diet. We aimed to assess if this approach is possible using eHealth.

Methods: The intervention comprised total diet replacement (825-853 kcal/day) for 12 weeks, stepped food reintroduction (6 weeks), and a weight stability phase (8 weeks). Antidiabetic and antihypertensive drugs were withdrawn during the study start. The participants were supplied with a scale, a finger-stick blood glucose meter, and a blood pressure monitor. They were advised to measure body weight, blood glucose, and blood pressure every morning during the study. The equipment was connected to their mobile phones by Bluetooth and the results were directly transferred to the study staff. Revisits and contact with the study staff were made only through video calls, phone calls, and chats. The primary outcomes were weight loss of at least 15 kg and remission of diabetes, defined as glycated hemoglobin (HbA_{1c}) of less than <48 mmol/mol without any diabetes medications. The study duration was 6 months.

Results: Ten people who had been diagnosed with type 2 diabetes within the past 6 years (age 58.5 ± 7.4 years, BMI 35.3 ± 5.9 kg/m^2, weight 114.3 ± 16.5 kg, HbA1c 50.5 ± 8.4 mmol/mol, nine male and one female) were recruited. At 6 months, 7 of 10 participants achieved a weight loss of 15 kg or more. Participants' body weight decreased by 16.9 ± 5.4 kg ($p<0.001$). Diabetes remission was achieved in all 10 participants. HbA1c decreased by 11.0 ± 8.4 mmol/mol ($p<0.01$).

Conclusion/Interpretation: Our findings show that, at 6 months, all participants achieved diabetes remission using eHealth. However, further research is needed to confirm these findings in larger populations over a longer period.

Session 5: Adipose, skeletal muscle metabolism

Beta-adrenergic stimulation and glucose homeostasis

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Adrenergic receptors, a class of G protein-coupled receptors, are widely distributed throughout the body and have a diverse range of physiological effects, including the regulation of glucose homeostasis in skeletal muscle and adipose tissue. Our recent in-vitro experiments have demonstrated that activation of the β_2-adrenergic receptor leads to increased glucose uptake in skeletal muscle cells, making this pathway an attractive target for treating metabolic diseases.

Interestingly, we have found that this effect occurs independently of the activation of AKT, a key signaling molecule involved in glucose metabolism, and is largely dependent on the activation of mTORC2 and the translocation of the glucose transporter GLUT4. Our in-vivo studies have demonstrated that administration of these compounds can lead to improvements in glucose homeostasis as well as beneficial effects in various organs implicated in the pathophysiology of type 2 diabetes. More recently, we and others have reported similar effects in healthy volunteers, where this pathway demonstrably improves insulin stimulated glucose disposal while increasing basal metabolic rate independently of substrate oxidation.

While this approach shows great promise for the treatment of metabolic disease, chronic administration of β_2-agonists is associated with well-documented cardiovascular side effects. Furthermore, the recruitment of beta arrestins can result in desensitization and loss of efficacy. To address these concerns, we have synthesized novel "targeted" β_2-agonists that limit unwanted signaling effects. These compounds stimulate glucose uptake in skeletal muscle without the production of cAMP, thus limiting potential cardiovascular side effects, and without the recruitment of beta arrestins, prolonging efficacy in-vivo resulting in long-term improvements in glucose homeostasis. Our targeted compounds are well tolerated and exhibit a better safety profile than conventional agonists highlighting the utility of this approach in developing agonists that can differentially regulate signaling pathways to counteract the development of various diseases.

White adipose tissue cytoarchitecture in obesity across depots

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Human white adipose tissue (hWAT) is a complex endocrine organ regulating a variety of physiological processes, which require the interaction between many cell types resident within the organ. It can be divided into subcutaneous and visceral depots, which have diverse and distinct functions and inter-organ communications. Visceral fat deposition is a known risk factor for cardiometabolic conditions such as insulin resistance/ type-2 diabetes, which lead to altered cellular and spatial organizations of hWAT. However, the cell types governing these changes and how they are perturbed in disease are to a large extent unexplored.

We performed single nuclei sequencing (snSeq) and spatial transcriptomics (STx) on paired hWAT samples collected from subcutaneous, omental, epiploic, and two mesenteric depots from eight individuals undergoing bariatric surgery. The results from the transcriptomics analysis were further validated by immunofluorescence staining and FACS analysis.

Using snSeq we show a high-resolution map of cell types highlighting depot-specific differences. One of the fibroblast clusters was unique to epiploic adipose tissue and characterized by the expression of LINC02147 and CTNNA2. The results were used to deconvolute STx data to define the cytoarchitecture of each depot. We furthermore performed ligand-receptor predictions, showing that epiploic and subcutaneous adipose tissue displayed enhanced cell-cell interactions compared to the other depots, and the pathways could be pinpointed down to the cell type level. For example, an M2-macrophage subtype was found to secrete VEGFA only in samples from the omental, epiploic, and mesenteric colon hWAT and to correlate negatively with HOMA-IR and Hba1c values.

The unique combination of both snSeq and STx on paired samples allowed us to identify tissue-specific cell type proportions and provide an in-depth characterization of hWAT cytoarchitecture across various depots and reveal new insights into local cell-cell communication and depot function and how they might contribute to cardiometabolic disease progression.

Interleukin 1β regulates white adipose tissue remodeling by targeting adipocyte precursors

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Obesity-associated metabolic diseases are closely linked to white adipose tissue (WAT) hypertrophy, whereas hyperplastic WAT expansion via differentiation of precursors into new adipocytes (adipogenesis) is protective. Although chronic WAT inflammation impairs metabolic health, some local inflammatory signals are required for maintained WAT function and insulin sensitivity. Postprandially, interleukin 1β (IL-1β) is acutely upregulated predominantly in WAT-resident macrophages, suggesting involvement in local response to energy influx. Thus, we aimed to investigate a physiological, metabolic role of IL-1β in WAT energy storage. Mice with adipocyte-specific IL-1 receptor (IL1R1) deletion displayed normal insulin sensitivity and WAT phenotype, whereas whole-body IL1R1-knockout mice had reduced subcutaneous and gonadal WAT mass on both chow and high-fat diet. In human WAT, progenitors were the major expressers of *IL1R1,* and reduced IL-1 signaling correlated to hypertrophy. *In vitro*, IL-1β potently increased lipid droplet formation, measured by high-throughput microscopy, in differentiating human adipose-derived stem cells. IL-1β upregulated several adipogenesis- and lipid handling-related genes during early, but not late differentiation (RNA-seq), and promoted adipogenesis exclusively when added to early-differentiation-stage cells. The pro-adipogenic, but not pro-inflammatory effect of IL-1β was blocked by chronic pre-treatment and potentiated by acute exposure. Mechanistically, IL-1β increased expression of the early adipogenic transcription factors CCAAT/enhancer-binding protein (C/EBP)δ and C/EBPβ, and their DNA-binding was required for its adipogenic stimulation. Flow cytometry analysis of EdU^+ adipocyte nuclei 9 weeks after EdU-injections showed a decreased high-fat diet-induced adipocyte formation in whole-body IL1R1-knockout mice. Here, we show that progenitors are the major targets of IL-1β in human WAT, where it promotes hyperplastic expansion via adipogenesis. This probably has a physiological role in allowing WAT to adapt during caloric excess, thus preserving metabolic health. We propose that IL-1β exerts this function via its acute postprandial surge, whereas the chronically high levels in obese WAT may counteract this effect, instead exacerbating inflammation.

Diacylglycerols in the interplay between peripheral insulin sensitivity, myofiber composition and ethnicity

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Individuals of African ancestry (AA) have lower insulin sensitivity compared to their European counterpart (EA). Studies show ethnic differences in muscle fat oxidation capacity, whilst no differences in total skeletal muscle lipids. We hypothesize that the link between muscle lipid subtypes, peripheral insulin sensitivity and myofiber composition differs between AA and EA, which may aid to explain ethnic differences in insulin sensitivity.

Muscle biopsies were obtained from individuals with AA (N=24, BMI=28.3±4.8) and EA (N=19, BMI=24.7±3.2). Ancestry was assigned via genetic admixture analysis; peripheral insulin sensitivity via hyperinsulinaemic–euglycemic clamp; and myofiber content via myosin heavy chain immunohistochemistry. Additionally, we harvested muscle types with high (soleus) and low (vastus lateralis) type I fiber content from F1-mice. Insulin sensitivity in mice was established via intraperitoneal glucose tolerance test. Mass spectrometry-based lipidomics was used to measure muscle lipids subtypes and magnetic resonance imaging for intramuscular lipids.

Compared to EA, AA had lower peripheral insulin sensitivity and oxidative type 1 myofiber content, without differences in total skeletal muscle lipids. Muscles with lower type I fiber content (AA and mouse vastus) showed lower levels of lipids associated with fat oxidation capacity, i.e., cardiolipins, triacylglycerols with low saturation degree and phospholipids, compared to muscles with a higher type 1 fiber content (EA and soleus from mice). Further, we found that muscle diacylglycerol content was inversely associated with insulin sensitivity in EA, who have more type I fiber, whereas no association was found in AA. Similarly, we found that insulin sensitivity in mice was associated with diacylglycerol content in the soleus (high in type I fiber), not in vastus (low in type I fiber). Our data suggest that lipid contribution to altered insulin sensitivity differs by myofiber composition, and that this needs to be considered to increase our understanding of the underlying mechanisms of altered insulin sensitivity.

Can PGC-1α1 counteract skeletal muscle capillary rarefaction and reduced glucose uptake induced by chronic inflammation?

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Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder with predominant musculoskeletal complications, including metabolic alterations. Patients with RA have a higher risk of developing type 2 diabetes (T2D) than the general population, however, the causality between RA and T2D is unknown. Skeletal muscle (SkM) is essential for locomotor activity and is the primary site for postprandial glucose disposal. Here we utilized a mouse model of arthritis to elucidate SkM metabolic and functional alterations induced by chronic inflammation.

Chronic inflammation was induced by complete Freund's adjuvant (CFA, ankle injection) in female wildtype or muscle-specific PGC-1α1 transgenic (MCK-PGC-1α1) mice. Glucose uptake (*in vivo* and ex *vivo*), immunofluorescence staining, and immunoblotting were assessed 14 days post-CFA injection. Gene expression was analyzed 3 days after CFA induction.

In vivo glucose uptake was reduced by 50-80% in limb muscle (extensor digitorum longus, tibialis anterior, soleus) of mice with arthritis as compared with control mice. Despite this, GLUT4 abundance and membrane translocation were enhanced by ~160% and ~70%, respectively, in limb muscles afflicted by CFA as compared with the control. However, no difference was observed in *ex vivo* glucose uptake between the two groups, suggesting the reduction in glucose uptake was due to an alteration in muscle blood perfusion. Expression of angiogenesis-associated genes, including PGC-1α (total and α1 isoforms), ERR (α and γ), and VEGF (A and B) was decreased by 50-70% in muscles afflicted by CFA, which was accompanied by a 25% reduction in muscle capillary density. However, the transgenic elevation of SkM PGC-1α1 prevented the increase in GLUT4 abundance and capillary rarefaction induced by arthritis. In conclusion, induction of chronic inflammation leads to reduced muscle capillary rarefaction and glucose uptake, which can advance the risk of developing metabolic disease. Strikingly, this pathophysiological development can be corrected by PGC-1α1.

Session 6: Inflammation and metabolism

ADAR1 suppresses meta-inflammation in myeloid cells

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ADAR1 (adenosine deaminase acting on RNA) influences the fate and function of several mRNA molecules either by catalyzing RNA modifications or acting as an RNA binding protein. While aberrant ADAR1 expression profiles have been associated with many diseases including neurological disorders and cancers, ADAR1 biology has not been extensively studied in the setting of non-alcoholic fatty liver disease (NAFLD) nor metabolic diseases. RNA-seq analysis of liver biopsy samples from two independent studies revealed that the ADAR1 expression levels as well as the enzymatic activity of NAFLD and NASH patients is considerably higher compared to lean and "healthy" obese. Immunofluorescent staining and western blot analysis of human livers corroborated a positive relationship of ADAR1 with NAFLD progression. Interestingly, $CD68^{+}$ cells were expressing remarkably higher ADAR1 levels compared to other liver cells suggesting that the main ADAR1-expressing cells are the myeloid cells. In parallel, protein analysis of monocytes from obese patients showed elevated ADAR1 levels compared to lean controls while a caloric restriction for 8 weeks in obese individuals led to a decrease of ADAR1 mRNA levels in monocytes. When human monocyte-derived macrophages were exposed to a metabolic syndrome cocktail (free fatty acids, insulin, high glucose and fructose), the protein levels and activity of ADAR1 increased recapitulating the ADAR1 induction *in vitro*. At last, loss-of-function experiments in human macrophages revealed an exacerbated proinflammatory phenotype in the absence of ADAR1 and a follow-up transcriptomic analysis uncovered a potential regulatory relationship between ADAR1 and immunosuppressive mediators such as IL10. These data suggest that the ADAR1 myeloid induction observed in obesity and NAFLD may provide a "brake" against metainflammation in a IL10-dependent manner. Understanding the downstream ADAR1 molecular cascade of immunosuppression in obesity and fatty liver disease may lay the foundation for the development of novel therapeutic strategies and new disease biomarkers.

The immune profile in adipose tissue of a mouse model of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is an endocrine-metabolic disorder, characterized by high circulating androgen levels and with a strong link to metabolic comorbidities, where 50% of women with PCOS develop type-2 diabetes (T2D) before the age of 40. Immune cells residing in adipose tissue play a central role in glucose homeostasis, and a low-grade inflammation is associated with PCOS as well as T2D. To determine how hyperandrogenism affects the immune populations in visceral adipose tissue (VAT), we characterized the immune profile of the dihydrotestosterone (DHT)-induced PCOS-like mouse model, with or without co-treatment with flutamide, an androgen receptor (AR) antagonist.

First, to assess if DHT-exposed mice (PCOS-mice) develop a metabolic phenotype, body composition was analyzed by EchoMRI, and glucose metabolism was assessed by oral glucose tolerance test. No effect was seen on fat mass, fasting blood glucose or glucose uptake. Next, the immune profile in VAT was analyzed by flow cytometry. The number of eosinophils in VAT was drastically reduced in PCOS-mice compared to controls. Multiplex analysis showed a trend of decreased eotaxin and IL-5 in VAT, which could affect the recruitment and survival of eosinophils. Moreover, NK cells in VAT of PCOS-mice displayed a higher expression of CD69, a marker of activation or tissue residency. Levels of IFN-γ were lower in VAT of PCOS-mice, contradicting a higher activation state of NK cells. Finally, macrophages in VAT of PCOS-mice displayed an altered phenotype, with lower MHC-II expression and a higher expression of CD11c compared to control. All effects of DHT exposure were prevented by co-treatment with flutamide, suggesting that the observed alterations are AR driven.

These findings show that androgen exposure affects immune populations in visceral adipose tissue, independent of metabolic status. If these alterations contribute to a higher susceptibility to metabolic dysfunction associated with PCOS remains to be elucidated.

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

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Background: Prostanoids, such as thromboxane and prostaglandins, are lipid mediators that activate cell surface receptors in various cell types, including skeletal muscle. Exercise is known to increase prostanoids levels in men. Levels of some prostanoids are dysregulated in people with type 2 diabetes (T2D). The aim of this project is to investigate the role of prostanoids in skeletal muscle remodeling and metabolism.

Methods: Blood samples were collected before and after an acute aerobic exercise bout from men and women with T2D and healthy controls. Plasma prostanoids levels were quantified using LC-MS. Primary human myotubes were treated with the thromboxane receptor agonist I-BOP, and glucose uptake, oxidation, and incorporation into glycogen were measured using radiolabeled substrates. Western blot was used to track signaling events. EDL muscles from male mice were treated ex-vivo with I-BOP, and glucose oxidation was measured using [14C]-glucose. Glucose tolerance tests were performed in mice after acute administration of I-BOP.

Results: Plasma thromboxane B2 levels were higher after exercise in healthy men (+106%,p=0.019,n=18) while it was unchanged in men with T2D and in healthy women. Treating primary human myotubes with the thromboxane receptor agonist I-BOP increased glucose uptake (+53%,p=0.0019,n=5), accompanied by actin cytoskeleton remodeling and GLUT4 translocation to the plasma membrane. I-BOP also increased glucose oxidation (+28%,p=0.017,n=5), and glycogen synthesis (+398%,p<0.001,n=4). In mice, I-BOP increased glucose oxidation in isolated EDL muscle (+118%,p<0.001,n=7-8), and i.p. administration of I-BOP improved glucose tolerance in male mice (iAUC-38%,p=0.0027,n=8).

Conclusions: The endogenous production of prostanoids represents a novel sex-dependent adaptation to exercise. Activating the thromboxane receptor in skeletal muscle improves whole-body glucose tolerance, indicating a potential role in promoting the metabolic health benefits of physical activity. These findings suggest that targeting prostanoids production and sex-specific exercise regimens may have therapeutic potential in improving glucose control and metabolic health in individuals with T2D.

Session 7: Circadian Rhythms and metabolism

Circadian Catecholamine Sensitivity: Does Time Matter?

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Circadian clocks control metabolism and energy homeostasis, and chronic circadian disruption is a risk factor for metabolic diseases, including type 2 diabetes. To investigate the functional role of the skeletal muscle clock we generated conditional and inducible mouse lines with muscle-specific ablation of Bmal1 (mKO), an essential clock gene. We previously reported mKO mice display normal 24-hr feeding and activity rhythms compared to wildtype littermates, yet mKO skeletal muscles show dramatically altered 24-hr gene expression, impaired insulin sensitivity, and major changes in glucose, lipid, amino acid and protein metabolism. Hormones and metabolites, including the catecholamines adrenaline and noradrenaline can modulate circadian genes and synchronize clocks in the periphery. We found that transcripts coding for adrenergic receptor β2 (Adrb2), the main receptor for catecholamines in skeletal muscle, oscillate ~2-fold over 24-hr and peak at the transition from sleep to awakening when circulating catecholamines are also highest. To investigate whether β2-adrenergic receptors (β2-AR) also oscillate at a functional level, we measured specific binding of β2-AR in membrane fractions isolated from mouse quadriceps across 24-hr. We found membrane-bound β2-AR followed 24-hr Adrb2 mRNA expression patterns by ~6hr, with lowest levels during the day, when mice are normally asleep and fasting, and ~3-fold higher membrane-bound β2-AR at night, when mice are normally awake, active and feeding.
24-hr Adrb2 mRNA oscillation is completely abrogated in muscles from muscle-specific Bmal1 knockout mice, including an inducible model in which muscle Bmal1 is inactivated during adulthood. In addition, some effects of acute β2-AR stimulation were severely blunted in mKO muscles, as shown by decreased phosphorylation of PKA substrates and decreased induction of β2-AR-responsive genes, while other responses appear normal. Our data suggest the muscle clock regulates aspects of 24-hr hormone sensitivity, and disruption of the muscle clock causes a condition of partial "catecholamine resistance" characterized by decreased muscle response to β-adrenergic agonists.

The transcriptional response to acute exercise is HIF1α dependent and time-of-day specific

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The regulation of metabolism in peripheral tissues is mediated by pathways under circadian control. A bi-directional relationship exists between the circadian clock and hypoxia-inducible factor-1 α (HIF1α), which is specific to time-of-day exercise. This study aims to elucidate the interplay between HIF1α and time-of-day in the skeletal muscle transcriptional response to acute exercise. Skeletal muscle specific HIF1α knock out (HIF1α-mKO) and wild type (WT) mice (N=8 per group) were subjected to 60 min treadmill running or sham treatment at zeitgeber time (ZT) 3 and ZT 15. RNA sequencing was performed on gastrocnemius tissue of mice 3 hr following the intervention. In exercised mice, a greater number of transcripts were differentially expressed between HIF1α-mKO and WT mice at ZT 3 (516) compared to ZT 15 (91). Within these differentially expressed transcripts, 477 transcripts were specific to exercise at ZT 3. Conversely, in sedentary mice, similar skeletal muscle transcriptional profiles were observed between HIF1α-mKO and WT mice at ZT 3 and ZT 15. Over-enrichment analysis of altered transcriptional profiles between HIF1α-mKO and WT mice in response to exercise determined a positive enrichment of gene ontology terms involved in oxidative metabolism (oxidative phosphorylation, aerobic respiration, cellular respiration) and mitochondrial function (mitochondrial respiratory chain complex assembly, ATP synthesis coupled electron transport and respiratory electron transport chain) at ZT 3, but not ZT 15. Collectively these results indicate that the transcriptional profile of skeletal muscle is time-of-day and HIF1α dependent in exercised, but not sedentary, mice. Specifically, following HIF1α KO, a greater transcriptional response is observed in exercised mice at ZT 3 compared to ZT 15, coupled with an overrepresentation of oxidative metabolism and mitochondrial function pathways. Thus, the time-of-day specific response of skeletal muscle to acute exercise may be partly driven by the interaction between the muscle circadian clock and HIF1α.

Posters

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Danai Zareifi	P20	Lorenzo Butto	P52
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Poster Abstracts

Insulin secretion and islet biology including type 1 diabetes

P1 - Adjudin improves beta-cell maturation, hepatic glucose uptake and glucose homeostasis

Lipeng Ren[1], Jérémie Charbord[1], Lianhe Chu[1], Aurino M Kemas[2], Jiarui Mi[1], Volker M Lauschke[2, 3, 4], Olov Andersson[1]

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Aims/hypothesis The aim of the present study is to investigate the effects of Adjudin, a small molecule identified in a beta-cell screen using zebrafish, on pancreatic beta-cells and diabetes conditions in mice and human spheroids.

Methods Insulin expression, glucose levels and distribution were examined in zebrafish. Pancreatic islets of wildtype postnatal day 0 (P0) and 3-month-old mice, as well as adult *db/db* mice, were cultured *in vitro* and analysed to examine their function. RNA sequencing was performed for islets of P0 and *db/db* mice. For *in vivo* assessment, *db/db* mice were treated with Adjudin and subjected to analysis of metabolic variables and islet cells. Glucose consumption was examined in primary human hepatocyte (PHH) spheroids.

Results Adjudin treatment increased insulin expression and decreased glucose levels after beta-cell ablation in zebrafish, and led to beta-cell maturation and glucose responsive insulin secretion in *in vitro* cultured P0 mouse islets. RNA sequencing of P0 islets indicated that Adjudin treatment resulted in increased glucose metabolism, mitochondrial function, as well as downstream signaling pathways involved in insulin secretion. In *in vitro* cultured islets from *db/db* mice, Adjudin treatment strengthened beta-cell identity and insulin secretion. RNA sequencing of *db/db* islets indicated Adjudin upregulated genes associated with insulin secretion, membrane ion channel activity and exocytosis. Moreover, Adjudin promoted glucose uptake in the liver of zebrafish in an insulin-independent manner, and similarly promoted glucose consumption in PHH spheroids with insulin resistance. *In vivo* studies using *db/db* mice revealed reduced nonfasting blood glucose, improved glucose tolerance and strengthened beta-cell identity after Adjudin treatment.

Conclusions/interpretation Adjudin promoted functional maturation of immature islets, improved function of dysfunctional islets, stimulated glucose uptake in liver, and improved glucose homeostasis in *db/db* mice. Thus, the multifunctional drug Adjudin, previously studied in various contexts and conditions, also shows promise in the management of diabetic states.

P2 - Lipidomics analysis of human and mouse pancreatic islets and the link to altered insulin secretion

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Type 2 diabetes (T2D) remission and restored islet function is possible and linked with the ability to reduce intra-pancreatic fat. Although the role of lipids is accepted, the molecular mechanisms by which islet lipids mediate β-cells dysfunction is still unknown.

This study aims to explore the link between bioactive lipid subtypes in pancreatic islets and altered insulin secretion capacity.

Islets were harvested from; i) transgenic mice that expresses human Islet Amyloid Polypeptide (hIAPP-TG, N=13); ii) wild type littermates (WT, N=17) and; iii) human donor islets (N=23) with varying insulin secretion capacity. hIAPP, is the major aggregating amyloid peptide in human β-cells, leading to reduced β-cell function and insulin secretion capacity and with time β-cell death. Insulin secretion was measured via intraperitoneal glucose tolerance test (mice) and insulin stimulatory index (human). All islet samples were subjected to mass spectrometry-based lipidomics for comprehensive lipid profiling and multivariate bioinformatics combined with univariate statistics for data evaluation.

Compared to WT, hIAPP-TG mice presented with significantly lower insulin secretion capacity, and no difference in insulin sensitivity, body fat composition or total islet lipids content. We found a significantly altered islet lipid signature between hIAPP-TG and WT mice that also appeared to correlate with lower insulin secretion capacity in human donor islets. Lower insulin secretion was associated with lower amounts of cardiolipins (mitochondrial lipids) and sphingomyelins, and higher levels of C16/C18-ceramides. Hyperglycemic exposure of *ex vivo* mouse cultured islets, which decreases β-cell functionality and insulin secretion, resulted in decreased sphingomyelin synthase 1, which can lower sphingomyelins and increase islet ceramide levels in Endoplasmic Reticulum/Golgi.

Our data suggest that bioactive lipids, specifically ceramides, and their regulatory pathway in Endoplasmic Reticulum/Golgi is associated with altered insulin secretion capacity. Further analyses of highlighted pathways in *ex vivo* cultured mouse and human donor islets is ongoing.

P3 - Surrogate measures of first phase insulin secretion vs. the reference methods IVGTT and HGC: a systematic review and meta-analyses

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Objective: In this systematic review, we investigated how established surrogate indices from fasting samples and the oral glucose tolerance test (OGTT) correlate to the first phase of insulin secretion calculated from the two gold standard methods, namely the hyperglycaemic clamp (HGC) and the intravenous glucose tolerance test (IVGTT***).***

Research Design and Methods: We conducted a search in the PubMed, Cochrane Central, and Web of Science databases. Studies measuring first phase insulin secretion with both reference (HGC or IVGTT) and surrogate measures in the same population were selected. The correlation coefficients between first phase measured with gold standard and surrogate methods were extracted from each study. Random-effects meta-analyses were performed to examine the correlation between all surrogate indices validated to either reference methods in at least three different studies.

Result: HOMA-beta and Insulinogenic index 30 (IGI(30)) were the surrogate measures that have been validated in the largest numbers of studies (17 and 13, respectively). HOMA-beta's pooled correlation to reference method was 0.50 (95% CI 0.48–0.53) The pooled correlation of IGI(30) to reference method was 0.60 (95% CI 0.57–0.62). The surrogate measures with highest correlation to reference method were Kadowaki (0.67 (95% CI 0.61–0.73)) and Stumvoll's first-phase secretion (0.65 (95%CI 0.62-68)).

Conclusions:

Surrogate measures from the first 30 minutes of an OGTT capture the first phase of insulin secretion and are a good choice for epidemiological studies. HOMA-beta, the most validated measure, has a moderate correlation to reference methods but is not a measure of first phase specifically.

P4 - Type III interferons are expressed in human pancreas at type 1 diabetes onset and induce immunostimulatory and antiviral activities in human β-cells

Emma Ringqvist[1], Soile Tuomela[1], Magdalena Mazur[1], **Fabian Byvald**[1], Kylie Burdsall[1], Svitlana Vasylovska[2], Anirudra Parajuli[1], Helena Sork[1], Lars Krogvold[3], Knut Dahl-Jorgensen[3], Clayton Matthews[4], Joey Lau Börjesson[2], Ivan Gerling[5], Malin Flodström Tullberg[1]
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Type 1 diabetes (T1D) results from progressive dysfunction and loss of insulin production by pancreatic islet β-cells. Coxsackievirus infections are more commonly present in children who develop T1D. The anti-viral immune response may contribute to β-cell destruction in human type 1 diabetes. Type III interferons (IFN-λ 1-4) constitute a group of IFNs that are produced by both immune and parenchymal cells during infection. We have previously shown that human pancreatic islets express type III IFNs when infected with Coxsackievirus B (CVB) in vitro. However, type III IFNs' role in T1D and direct effect on β-cells remains unexplored.

In the present study, we aimed to investigate if IFNλs are expressed in the human pancreas at diabetes onset and to describe the effects that IFNλ have on β-cells by in-depth proteome, islet transcriptome and immune marker analysis of a human β-cell line (EndoC-bH1), primary human islets and stem cell derived islet like clusters. We also investigated the function of IFNl-induced pathways in β-cells regarding immune status and antiviral defense.

The proteome of IFNλ1- or IFNλ2-exposed EndoC-bH1 cells revealed 93 and 119 differentially expressed proteins. The cell surface expression of MHC class I was induced on both EndoC-bH1 cells and stem cell derived islet-like clusters, which was prevented by drugs blocking JAK/STAT signaling. IFNλ1/2 treatment strongly reduced permissiveness to CVB infection, as did blocking of the viral receptor (CAR). Finally, we discovered that the genes encoding IFNλ1/2 showed increased expression in islets from diabetic individuals compared to healthy controls.

In conclusion, we found that type III IFNs are expressed in the human pancreas at T1D onset. Type III IFNs increase MHC-class I expression and activate antiviral defense in human β-cells. We also show that IFNλs have antiviral activity. In summary, these results highlight an immunomodulatory function of type III IFNs during T1D development.

Skeletal muscle metabolism, growth and (patho)physiology

P5 - A rapid muscle fiber type identification protocol allows for muscle fiber type-specific mitochondrial respiration in children with cerebral palsy

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Human skeletal muscle consists of slow- and fast-twitch fibers with diverse metabolic properties. Despite this, investigations into human muscle physiology are often conducted in mixed muscle samples due to the laborious methods associated with fiber type-specific analysis. This may skew interpretations, especially when comparing populations with known variations in fiber type distribution. For example, it has been reported that children with cerebral palsy (CP) have a higher proportion of fast-twitch fibers than typically developed (TD) children. We and others report that in mixed-muscle samples, children with CP exhibit impaired mitochondrial function and decreased mitochondrial mRNA and protein levels. These alterations are suggested to predispose individuals with CP to chronic diseases such as cardiovascular disease and type II diabetes. However, it remains to be elucidated whether these findings are driven by the aforementioned fiber type switch, or by intrinsic changes to the muscle fibers themselves.

Therefore, we aim to assess the fiber type-specific mitochondrial respiratory capacity of slow- and fast-twitch muscle fibers separately in children with CP and TD control children.

For this investigation, we utilized a new method for rapid fiber type identification, allowing for fiber typing of approximately 100 fresh living fibers within hours following muscle sampling[1]. Muscle biopsies were obtained during planned orthopedic surgery. Immediately following surgery, individual fibers were isolated in mitochondrial preservation media. Thereafter, small pieces were cut from one end of the fibers, mounted on a microscopy slide with a custom grid print, and stained for myosin heavy-chain isoforms using standard immunohistochemistry methodology. Fibers were subsequently pooled according to their respective fiber type based on the fluorescence staining. Finally, mitochondrial respiratory capacity was assessed using an oxygraph-2k (OROBOROS).

Understanding the mechanism behind the impaired skeletal muscle metabolic phenotype in individuals with CP will aid in counteracting the development of metabolic disease in this population.

1)PMID:36069036

P6 - Cell-type-specific disease signatures and mechanisms regulating skeletal muscle in women with polycystic ovary syndrome

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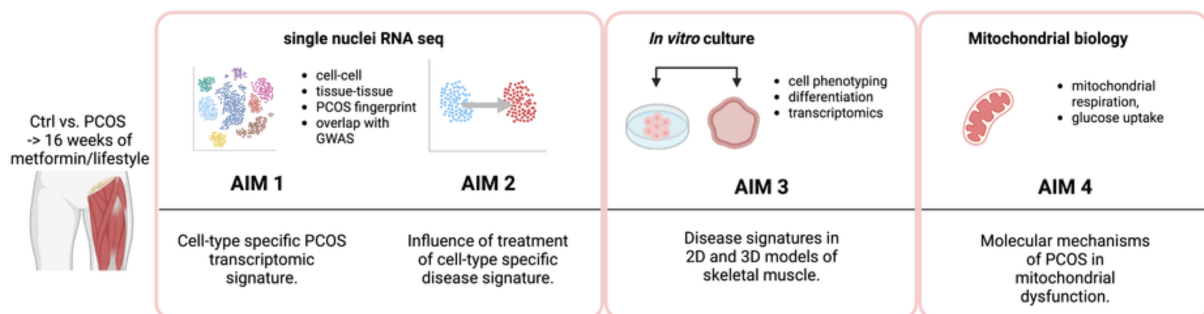
Polycystic ovary syndrome (PCOS) is associated with a high degree of metabolic comorbidities: 75% have insulin resistance (IR) and 40% develop type 2 diabetes before the age of 40. Lifestyle management including diet and exercise is the first-line treatment, followed by prescription of antidiabetic drugs (metformin) to regulate glucose. Skeletal muscles play a central role in energy and glucose metabolism and their function is impaired in PCOS, due to elevated testosterone and insulin levels. Fibrosis, low-grade inflammation, and mitochondrial dysfunction in muscle further exacerbate IR. How cellular complexity and heterogeneity are altered in skeletal muscle dysfunction and IR in PCOS and whether alterations can be reversed by lifestyle/metformin remain unclear.

We aim to uncover the cell-type specific fingerprint in skeletal muscle of healthy women and hyperandrogenic and insulin-resistant women with PCOS using single-nuclei RNA sequencing (snRNA-seq). In addition, women with PCOS are randomized to a 16-week lifestyle management intervention, with or without metformin, allowing us to uncover whether identified alterations can be reversed. In a subset of participants, muscle satellite cells are isolated and will be used to validate the cellular and molecular mechanisms of the cell-type-specific disease signatures identified by snRNA-seq. To define the myogenic capacity and mitochondrial function of satellite cells and myotubes *in vitro,* we will perform bulk RNA-seq, Seahorse metabolic flux assays, and glucose uptake using fluorescent D-glucose analogue (2-NBDG). The transcriptomic data from the *in vitro* studies will be deconvoluted with snRNA-seq data to explain the phenotypic fingerprint of PCOS.

This project is just getting started, and we are currently establishing and optimizing a nuclei extraction protocol for 10X single nuclei 3' RNA sequencing. We have successfully sequenced two samples and will present preliminary analyzes, along with preliminary results of *in vitro* experiments.



P7 - Characterisation of Peripheral Artery Disease Myopathology

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Comorbidities of type II diabetes (T2D) are major health concerns worldwide, including peripheral artery disease (PAD). The prevalence of PAD is four times higher in diabetic individuals compared with nondiabetic individuals and is a major cause of nonhealing ulcers, lower limb amputation, and mortality. PAD lowers blood flow to the lower limb, causing muscle ischemia and dysfunction, which can ultimately lead to tissue necrosis. Interventions that improve distal arterial pressures (ie. bypass surgery or endovascular procedures) generally fail to normalise the structural and functional abnormalities of muscles, which point towards pathophysiological mechanisms within the myofibers. However, despite serious muscle abnormalities, the field is lacking molecular understanding of PAD-induced muscle dysfunction. Moreover, research often uses muscle tissue from the same patients as intraindividual controls. Given the systemic nature of the disease, this methodology may confound resulting data.

This project will use classic histological techniques, clinical data, and state-of-the-art single nuclei sequencing (snSEQ), to characterise PAD myopathology. The affected ischemic muscles (distal of the arterial occlusion; gastrocnemius muscle) and ´non-ischemic´ muscles (proximal of the arterial occlusion; thigh muscle) will be compared to healthy control tissue from subjects with no history of T2D or PAD. Initial data from these analyses indicate significant fibre atrophy, fat accumulation, fibrosis, and centralised myonuclei in both ischemic and non-ischemic tissues of PAD patients compared to healthy controls. This suggests that even muscle not directly affected by ischemia can exhibit significant abnormalities. By performing these in-depth analyses, we aim to create a gold-standard set of data that can be used to elucidate pathways that underlie the disease process. Next step is to perform comprehensive cellular analyses with snSEQ and the top candidate pathways will be selected for *in vivo* analysis in mouse models of PAD. The expected results will provide more specific mechanistic insight into the disease myopathology.

P8 - Exercise induced epigenetic modification through histone lactylation in skeletal muscle cells

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Acute physical activity induces a wide variety of molecular adaptations, in gene expression, proteins, and metabolites. The temporal molecular response to exercise exerts beneficial effects on metabolic control, which differs between males and females. Lactate is a metabolic intermediate mainly produced in skeletal muscle under anaerobic conditions, such as physical activity. Histone lysine lactylation represents a novel epigenetic modification involved in the regulation of gene expression. This mechanism establishes a direct link between intermediates of cellular metabolism to epigenetic regulation. Exercise causes epigenetic changes affecting both gene expression and histone modifications, however the involvement of lactate in this process remains unknown. Lactate production and histone lactylation are regulated by glucose in a dose dependent manner. Fasting lactate levels are elevated in type 2 diabetes (T2D) compared to healthy individuals at rest, but it remains unclear if is a consequence or a cause of this metabolic disease. The aim of the study is to determine the effects of muscle contraction on protein and histone lactylation.

We will use electrical pulse stimulation (EPS) as an *in vitro* exercise model in primary human skeletal muscle cells (HSMC) from male and female normal glucose tolerant or T2D donors. Lactate will be measured in cell medium from HSMCs at baseline, and at 1, 3 and 24 hour following EPS using a colorimetric assay. Total protein lactylation and histone lactylation will be assessed by western blot. Histone lactylation will be evaluated at H3K18la, a region which marks active CpG island-containing promoters of highly expressed genes.

These experiments will allow us to determine whether muscle EPS alters lactylation to probe how this may be involved in the regulation of metabolism and differences in gene expression observed in skeletal muscle cells between men and women with normal glucose tolerance or T2D.

P9 - Exercise-responsive serum- and skeletal muscle-derived extracellular vesicle miRNAs in the regulation of skeletal muscle metabolism in type 2 Diabetes

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Skeletal muscle plays a key role in type 2 diabetes (T2D) by maintaining glucose homeostasis and modulating insulin resistance. Upon exercise the physiology of skeletal muscle cells is altered, releasing vesicles and metabolites into the peripheral blood to facilitate the crosstalk between tissues. Extracellular vesicles (EVs) are one of these factors and have been shown to carry proteins, lipids and small non-coding RNAs, such as miRNAs. Although recent evidence shows exercise can alter the levels of the circulating EV miRNAs, the mechanistic understanding of how serum- and skeletal muscle-derived EV miRNAs could mediate changes in skeletal muscle metabolism in the context of T2D or acute exercise remains unclear.

In this study, serum-derived EVs were isolated by precipitation from serum samples of individuals with normal glucose tolerance and T2D before (pre), immediately after (post) and 3 hours-post exercise (recovery). The presence of EVs was confirmed by western blot and the differentially expressed miRNAs were determined by small-RNA-sequencing. To determine the skeletal muscle-specific EV miRNAs, *in-vitro* murine skeletal muscle cells (C2C12) and *ex-vivo* murine skeletal muscle models were used and EV miRNAs were extracted from media after contraction by electrical pulse stimulation. The role of selected candidates in the regulation of skeletal muscle metabolism are being investigated with functional validations.

Differentially expressed EV microRNAs in T2D could be a novel approach on exercise ability to modulate skeletal muscle post-transcriptional gene regulation and highlight communication pathways between skeletal muscle and other organs.

P10 - Insight into how chronic inflammation alters capillary number in skeletal muscle

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Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease, which is known to induce vascular dysfunction, including accelerated atherosclerosis and endothelial dysfunction. This reduced vascular function, may contribute to muscle hypoxia and reduced nutrient uptake (e.g. glucose) which together may increase the risk of metabolic disturbances and abnormal anabolic response. The transcriptional co-activator PGC-1α is known to regulate expression of vascular endothelial growth factor (VEGF), promoting angiogenesis and healthy vascular function in muscle. Here, we aimed to investigate how chronic inflammation impacts the small blood vessels in skeletal muscle.

To induce chronic inflammation, Complete Freund's adjuvant (CFA) was injected into the ankle of female wild-type (WT) or muscle-specific PGC-1α1 transgenic (MCK-PGC-1α1) mice. mRNA levels were measured in tibialis anterior (TA) at day 3 post-CFA injection and immunofluorescence staining was performed after 14 days. VEGFa was analyzed by ELISA at days 3 and 14 after CFA injection. In WT mice, CFA induced a 25% decrease in muscle capillary density, which was associated with decreased gene expression of PGC-1α1 (-60%), ERRα (-70%), and VEGFa (-78%) compared to the control group. The CFA-induced capillary rarefaction was prevented in MCK-PGC-1α1 mice, as well as the decreased gene expression of PGC-1α1 and ERRα. However, the CFA-induced decrease in VEGFa gene expression (-67%) was also observed in the MCK-PGC-1α1 mice. Intriguingly, the protein levels of VEGFa were higher in muscle from WT mice with chronic inflammation than in control mice at days 3 and 14 post-injection, implying an uncoupling between VEGFa gene and protein expression. In the upcoming experiments, we will measure VEGFa protein levels in muscle from MCK-PGC-1α1 mice with and without CFA-induced inflammation, as well as VEGFa downstream signaling (e.g. receptor levels and phosphorylation status).

P11 - Long 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. 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. In this study, we identify differentially expressed skeletal muscle-derived lncRNAs in individuals with normal glucose tolerance (NGT) and T2D at rest and following an acute exercise bout and investigate the role of these lncRNAs in the regulation of skeletal muscle metabolism.

19 individuals with T2D and 17 individuals with NGT underwent an acute bout of cycling exercise. Skeletal muscle biopsies were collected before (basal), immediately after, and 3 hours after (recovery). Total RNA samples were isolated and RNA-SEQ was performed for the discovery of differentially expressed skeletal muscle-derived lncRNAs.

A single bout of exercise changed the expression of more than 200 lncRNAs significantly in the T2D group ($FDR<0,05$), whereas this number was much lower in the NGT group. Using bioinformatic analysis, we narrowed down the candidate lncRNA list for the functional characterization. Moreover, at rest state, we identified and validated the function of a novel antisense lncRNA, which was significantly downregulated in T2D ($p=0,013$). We show that this lncRNA regulates protein synthesis through the modulation of ribosomal biogenesis by mediating the translation of the master regulator of ribosomal biogenesis, MYC. Several more *in vitro* and *in vivo* experiments are ongoing to validate the function of these lncRNAs on skeletal muscle metabolism, mass, and function to further support our findings. The functional characterization of these candidates could provide mechanistic insights into the regulation of skeletal muscle metabolism by exercise and/or in T2D.

P12 - miR-136-3p is a potential regulator of skeletal muscle adaptations to exercise via Nardilysin Convertase

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Background: microRNAs (miRNAs) are non-coding RNAs that regulate gene expression. miRNAs are carried by extracellular vesicles (EVs) to mediate intercellular communication. EV-associated miRNAs may mediate the effects of exercise on skeletal muscle. Here, we aimed to (1) identify serum EV-associated miRNAs that are responsive to training, and (2) understand if they contribute to training adaptations in skeletal muscle.

Methods: Twelve volunteers underwent three weeks of aerobic training. Serum was collected before and after, and EVs isolated using a precipitation-based method. Differentially expressed miRNAs were identified using a miRNA PCR panel. Functional characterisation was performed through transfection of miRNA mimics in human skeletal muscle cells (HSMC), followed by radiolabelled substrate assays. Microarray analysis and luciferase assay were used for target identification and validation. MetaMEx (Meta-analysis of skeletal muscle transcriptomic response to exercise and inactivity) was consulted to draw physiological relevance. Immunocytochemistry was used to assess myoblast differentiation and growth.

Results: miR-136-3p was increased in serum EVs after training. Microarray analysis and luciferase assay revealed Nardilysin Convertase (NRDC) as a direct target. NRDC is decreased with training and increased with inactivity in MetaMEx. Overexpression of miR-136-3p in HSMC led to increased glucose uptake, but this was not recapitulated by NRDC silencing. Glucose oxidation, fatty acid oxidation, glycogen synthesis, protein synthesis and lactate release were also unaltered. Silencing of NRDC affected myoblast differentiation and growth as shown by immunocytochemistry for desmin, myosin heavy chain and marker of proliferation Ki-67.

Conclusions: miR-136-3p is increased in serum EVs after training and targets NRDC in HSMC. NRDC is regulated by exercise and inactivity *in vivo*. Our findings suggest that NRDC is not involved in metabolic regulations, but may play a role in myoblast proliferation and differentiation. Current research aims to understand how miR-136-3p and NRDC contribute to skeletal muscle remodelling in response to exercise and inactivity.

P13 - Mitochondria as a possible therapeutic target to improve muscle function in chronic inflammation.

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Background: Chronic systemic inflammation is present in many non-communicable diseases including type II diabetes, rheumatoid arthritis, and cardiovascular disease. Clinical and preclinical studies have shown that these diseases are associated with oxidative stress which contributes to muscle dysfunction and weakness. Being mitochondria the major source of reactive oxygen species we hypothesize that chronic inflammation induces mitochondrial dysfunction in skeletal muscle which impairs muscle metabolism and contractile function. **Methods:** Complete Freud's adjuvant-induced arthritis (CFA) was used as a mouse model of chronic inflammation. CFA was injected in the knee or ankle joints of C57BL/6JRj and hNGFR100E knock-in mice. hNGFR100E mice have decreased pain sensitivity, and thus lack reduced pain-induced changes in locomotor activity and gait. After two weeks of CFA injection, mitochondrial respiration was evaluated by high-resolution respirometry in permeabilized soleus fiber bundles. Muscle mitochondrial ultrastructure was analyzed by electron microscopy, while mitochondrial-related gene and protein expression was accessed by RT-PCR and immunoblotting, respectively. **Results**: The mitochondrial OXPHOS capacity was reduced by 17% ($p<0.05$) and complex I- and complex II-driven respiration was reduced by 28% ($p<0.05$) and 18% ($p<0.01$), respectively, in mice with CFA as compared with controls. These results were accompanied by a reduction of mitochondrial density (~20%, $p<0.05$), an increased distance between mitochondria and sarcoplasmic reticulum (~29%, $p<0.01$), and 40% ($p<0.01$) lower protein expression of subunits of the electron transport chain as compared with control muscle. Furthermore, muscle inflammation was confirmed by increased IL-1β levels (~83%, $p<0.05$) and NF-kβ p65 protein expression (~50%, $p<0.05$). The mitochondrial impairments persisted in the hNGFR100E knock-in transgenic mice with chronic inflammation, showing that the inflammation-induced alterations are not the result of disuse but rather inflammation-induced. **Conclusion:** Our data show that a chronic inflammation promotes altered muscle mitochondrial function and structure which might be induced by NF-kB pathway activation and upregulation of pro-inflammatory cytokines.

P14 - NOR1 regulates protein synthesis and glucose metabolism in human skeletal muscle

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Sedentary behaviour is a risk factor for metabolic disease. Conversely, physical activity improves metabolic health and is a potent adjunct therapy for the treatment of type 2 diabetes. NOR1 (also known as NR4A3) regulates exercise adaptation and metabolism in skeletal muscle. However, whether a downregulation of NOR1 contributes towards inactivity-associated detriments in skeletal muscle metabolism is unclear. Here we investigated the consequence of *NOR1* silencing in human skeletal muscle.
siRNA silencing of *NOR1* was studied in primary human skeletal muscle myotubes. Myotube area was determined by immunocytochemistry. Protein synthesis and signal transduction were assessed by immunoblot analysis. Glucose and long-chain fatty acid metabolism were interrogated using radiolabelled substrate assays. Lactate in culture medium was measured by colourimetry and gene expression was quantified by RT-qPCR. Overexpression of *NOR1* transcript variant 1 (isoform *NOR1-a*) was achieved by lentiviral transduction. The human skeletal muscle response to bed rest and limb immobilisation inactivity was investigated through meta-analysis of published transcriptomic datasets (https://metamex.serve.scilifelab.se/).
A 36% depletion of NOR1 protein associated with reduced myotube area (-33%), attenuated protein synthesis (-25%), and impaired mTORC1 signalling. Glucose oxidation decreased by 18%, whereas lactate production increased 24% alongside a 40% downregulation of the *LDHβ* isoform. *NOR1*-silencing potentiated AMPK signalling, which coalesced with greater basal (+26%) and uncoupled (+54%) rates of fatty acid oxidation. A 120-fold overexpression of *NOR1*-a mRNA augmented protein synthesis (>2-fold) but did not affect glucose or fatty acid metabolism. After human interventions of inactivity, *NOR1* gene expression was decreased by 18% in skeletal muscle and gene ontology analysis of *NOR1*-correlated transcripts was enriched for 'muscle system', 'glucose metabolism', and 'cellular respiration' biological terms.
Our data implicate NOR1 in the regulation of protein synthesis in human skeletal muscle. Interventions that stimulate NOR1 could prove an effective strategy to combat skeletal muscle atrophy during periods of chronic inactivity.

P15 - Regulation of muscle function by E3 ubiquitin ligase HECTD1

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Skeletal muscle is an essential tissue for human movement and metabolism, as it accounts for a large proportion of body mass and plays a key role in energy expenditure. Skeletal muscle also has critical implications for health and disease, with alterations in muscle mass, strength, and function linked to numerous metabolic, cardiovascular, and musculoskeletal disorders. Therefore, understanding the biology and physiology of skeletal muscle is crucial for developing effective strategies to prevent and treat these conditions, as well as to promote healthy aging and longevity.

We have compiled several publicly available datasets to identify novel regulators of muscle function and found that E3 ligase HECTD1 is commonly deregulated in conditions of atrophy and myopathies. We have characterized the effect of HECTD1 during myotube differentiation, discovering profound changes in expression of both transcription factors responsible for myotube maturation as well as calcium signaling and structural components. Mass spectrometry and overexpression studies place HECTD1 as a component in the Klhl40/41-Nebulin axis, which regulates templating for actin assembly in actomyosin contractile apparatus. HECTD1 conditional skeletal muscle knock-out animals (HECTD1-mKO) have compromised both mitochondrial and muscle function as well as structure. Additionally, HECTD1-mKO muscles show increases in signatures of protein refolding and protein aggregate clearing, reminiscent of several human myopathies.

Understanding the role of HECTD1 can shed light on new players and pathways regulating sarcomeric integrity. Additionally, since enzymes such as E3 ligases are amenable to pharmacological interventions it could be a potential strategy for combating loss of muscle function during aging, atrophy or in musculoskeletal disorders.

P16 - Ribosome specialization during skeletal muscle hypertrophy

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Skeletal muscle (SkM) plays a key role in whole-body metabolism and is the most important organ for insulin-stimulated glucose clearance from circulation. Maintenance of SkM homeostasis is paramount to counteract metabolic diseases. The ribosome is the ribonucleoprotein complex responsible for cellular protein synthesis and ribosome biogenesis has emerged as a crucial regulator of SkM growth. Recent studies suggest new ribosomes made in response to mechanical loading differ from the resting state in SkM. Moreover, it's been suggested that changes in ribosome composition could influence the proteome e.g., by modulating translation fidelity, translation speed and/or by influencing mRNA selectivity. Ribosome biogenesis involves the transcription of two pre-ribosomal RNA (rRNA)s: one common to 18S (SSU), 5.8S, and 28S (LSU) rRNA, while the other exclusively for 5S rRNA. Especially, rRNAs are heavily modified by 2'-O-methylation (2'-O-Me) post-transcriptionally. Thus, this project aims at determining ribosome 2'-O-Me heterogeneity in SkM by a dedicated sequence-based profiling method (RiboMeth-Seq) in response to mechanical loading.

Preliminary results indicate that rRNAs from adult human SkM have a highly methylated 2'-O-Me pattern. In contrast, proliferating human myoblasts significantly differ with respect to % of rRNAs methylation at 10 sites: (i) sites with decreased methylation (SSU-U354, SSU-G797, SSU-G867, SSU-C1272, SSU-G1442, LSU-G1303, LSU-A3846, LSU-G4607, 5.8S-U14); (ii) one site with sharply increased methylation (LSU-G4593). Using a mechanical overload mouse model (synergist ablation), we have determined the dynamic response of 2'-O-Me in SkM at 3, 7, and 14 days during the hypertrophic response. We observed marked hypomethylation on days 3 and 7 for 17 sites (SSU-U121, SSU-U354, SSU-G436, SSU-A512, SSU-A590, SSU-C797, SSU-G867, SSU-C1272, SSU-1442, LSU-G1303, LSU-C2409, LSU-G2411, LSU-C2848, LSU-G4020, LSU-U4276, LSU-A4541, 5.8S-U14), followed by a recovery of 2'-O-Me levels at 14 days. The same trend was observed at hypermethylation site(LSU-G4593) and recovery at 14 days. Future experiments will characterize the functional significance of these changes.

P17 - Sarcomeric mitochondrial creatine kinase 2 modulates mitochondrial function and is downregulated in men with type 2 diabetes

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Hypothesis: Mitochondrial dysfunction is implicated in the pathogenesis of insulin resistance, whereas high plasma creatine levels are associated with increased risk of type 2 diabetes. We hypothesize that alterations in the sarcomeric mitochondrial creatine kinase 2 (CKMT2) are linked to impaired mitochondrial function and insulin resistance. **Methods:** We silenced or overexpressed *Ckmt2* in C2C12 myotubes and incubated them with oleate/palmitate (0.5 mM) to assess the role of CKMT2 against metabolic stress. Glucose oxidation, reactive species production and mitochondrial respirometry were assessed. Next, we fed male C57BL/6 mice with a high-fat diet for 8 weeks, overexpressed *Ckmt2* in the *tibialis anterior* muscle, and measured glucose uptake and mitochondrial respiration. Finally, we collected plasma and *vastus lateralis* biopsies from men with normal glucose tolerance (n=25) or type 2 diabetes (n=25). Creatine and phospocreatine were assessed by LC-MS. Expression of *CKMT2* and creatine transporter *SLC6A8* was obtained from a microarray. **Results:** Silencing *Ckmt2* in C2C12 myotubes reduced glucose oxidation (-66%, p=0.014), downregulated *Ppargc1a* (-12%, p=0.038) and *Sod2* (-13%, p=0.022) and increased hydrogen peroxide production (+5.1%, p=0.04). *Ckmt2* silencing led to lower mitochondrial respiration (-54%, p=0.007) and higher uncoupling ratio (+31%, p=0.045). *Ckmt2* overexpression reversed oleate/palmitate-induced downregulation of *Sod2* (-2% vs. -18%, p=0.06) as well as the activation of the stress-responsive P38 kinase (+11% vs. +40%, p=0.007). Consistently, overexpressing *Ckmt2* in the *tibialis anterior* muscle of high-fat diet fed mice attenuated P38 activation (-36%, p=0.003) and enhanced mitochondrial respiration (+14.1%, p=0.004). *CKMT2* and *SLC6A8* expression was reduced in *vastus lateralis* muscle from men with type 2 diabetes (-47%, p=0.011; -18%, p=0.022; respectively), and it was associated with lower intramuscular phosphocreatine (-32%, p=0.02). **Conclusions:** Lower *CKMT2* expression in type 2 diabetes might contribute to mitochondrial dysfunction and exacerbated oxidative stress. Therapeutic strategies aiming at upregulating *CKMT2* could improve mitochondrial function in type 2 diabetes patients.

P18 - The CXCL12/CXCR4 axis regulates skeletal muscle immunometabolic responses in aging

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Background
Aging is associated with decreased skeletal muscle mass and adaptability. Transient exercise-induced inflammation promotes muscle regeneration, while chronic low-grade inflammation is associated with decreased muscle mass and insulin resistance. Previous results showed differential effects of exercise on cytokine production in type 2 diabetes. Here we aim to characterize the association of the chemokine CXCL12 and its cognate receptor CXCR4 with skeletal muscle inflammation and aging to provide molecular insight into the role of this axis in cell senescence and metabolism.

Methods:
Plasma CXCL12 concentration was determined by ELISA. *In vitro* analyses were performed using myotubes differentiated from mouse C2C12 or primary human (HSMC) skeletal muscle cells. Following exposure to alpha or beta isoforms of recombinant CXCL12, glucose uptake was measured using radiolabeled 2-deoxy-d-glucose. Protein phosphorylation was measured by immunoblot analysis, and cAMP was quantified by ELISA. Gene expression was measured by qPCR.

Results:
The Genotype-Tissue Expression dataset shows increased *CXCL12* and *CXCR4* mRNA expression in skeletal muscle with age. ELISA showed a 2-fold increase in circulating CXCL12α immediately after acute exercise and decreased circulating CXCL12α with age. *CXCR4* mRNA expression in HSMCs increased 3.5-fold across 10 days of differentiation, while *CXCL12* expression remained unaltered. Exposure to CXCL12 during differentiation increased the expression of the proliferation marker KI67 in HSMC and impaired C2C12 myotube formation. In C2C12 myotubes, acute exposure to mouse CXCL12 increased glucose uptake independently of insulin signaling. Cxcl12 activated G protein-coupled receptors coupled to Gi proteins, leading to a 2.5-fold increase in ERK1/2 phosphorylation and inhibition of forskolin-induced cAMP production.

Conclusion:
CXCL12 modulates skeletal muscle cell differentiation and glucose metabolism *in vitro*, potentially playing a role in the regulation of skeletal muscle adaptation to exercise and the etiology of metabolic diseases. CXCL12 production and release are dysregulated with aging, suggesting involvement in skeletal muscle senescence.

P19 - The role of Mustang in smooth-to-skeletal muscle crosstalk

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Skeletal muscle is a very adaptive tissue that changes with physiological stimuli such as exercise, disuse, and age, and deregulation of its physiology can lead to pathologies affecting the whole organism. It is a highly vascularized tissue and the cells composing its microvasculature play an important role in muscle injury, regeneration and disease. Among those, smooth muscle cells (SMC) play a central role in regulating blood flow to the muscle, but little is known about how they communicate with their neighboring cells – skeletal muscle fibers, endothelial cells etc. – and how these processes are affected by muscle injury and disease.

Using transcriptional analysis of muscle in a mouse model of hindlimb unloading/reloading we identified *mustn1* to be strongly regulated at the onset of hindlimb reloading. *Mustn1* encodes a conserved micropeptide of 82 amino acids called Mustang, whose function is still largely unknown. Single-cell transcriptomic data places the origin of *mustn1* expression in SMC and skeletal muscle fibers. We observed Mustang protein expression in blood vessels of the muscle, and bioinformatics predictions indicate it might be non-classically secreted. Indeed, we found that muscle cells could secrete ectopically expressed Mustang into the extracellular milieu. Furthermore, using the LigandTracer system, we found that recombinant Mustang could interact with a (still unknown) receptor expressed on specific target cells, including skeletal muscle cells.

The next steps in this project include investigating the role of SMC in secreting Mustang under conditions such as exercise and muscle injury and the cellular effects of Mustang on target cells.

Adipose tissue metabolism, growth and (patho)physiology

P20 - Computational identification of transcriptional regulators affecting lipid droplet morphology in adipocytes

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Lipid droplets (LDs) are crucial storage organelles that maintain energy balance in response to fluctuations. The size and number of LDs directly reflect lipid synthesis and degradation, yet the underlying mechanisms remain poorly understood. A thorough understanding of the regulation of lipid droplet morphology is critical for developing effective therapeutic strategies for metabolic disorders, including obesity and type 2 diabetes. This project aims to investigate the transcriptional regulation of lipid droplet morphology in white adipose tissue. A large-scale RNAi screen was conducted to silence 700 genes that control multiple target genes and pathways involved in lipid turnover. High-content image analysis was performed on in-vitro differentiated human adipocytes from three donors to assess fat cell metabolism. Aiming to deduce changes in LD morphology, 380 image features from more than 1 million cells features were extracted. Using a feature selection pipeline and an SVM classifier, transcriptional regulators affecting LD morphology were identified. The future direction of this project includes validating the computationally identified hits.

P21 - Exploring the contribution of DNA damage and repair to senescence induction in mature human adipocytes

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Aim: To elucidate the role of DNA damage and impaired DNA damage repair, in conjunction with hyperinsulinemia, on the induction of senescence in mature human adipocytes.

Methods: To directly assess the extent of DNA damage, we employ the comet assay, while to investigate the efficacy of DNA damage repair mechanisms, we utilize western blot and immunocytochemical staining for the DNA repair proteins 53BP1, yH2AX and APE1. To quantify senescence in adipocytes, we use senescence-associated beta-galactosidase staining.

Preliminary results/planned studies: Previous studies in the lab have revealed elevated levels of senescence in subcutaneous human adipocytes relative to omental adipocytes. Senescent cells are considered detrimental to healthy adipose tissue function, secreting factors that promote inflammation and fibrosis. Preliminary data from our group suggests a decrease in DNA damage in subcutaneous adipocytes but an increase in DNA repair proteins, yH2AX and APE1, compared to omental adipocytes. These findings suggest that reduced senescence in omental adipocytes may be due to an impaired capacity for DNA damage repair, rather than DNA damage itself. The lack of a DNA damage response (DDR) may enable omental adipocytes to avoid the initiation of a senescence program. Obesity associates with increased oxidative stress, a main contributor of DNA damage. Whether obesity-associated differences in DNA damage and DDR exists between omental and subcutaneous adipocytes will be in investigated.

P22 - Functional characterization and response to metformin or flutamide of in vitro differentiated adipocytes from women with polycystic ovary syndrome

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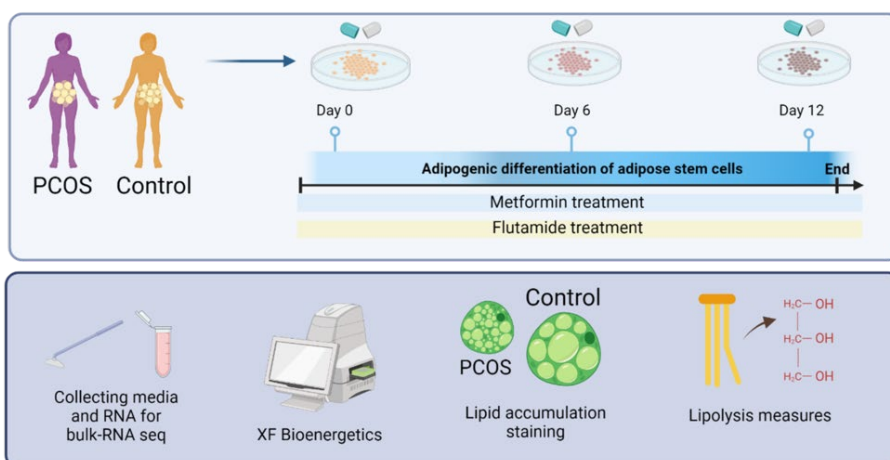
Background & Aim: Adipose tissue plays a key role in metabolic dysfunction in hyperandrogenic women with polycystic ovary syndrome (PCOS). Enlarged adipocytes and low levels of adiponectin are strong factors driving their insulin resistance (IR) and type 2 diabetes. Global gene expression studies have identified genes that may contribute to PCOS-specific adipose dysfunctions. However, detailed functional characterization of PCOS adipocytes is lacking, and although drug treatment with metformin or androgen receptor blocker flutamide alleviates symptoms of PCOS, the effect and mechanisms of these drugs on adipocytes remain unclear. Therefore, the main aim is to characterize metabolic differences of PCOS adipocytes throughout *in vitro* differentiation and investigate how metformin and flutamide affect these cells.

Preliminary Results: We have isolated adipose stem cells from hyperandrogenic and IR women with PCOS and from BMI-matched controls. Our preliminary data show that *in vitro* differentiated mature adipocytes from IR-PCOS are enlarged, mimicking the adipocytes from tissue biopsies. The transcriptomic PCOS profiling shows genes related to cell cycle are downregulated in adipose stem cells, whereas genes related to fatty acid metabolism and tricarboxylic acid cycle are upregulated in preadipocytes and mature adipocytes. Measurements of cellular bioenergetics, lipolysis, and the effect of metformin and flutamide on observed alterations are currently ongoing. For further insight, we will also perform sex steroid analyses in the cell secretome as well as bulk RNA-sequencing of cells during adipocyte differentiation and simultaneous drug treatment.

Conclusion: Our findings suggest that adipose stem cells of women with PCOS have an increased adipogenic potential and decreased proliferative capacity supported by increased lipid accumulation and hypertrophy. As the *in vitro* differentiated adipocytes keep the phenotypic features of PCOS-derived adipose tissue, these findings will pave the way for further mechanistic studies on how the disordered adipose tissue function in PCOS can be ameliorated therapeutically with a disease-specific approach.



P23 - Functional characterization of fibroblast heterogeneity in human subcutaneous adipose tissue

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Background: With up to 40% of all cells, fibroblasts constitute the largest cell class in human white adipose tissue (WAT). Despite this, how specific fibroblast subtypes contribute to health and metabolic disease is not well characterized.

Aim: The purpose of this project is to systematically characterize WAT fibroblast heterogeneity and microarchitectural organization, and to understand how these features are influenced by body weight changes and contribute to metabolic disease both in vitro and in vivo.

Methods: We analyzed single cell and spatial transcriptomics data sets across metabolic conditions and WAT depots to determine fibroblast heterogeneity. Identified fibroblast population are isolated by FACS and studied for their differentiation and functional potential in vitro and in vivo. For this, cells are cultured in 3D spheroids or transplanted into a humanized mouse model. At termination, the transplanted fat pads will be excised and tested by using H&E staining, IHC and ELISA. Following the first engraftment, FACS-sorted cells labeled with different fluorophores will be transplanted for studying the microarchitecture organization and tracing linages of fibroblasts.

Results: We identified 16 subcutaneous fibroblast subpopulations of which only two have been consistently described in the scientific literature. These are CFD+ committed preadipocytes and CD55+ adipogenic precursor cells. In general, fibroblast showed a bimodal distribution across WAT, while specific fibroblasts subtypes exhibited unique localization patterns, as shown by SLIT2+ fibroblasts, that are only found around vessel structures in WAT, as confirmed by immunofluorescence staining. Deconvolution of bulk transcriptomics data suggest that amount of CD55+ are strongly linked to adverse metabolic states. To study the role of specific cell states and their respective marker genes, wehave established and optimized a FACS panel to sort at least four different fibroblast populations that will be characterized by bulk sequencing. We established protocols to perform transplantation.

P24 - Is altered adipocytes cholesterol acquisition promoting adipose tissue dysfunction?

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Obesity is associated with several detrimental comorbidities such as increased risk for cardiovascular disease, but their interconnectivity remains poorly understood. Human adipocytes become hypertrophied during obesity and can grow 200- to 1000-fold in volume. Interestingly, obesity has been shown to induce a cholesterol imbalance in hypertrophied adipocytes. While triglycerides are the main constituents of adipocyte lipid droplets, they also contain cholesterol-esters, which can be hydrolyzed to free cholesterol that is an important constituent of cellular membranes. How adipocytes handle their cholesterol, storing it in the lipid droplets or in their membranes, is still debated in literature, and is of great importance as membrane cholesterol levels significantly affect several adipocyte functions such as signalling sensitivity and inflammatory activation. The aim of this project is to explore the effects of altered cholesterol acquisition on adipocyte dysfunction.

Our preliminary sequencing data suggested that adipocytes from insulin resistant obese subjects may have a reduced capacity to synthesize free cholesterol and therefore instead increase their uptake of lipoprotein-associated cholesterol esters via upregulation of the LDLR. Importantly for systemic disease development, these changes seemed to be coupled to a compensatory lowered triglyceride uptake instead. By using our Human Unilocular Vascularized Adipocyte Spheroid model (HUVAS, Ioannidou et al, 2022), we investigated how cholesterol loading impacts adipocyte dysfunction in lean (control) and obese (fattened) adipocyte cultures. In fact, supplementation with free cholesterol leads to significantly increased multilocularity and increased cholesterol ester content in lean adipocytes, but not in obese adipocytes. The results moreover indicate that obese adipocytes take up higher levels of cholesterol-dense lipoproteins, potentially to allow for membrane synthesis and growth. Herein, we suggest that it is not the storage location of the cholesterol, but the model of cholesterol delivery (synthesis versus uptake) that is the critical factor for maintaining adipocyte functionality during obesity.

P25 - Revealing the Hidden Signals: A new Approach to Detect Senescence in Mature Human Adipocytes

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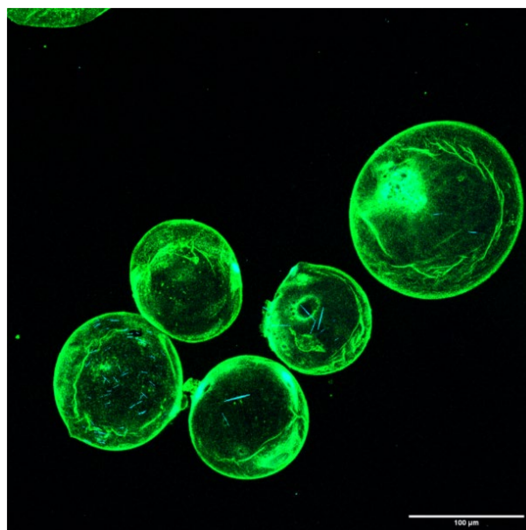
Aim: To develop a more sensitive, objective, and high-throughput method to detect and quantify senescence associated beta-galactosidase activity in mature adipocytes.

Methods: We adopted a confocal, reflected-light microscope approach to detect the presence of x-gal metabolites in adipocytes with active beta-galactosidases. This method allows for a whole-cell scan of the adipocytes and can be coupled with fluorescence stains to investigate additional senescence associated proteins.

Results and conclusions: Senescence is characterized by a gradual decrease in cellular function and the acquisition of a pro-inflammatory signature. Senescent cells exhibit enzymatically active senescence associated beta-galactosidase (SABG) at pH 6. When x-gal is added to these cells, SABG cleaves the x-gal and produces an opaque chromogenic compound that can be detected using reflected light. To quantify this signal, we used a microscope fitted with reflected-light detection capabilities that effectively identifies opaque materials. However, other complex structures, such as highly folded membranes or organelle dense areas can also reflect light and cause noise and/or false positive signal. To combat this, we have adopted a machine learning approach to classify opaque pixel signals resulting from SABG activity with high fidelity. Furthermore, these staining protocols can be combined with traditional fluorescence-based antibody labeling to investigate multiple senescence-associated parameters. This new approach is more sensitive, less subjective, and high throughput compared to previous SABG quantification methods that rely on manual classification with brightfield microscopes.



P26 - Sensory neuron-derived calcitonin gene-related peptide (CGRP), but not substance P, impairs adipocyte differentiation

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Adipose tissue plays a critical role in whole-body energy homeostasis and constitutes an organ system capable of information exchange. Information from adipose tissue is secreted into circulation, but also to nearby cell types including sensory neurons. Sensory neurons receive these signals to send distally, but at the same time, secrete factors locally, where the information is gathered. Despite significant understanding about how sensory neurons send signals to the brain, the role of neuropeptides secreted from sensory neurons in adipose tissue remains undetermined. Here, we sought to determine if sensory neuron-derived calcitonin gene-related peptide (CGRP) and substance P (SP) play a role in the expansion of adipose tissue seen in obesity. To study this, we used an in vitro model of adipogenesis using primary murine preadipocytes. We observed that the presence of CGRP at specific time points during differentiation leads to impaired accumulation of lipids. In support of this, CGRP represses key adipogenic genes including CCAAT-enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma. CGRP also increases the expression of col1a1 and col3a1, both of which are associated with fibrosis. CGRP signals in part through p-CREB, which remains elevated compared to controls. Interestingly, treatment with SP during adipogenesis leads to normal lipid accumulation, suggesting that the inhibition of adipogenesis is CGRP specific. Taken together, these data indicate that CGRP, but not SP, impairs adipocyte differentiation; this effect seemingly occurs through increased CREB activation leading to dysregulated gene expression. Further research is required to fully understand the peripheral effect of sensory neuron-derived neuropeptides on adipose tissue.

P27 - Single-cell transcriptomics of white adipose tissue from women with polycystic ovary syndrome

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Hypertrophic adipocytes together with low adiponectin are strong factors driving insulin resistance (IR) in women with polycystic ovary syndrome (PCOS). Moreover, altered production and release of lipids and adipokines, and chronic low-grade tissue inflammation are associated with pathological white adipose tissue (WAT) function with altered production and release of lipids and adipokines and chronic low-grade tissue inflammation. WAT is a dynamic and heterogenous organ composed of different cell types involved in a wide array of biological processes but how cell-type-specific WAT dysfunction in women with PCOS contributes to IR and subsequent development of T2D is unclear, and whether the treatments aimed at improving insulin sensitivity and reducing androgen excess could reverse these alterations.Single nuclei were extracted from our frozen WAT tissues collected from controls (n=4) and from hyperandrogenic and insulin-resistant women with PCOS (n=10) at baseline and in PCOS after 16 weeks of metformin (n=6) or lifestyle management (n=3). The nuclei RNA libraries were prepared following the 10x genomics protocol and 200 million reads were sequenced from ~5,000 nuclei/sample. Pre-processing and quality control (QC) of the data was performed using CellRanger and Seurat 4.0 was used for downstream QC, filtering, integration, clustering, and differential gene expression analyses.

A total of 109,739 nuclei were captured and four main canonical cell types were identified based on known markers: 1) adipocytes (n=17,896); 2) adipose stem and progenitor cells (ASPCs) (n=12,438); 3) immune cells (n=15,925); and 4) vascular cells (n=20,486). Subclustering and differential gene expression within each subcluster, pathway- and functional analyses as well as defining the effect of metformin and lifestyle management are ongoing.

These comprehensive analyses will significantly increase our understanding of the cellular complexity and heterogeneity in specific cell types underlying WAT dysfunction in PCOS and define whether cell-type-specific molecular dysfunctions can be reversed by current first-line treatment.

P28 - The regulation of fatty acid transport over the endothelial barrier of white adipose tissue

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By taking up fatty acids (FAs), the white adipose tissue (WAT) acts as a nutrient buffer that protects other organs from ectopic lipid accumulation. In WAT the microvascular endothelial cells (ECs) form a continuous layer that creates a physical barrier between the blood stream and the parenchyma, where FAs actively must be transported over. In contrast to most metabolically active organs, the WAT only takes up FAs during the postprandial state, suggesting the need for a WAT-specific regulatory mechanism. This is in line with capillary beds often adapting their function towards the tissue they reside in. However, not much is known about such adaptations within the WAT. The aim of this project was to explore the mechanisms that are required for nutrient transport over the WAT EC barrier and whether they are modulated by the fed and fasted state at the circulatory level.

To mechanistically investigate WAT endothelial barrier integrity and FA uptake, we have developed an organotypic *in vitro* transwell system using human WAT-derived microvascular ECs (hAMECs) and human sera. By simultaneously incubating hAMECs with BODIPY-C12 and FITC-Dextran to measure FA transport and barrier integrity, respectively, we have developed a system where both parameters in parallel can be monitored in response to changes in cellular environment. The effects of fasting and (over) feeding on hAMEC barrier function and FA transport are assessed by exposing the cells to sera from overnight fasted and non-fasted individuals as well as single nutrients. Besides functional readouts from the transwell system, transcriptional analysis will be performed. This transwell system has been adapted to other capillary beds, allowing us to compare the functional responses of hAMECs to that of primary human microvascular ECs from heart and liver. This has shown large differences between capillary beds, and the preservation of tissue-specific endothelial functions in culture.

P29 - VEGF-B signaling in the white adipose tissue regulates hepatic steatosis, development of diabetic fatty liver disease and hepatocellular carcinoma.

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Diabetes mellitus (DM) is associated with the development of several vascular diseases, that are the major causes of disability and death in diabetic patients. One of the most common complications in type 2 DM is non-alcoholic fatty liver disease (NAFLD). NAFLD is associated with a range of pathologies ranging *from steatosis* to non-alcoholic steatohepatitis (NASH), and finally to hepatocellular carcinoma (HCC). Today there is no established pharmaceutical way to treat NAFLD and NASH, and the established standard of care therapy for HCC is limited. The underlying mechanisms of NASH-mediated HCC are not known, however abnormal lipid accumulation in hepatocytes is a pathological characteristic of NAFLD. We have previously shown that Vascular Endothelial Growth Factor B controls tissue lipid accumulation by targeting endothelial fatty acid transport. Herein we used combinatorial diet regimes to induce the different consecutive phases of NASH in experimental models with systemic reduction of VEGF-B levels. Inhibiting VEGF-B signalling, using whole-body genetic knockouts and pharmacological tools, in choline deficient high fat diet (CD-HFD) fed mice decreased steatosis and ameliorated several of NAFLD/NASH associated pathologies and, importantly, reduced HCC development. Mechanistically we show, by using mice with specific decrease or overexpression of VEGF-B in adipocytes, that systemic inhibition of VEGF-B signaling targets the lipolytic activity in the white adipose tissue (WAT). We suggest that VEGF-B antagonism represents a novel approach to treat NASH-mediated HCC by targeting WAT lipolysis and thereby preventing liver steatosis and lipotoxicity.

P30 - The Liver Clock Drives Circadian Regulation of Adipogenic Genes in White Adipose Tissue

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Background and aim: Circadian rhythms are tightly coupled with energy homeostasis and are sustained by inter-organ clock communication. White adipose tissue (WAT) is the main energy reservoir of the body and controls metabolism by storing and releasing lipids. The transcription factors Cebpα and Pparγ regulate adipogenesis (fat cell differentiation), and disruption of adipogenic rhythms has been proposed to contribute to the pathophysiology of metabolic diseases. Yet, little is known about how the circadian system controls adipogenic rhythms. This project aims to investigate the role of the hepatic clock in regulating WAT adipogenic gene expression.

Material and methods: *Animal samples*: WAT samples were collected every 4 hours over the circadian period from whole-body *Bmal1* (KO) mice, mice with reconstituted *Bmal1* in the liver (LRE) and their corresponding littermates (WT). These animals lack the central clock in the suprachiasmatic nucleus and therefore lack behavioural feed-fasting rhythms. All genotypes were therefore subjected to 12-hr time-restricted feeding and thus, eating rhythms were re-instated.

Cell culture experiments: Immortalised murine liver (Aml-12) and adipose tissue (3T3-L1) cell lines were used to dissect the underlying mechanisms involved in liver-to-WAT communication. This was achieved by synchronisation of hepatocytes using a dexamethasone shock followed by the transfer of conditioned media to the adipocyte cultures.

Results and Conclusions: We demonstrate that, in the presence of feed-fasting rhythms, the hepatic clock is sufficient to drive adipogenic gene expression in the WAT. Our *in vitro* data suggest that *Bmal1* in hepatocytes mediate these signals via circadian control of hepatokines (proteins secreted from hepatocytes). Taken together, our data indicate that the local WAT clock is not required for circadian adipogenic gene regulation and that the hepatic clock is sufficient to mediate these rhythms. Future experiments will aim to characterise the underlying mechanisms and their role in the pathophysiology of metabolic disease.

Liver metabolism, growth and (patho)physiology

P31 - Uncovering Zfp697 as a potential novel target in non-alcoholic fatty liver disease

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Background and aim: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world affecting 25% of the adult population. The global prevalence of NAFLD is expected to increase in consistence with the increase in type 2 diabetes and obesity, which are well-known risk factors for NAFLD.

We have identified a zinc finger protein of previously unknown function (mouse: Zfp697, human: ZNF697) and discovered that it operates as a regulator of inflammation, extracellular matrix remodeling, and fibrogenesis. In this project we investigate the function of Zfp697 in NAFLD and evaluate its potential as a novel therapeutic target.

Method: We are using genetic and viral approaches to modulate Zfp697 expression in mice and in cell cultures. This is combined with NAFLD/liver injury inducing diets and treatments to investigate the role of Zfp697 in liver disease and uncover the NAFLD-driven pathways regulating Zfp697 expression.

Results: Zfp697/ZNF697 expression is elevated in human liver disease and NAFLD-mouse models. Importantly, we have found that increasing liver Zfp697 expression, within pathophysiological levels, is sufficient to drive a strong NAFLD-like phenotype. This includes Zfp697-induced expression of genes associated with immune response, extracellular tissue remodeling and fibrosis. Interestingly, Zfp697 gene ablation in hepatocytes ameliorates glucose homeostasis and energy expenditure in NAFLD-diet fed mice. In cell cultures, NAFLD-associated conditions including oxidative stress, lipid accumulation as well as TGFb1, IL-6 and IFNg activated pathways drive Zfp697 expression. Moreover, we have found that Zfp697 associates with RNA binding proteins and binds a specific set of RNAs.

Conclusion: Zfp697 is a novel regulator of inflammation involved in some of the key molecular and cellular events of NAFLD development and progression. Research in this project could form the foundation for finding a new class of pharmacological therapies for NAFLD and potentially other liver inflammatory diseases, such as alcoholic hepatitis.

Central nervous system and metabolism

P32 - Characterizing the effects of chronic stress on circadian rhythms in liver and brain.

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Background and aim: Disruptions in circadian rhythms are highly correlated to stress exposure and considered as risk factors for developing mental and metabolic disorders such as obesity and depression. The underlying mechanisms explaining the relationship between mental- and metabolic- morbidity remains elusive. The aim of this study is to dissect the circadian processes linking chronic stress to liver and brain transcriptional rhythms.

Material and methods: Mice were co-housed with an aggressor and divided into groups depending on their response. This resulted into three groups: controls, co-housed with a mouse of the same breed; stress-susceptible, that presented aversion to the aggressor; and stress resilient, that did not present aversion to the aggressor. Analyses of RNA-seq data has been performed on tissues (liver, hypothalamus, hippocampus and prefrontal cortex) collected every 4h over the circadian cycle.

Results: Both resilient and susceptible groups showed increased number of rhythmic genes in hypothalamus and liver, and less rhythmic genes in hippocampus and prefrontal cortex compared to the controls. Interestingly, the resilient group displayed a unique transcriptional fingerprint that was different from both the susceptible mice and controls.

Conclusion: This study explores associations between processes regulating cognitive functions and metabolism. The induced rhythms in hypothalamus and liver in combination with dampened rhythms in higher brain regions indicates a priority for circadian regulation of metabolic processes in response to stress. Our data suggest that the resilience to stress is not reflected by a transcriptional fingerprint more similar to non-stressed mice but rather a unique transcriptional response.

P33 - LisdexAmphetamine versus Methylphenidate for Patients with ADHD and Type 1 Diabetes (LAMA_in_Diab) – randomized cross-over clinical trial protocol

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Attention deficit hyperactivity disorder (ADHD), which affects 5-10% of the general pediatric population, was reported to be more common in children with type 1 diabetes (T1D). Patients with T1D and ADHD encounter problems with reaching proper metabolic control. A question arises would the reduction of ADHD symptoms by optimal treatment in children with T1D lead to the improvement of metabolic control?

The Study is a multicenter, randomized, open-label, cross-over clinical trial in children with ADHD and T1D. The Trial will be conducted in four Polish reference diabetes centres (Lodz, Katowice, Gdansk, Opole) that provide care for ~25% of Polish pediatric population with T1D. Over 36 months, all eligible patients with both diagnoses of T1D and ADHD (aged 8-16.5 y.o., T1D duration >1 year) will be offered participation in the project. Patients will be enrolled in a parental training in behavioral management (PT) and subsequent allocation to pharmacotherapy groups: methylphenidate (MPH, long-release capsule, standard) versus lisdexamphetamine (LDX, investigated treatment).

Enrolled participants will take part in parental training in for ten weeks. Afterward, the children will be randomized to either LDX (30, 50, 70mg/day) or MPH (18, 36, 54mg/day). After dose escalation is performed over the initial 5 weeks, the treatment will continue for 6 months. Switch to the alternative medication will be performed after up to one week of wash-out. Throughout the Trial, the participants will be evaluated every three months by their diabetologist and concurrently performed, online psychological assessments. The Trial primary endpoint (ADHD symptom severity) will be assessed using the Conners 3.0 questionnaire by an investigator blinded to current treatment. Secondary endpoints will include metabolic control assessed with HbA1c and continuous glucose monitoring, and Quality of Life (QoL, measured by PedsQL).

The study results will be used to improve ADHD treatment in children with T1D.

P34 - Thrombolytic tPA-induced intracerebral hemorrhage in ischemic stroke is mediated by increased adipose tissue lipolysis

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Intravenous thrombolytic therapy (IVT) with tissue plasminogen activator (tPA) can significantly improve neurologic outcomes after ischemic stroke; however, tPA treatment is associated with an increased risk of intracerebral hemorrhage (ICH). ICH connected to tPA treatment increases with time after stroke onset and is exacerbated in obese and diabetic patients, considerably limiting the use of thrombolytic therapy beyond 4.5h after stroke onset. Identifying pathways induced by tPA that increase this risk could thus provide new therapeutic options to extend thrombolytic therapy to a wider patient population.

ICH and infarct volume increased after middle cerebral artery occlusion (MCAO) in mice fed with high-fat diet (HFD) for 20 weeks, compared to age-matched littermate mice on normal chow, thus serving as a suitable model to study adverse events such as ICH after tPA IVT.
tPA IVT initiated 5h post-MCAO in HFD mice led to a significant rise in non-esterified fatty acid (NEFA) content in blood and buildup of neutral lipids in liver and brain. Further mechanistic studies determined that intravenous tPA administration, within 30 min, specifically promoted visceral adipose tissue lipolysis and blood NEFA rise; correlating with enhanced ICH after stroke. The effect on blood NEFAs were also confirmed in stroke patients undergoing thrombolytic tPA treatment.

We hypothesized that co-treatment with an anti-lipolytic therapy could prevent tPA-induced lipolysis, and potentially the adverse events coupled to IVT. Indeed, co-treatment with neutralizing anti-VEGF-B antibodies improved the efficacy and safety of a 5h delayed tPA IVT after MCAO in HFD mice, correlating with decreased infarct volume and prevention of ICH.

These results point to an unexpected involvement of adipose tissue lipolysis in ischemic stroke outcome and the potential use of an anti-VEGF-B monoclonal antibody as adjuvant therapy extending the therapeutic window and use of tPA for thrombolytic therapy in ischemic stroke.

P35 - Endothelial cell lipolysis regulates vascular fatty acid and glucose transport: implications for cerebrovascular energy substrate uptake.

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Abstract text: Cellular substrate utilization will depend on substrate availability as dictated by the balance between uptake, self-utilization, and transport of energy substrates over the endothelial cell (EC) barrier to the underlying parenchyma. However, the processes that regulate the metabolic flux of energy substrates in ECs remain poorly understood. We have previously reported that vascular endothelial growth factor B (VEGF-B) increase EC fatty acid (FA) uptake but decrease glucose uptake, and therefore aimed to further investigate VEGF-Bs effect on EC energy substrate selection to elucidate pathways involved in the regulation of EC metabolic flux.

Human umbilical vein endothelial cells were used as primary EC model system. The cells were allowed to reach confluency prior to oleic acid supplementation, pharmacological treatment, or VEGF-B stimulation. BODIPY neutral lipid dye, C_{12}-BODIPY and 2-NBDG tracer were used to assess cellular neutral lipid content, FA uptake and glucose uptake respectively. 2-photon imaging of WT C57BL/6J mice with cranial window implants was used as primary *in vivo* model system to assess cerebrovascular energy substrate transport.

BODIPY neutral lipid staining revealed that VEGF-B reduces neutral lipid content in primary ECs supplemented with oleic acid. Pharmacological inhibition of lipolysis both restored the neutral lipid content and further blunted VEGF-Bs effect on both EC FA and glucose uptake as assessed by C_{12}-BODIPY and 2-NBDG uptake respectively, while pharmacological induction of lipolysis had the opposite effect. Consistently, cerebral 2-photon imaging of cranial window mice revealed an increased and prolonged cerebral uptake of intravenously administrated C_{12}-BODIPY in fasted conditions with higher systemic lipolysis as compared to non-fasted conditions. Collectively, our data suggest lipid metabolism and lipid turn-over as key factors for EC energy substrate selection and transport. Therefore, targeting EC lipid metabolism through inhibition of VEGF-B may help to restore vascular and tissue nutrient homeostasis thus prohibiting metabolic and CNS disease progression.

Diabetes complications

P36 - A novel reno-cardio-metabolic disease model to study diabetic nephropathy

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Background: In diabetes, progressive nephropathy is a major clinical problem affecting up to 40% of all patients. Not only being a main cause of renal failure; diabetic nephropathy is also strongly associated with increased cardiovascular morbidity and mortality. However, understanding the pathophysiology of the disease has been a major challenge due to its complexity. Here, we aimed to establish a novel reno-cardio-metabolic disease model, resembling the pathophysiology of diabetic nephropathy, by combining surgical and unhealthy dietary approaches.

Method: Young male C57BL/6 mice were subjected to unilateral nephrectomy (UNX) or sham surgery, followed by chronic intake of regular diet (RD) or a special Western Diet (WD) with high fat and sugar but also salt for 12 w. Reno-cardio-metabolic functions were monitored *in vivo* and organs (incl. blood, kidney, heart, aorta, fat, urine) were collected for *ex vivo* functional studies as well as histology/ biochemistry analyses.

Results: UNX+WD mice had increased fat and reduced lean masses, insulin resistance and impaired glucose clearance compared to the Sham+RD group. The UNX+WD group also developed high blood pressure and weakened cardiac contractility alongside with significant endothelial dysfunction. Glomerular filtration rate together with histological and urinary biomarkers analyses clearly showed kidney dysfunction and injuries. Mitochondria from heart and kidney of UNX+WD mice had impaired respiration.

Conclusion and Significance: Our dual approach, combining reduction of nephron number with chronic intake of an unhealthy diet, successfully induced clinically relevant reno-cardio-metabolic dysfunctions associated with diabetic nephropathy. This new model is valuable for future studies to dissect underlying mechanisms of disease development and progression as well as to investigate novel therapeutic targets.

P37 - Activation of Vascular Endothelial Growth Factor B Signalling and Renal Lipotoxicity are Novel Pathological Hallmarks of Diabetic Kidney Disease

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Diabetic kidney disease (DKD) rates among the most fatal long-term complication of Type 2 diabetes (T2DM). The most common hallmark of T2DM is the development of insulin resistance. One pathological characteristic in DKD and among other T2DM complication is the abnormal accumulation of lipids in different tissues (lipotoxicity), which often associates with insulin resistance. We identified a novel approach to ameliorate insulin resistance by targeting Vascular Endothelial Growth Factor B (VEGF-B) signaling. Our studies have shown that VEGF-B can exert lipid-lowering effects in the peripheral tissue either locally by regulating endothelial lipid transport or systemically by controlling lipolysis in the adipose tissue. We have shown that VEGF-B expression is up-regulated in DKD subjects and anti-VEGF-B therapy in animal models of DKD prevented lipid accumulation and disease progression. Herein, we characterised the VEGF-B signalling pathway using renal biopsies from both DKD patients and mouse models. IHC analyses of renal biopsies showed that VEGF-B expression was detected in both glomeruli and tubular cells in human DKD patients. Interestingly, the increase in VEGF-B activity in DKD was confined to activation of the VEGF-B signalling in the glomeruli. By using clinical records and measurements of VEGF-B expression in the same patient we could show that VEGF-B expression positively correlated to lipid accumulation and renal dysfunction. We will explore the possibility of using VEGF-B expression and/or renal lipid accumulation as marker(s) for DKD development by measuring VEGF-B levels in collected renal biopsies and compare with clinical following up measurements of renal function.

P38 - Early-Onset Type 2 Diabetes and Mood, Anxiety, and Stress-Related Disorders: A Genetically Informative Register-Based Cohort Study

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Objective: To assess the association and familial co-aggregation between early-onset type 2 diabetes (diagnosed before age 45) and mood, anxiety, and stress-related disorders, and estimate the contribution of genetic and environmental factors to their co-occurrence.

Research Design and Methods: This population-based cohort study included individuals born in Sweden 1968-1998, from whom pairs of full-siblings, half-siblings, and cousins were identified. Diagnoses of early-onset type 2 diabetes and mood (including unipolar depression and bipolar disorder), anxiety, and stress-related disorders were obtained from the National Patient Register. Logistic and Cox regression models were used to assess the phenotypic association and familial co-aggregation between type 2 diabetes and psychiatric disorders. Quantitative genetic modeling was conducted in full- and maternal half-sibling pairs to estimate the relative contributions of genetic and environmental factors to the association.

Results: Among a total of 3,061,192 individuals, 7,896(0.3%) were diagnosed with early-onset type 2 diabetes. These individuals showed higher risks of any diagnosis (Odds Ratio [OR][95%CI]: 3.62[3.44,3.80]) and specific diagnosis of unipolar depression (3.97[3.75,4.22]), bipolar disorder (4.17[3.68,4.73]), anxiety (3.76[3.54,3.99]) and stress-related disorders (3.35[3.11,3.61]). Relatives of individuals with early-onset type 2 diabetes also had higher overall risks of the examined psychiatric disorders (ORs:1.03-1.57). These associations are largely explained by genetic factors (51%-78%), with the rest explained by non-shared environmental factors.

Conclusion

Our findings highlight the burden of mood, anxiety, and stress-related disorders in early-onset type 2 diabetes and demonstrate that shared familial liability may contribute to their co-occurrence, suggesting that future research should aim to identify shared risk factors and ultimately refine preventive and intervention strategies.

P39 - Association between data-driven clusters and complications of type 2 diabetes

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There is a need to apply precision medicine in disease prevention. Data-driven methods have been used to stratify individuals' risk of type 2 diabetes. However, their utility to predict complications of type 2 diabetes remains unknown.

We used data from 7,317 diabetes-free participants of the Stockholm Diabetes Prevention Program. Follow-up included examinations after 10 and 20 years and linkage to regional and national quality registers. A total of 1,276 incident cases of type 2 diabetes were detected.

Partitioning cluster analysis was done using baseline data on sex, family history of diabetes, educational attainment, fasting plasma glucose and insulin, estimated insulin resistance and β-cell function, systolic and diastolic blood pressure, and body mass index. The resulting clusters were labeled: very low-risk (VLR), low-risk low β-cell function (LRLB), low-risk high β-cell function (LRHB), high-risk high blood pressure (HRHBP), high-risk β-cell failure (HRBF), and high-risk insulin-resistant (HRIR).

The outcomes in this study were suboptimal glycemic control, ischemic disease and stroke, chronic kidney disease and retinopathy. The association between risk clusters and the different complications of diabetes was estimated using logistic regression.

We found a significant association of risk clusters with suboptimal glycemic control, chronic kidney disease and ischemic disease including stroke, but not with retinopathy. Compared to the VLR cluster, the HRIR cluster had the highest risk of suboptimal glycemic control (OR: 3.0, 95%CI: 1.8, 5.3) and ischemic disease including stroke (OR: 4.6 95%CI: 2.1, 9.9). The risk of chronic kidney disease was highest in the HRHBP cluster (OR: 2.5, 95%CI: 1.08, 5.59). Risk of ischemic disease and stroke was also high among a cluster with low risk for diabetes (LRHB, OR: 3.8, 95% CI:1.6, 8.7).

Data-driven methods can be used to predict the risk of complication of type 2 diabetes, and might be used to better target prevention and treatment.

P40 - Association between impaired glucose metabolism and pulmonary dysfunction

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Aims We assessed a putative association between altered glucose metabolism and pulmonary function, since growing evidence indicates that the lung is a target organ for type 2 diabetes complications.

Methods In the cross-sectional Swedish CArdioPulmonary bioImage Study (SCAPIS), a total of 2507 study participants aged 50-64 years from the greater Umeå area were examined with spirometry including diffusion capacity and an oral glucose tolerance test (OGTT). Normoglycemia, prediabetes and type 2 diabetes were defined based on fasting glucose, 120-minute samples after OGTT and HbA1c according to WHO criteria.

Results The proportion of prediabetes, screening detected type 2 diabetes (T2D) and previously diagnosed T2D were 20,5, 2,4 and 4,3% respectively. Compared to participants with normoglycemia, those with prediabetes, screening-detected type 2 diabetes and T2D were older and had higher body mass index (BMI.) Using Spearman correlation test, we found significant negative correlations between forced vital capacity (FVC) and fasting glucose, HbA1c and 120 minutes blood glucose ($p < 0.001$ for all). A significant negative association between FVC and HbA1c remained after inclusion of BMI in a linear regression analysis ($p=0.009$). A significant negative association was also found between diffusion capacity and HbA1c ($p=0.003$) and blood glucose 120 minutes after OGTT ($p=0.028$), but not fasting glucose.

Conclusion Impaired glucose metabolism is associated with reduced lung volume and impaired diffusion capacity. Possible mechanisms behind the association between impaired lung function and altered glucose metabolism should be explored further.

P41 - Elucidating the role of mitochondria encoded circular RNA circ_1690-2254 in Diabetic Foot Ulcer

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Background: Diabetes is a widespread metabolic disease that has become a global burden. Up to 20% of diabetic patients are prone to impaired wound healing, among which Diabetic Foot Ulcer (DFU) is the most common. Mitochondria dysfunction has been involved in the pathogenesis of diabetes and understanding its underlying molecular mechanism may help to reactivate the healing program in DFU. Recent observations about the downregulation of Mitochondria-encoded circular RNA (mecciRNAs) in human DFU has urged us to explore their potential role in DFU.

Methods: Wound skin was collected from 5 healthy donors (Day 1, Day 7 and Day 30 post wounding), paired with healthy intact skin. Keratinocytes and fibroblasts were isolated. The mecciRNA of interest, hsa_circ_1690-2254, was knocked down in Human Dermal adult Fibroblasts (HDFa) and Human Epidermal adult Keratinocytes (HEKa). Its subcellular localization was determined by fractionation assay. Moreover, scratch wound migration assay and proliferation analysis were performed to uncover the function of hsa_circ_1690-2254 in HDFa and HEKa. To mimic the DFU wound environment, HDFa were cultured in hypoxic conditions and treated with high or low glucose medium.

Results: Compared to normal skin, hsa_circ_1690-2254 showed lower expression in DFU. Its expression in dermis is higher than epidermis and it is downregulated in normal skin wound healing stages. Moreover, this mecciRNA is mainly located in the cytoplasm. hsa_circ_1690-2254 knockdown reduces keratinocyte and fibroblast growth but stimulates their migration. As expected, high glucose levels decreased hsa_circ_1690-2254 expression under hypoxia.

Conclusions: Since keratinocyte proliferation and movement capacity are crucial for wound closure, hsa_circ_1690-2254 acts as a positive regulator, endorsing a potential role in human skin wound healing. Future experiments will focus on assessing how hsa_circ_1690-2254 affects mitochondrial function and which signalling pathways it regulates. The findings from this project will have a direct impact on both clinical diagnosis and novel treatment options.

P42 - Maternal obesity perturbs placental cell-specific transcriptomes related to baby birthweight and sex

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Background/Aim: Maternal obesity is associated with increased birthweight, raising risks of offspring long-term diseases. We aim to study whether and how maternal obesity affects placental cell types related to baby birthweight and sex.

Methods: Thirteen term placentas including both baby sex obtained from a Chile cohort were subdivided into normal maternal BMI and appropriate baby birthweight for gestational age (N-AGA, n=4) defined as 18.5≤BMI<25 and birthweight within 25th-75th percentiles for gestational age; obese AGA (Ob-AGA, n=4) defined as maternal 30≤ BMI<35; obese and large baby birthweight for gestational age (Ob-LAGA, n=5) defined as birthweight over AGA groups. Single-nuclei RNA-sequencing was performed and low-quality nuclei (detected genes<200 or UMIs<400), doublets were removed. After clustering and cell-type annotation, differentially expressed genes (DEGs) were detected by MAST (adjusted p-value <0.05).

Results: Of the 13 placentas, 39,404 cells clustered into 14 cell types with syncytiotrophoblast being the most abundant type. In male Ob-LAGA, the syncytiotrophoblast proportion was significantly reduced compared to others (FDR<0.05). In Ob-AGA *vs* N-AGA, more DEGs were obtained from females vs males (437 and 378 DEGs, respectively). In Ob-LAGA *vs* N-AGA, further unique DEGs (up to 205 in females and 126 in males, depending on cell types) were observed. In male extravillous trophoblasts, 111 DEGs were unique to Ob-LAGA but not Ob-AGA *vs* N-AGA. Interestingly, two genes involved in growth hormone (GH) and chorionic somatomammotrophin hormone (CSH) pathways, acting as placental metabolic adaptors during pregnancy, were perturbed regardless of baby sex, with over-expression of GH receptor (*GHR*) in cytotrophoblast of both obese groups *vs* N-AGA, and *CSH1* over-expression in syncytiotrophoblast of Ob-LAGA only *vs* both N-AGA and Ob-AGA.

Conclusions: Maternal obesity perturbs the syncytiotrophoblast proportion and the gene expression in cell-type specific manner. Babies with larger birthweight born to obese mothers present distinct placental transcriptomes compared to ones with appropriate birthweight.

P43 - Tubule-specific VHL gene ablation protects against diabetic kidney disease

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Diabetic kidney disease (DKD) is the major cause of mortality in patients with diabetes. Hypoxia in renal tubules is the driving force for tubular atrophy and interstitial fibrosis, which can further reinforce glomerular pathology during the development of diabetic nephropathy. Hypoxia-inducible factor-1 (HIF-1) is the major regulator of adaptive responses to hypoxia. However, HIF-1 signaling is inhibited in diabetic kidney, contributing to the progression of DKD. The destabilization of HIF-1 in diabetes is mainly through the von Hippel-Lindau protein (VHL) - mediated degradation of HIF-1a. However, the role of tubular VHL in DKD is still not clear.

In this study, we first evaluated the effects of VHL gene silencing in mouse tubular epithelial mIMCD3 cells. VHL gene silencing increased the expression of HIF-1a protein and reduced ROS levels in cells that were exposed to high glucose levels in hypoxia. We then generated tubule - specific VHL knockout (VHL-KO) mice. Diabetes was induced in the mice with streptozotocin *i.p.* injections. Kidney pathology and renal function were analyzed 6 weeks after the induction of diabetes. Tubule-specific VHL ablation increased expression of HIF-1a and HIF-1 target genes and reduced ROS levels in the kidneys from diabetic mice. This is accompanied by reduced kidney injury demonstrated by decreased expression of Kidney Injury Marker–1 (KIM-1) and reduced TUNEL-positive apoptotic cells. Albuminuria was significantly ameliorated in diabetic VHL-KO mice, indicating that the development of DKD was inhibited by tubule-specific VHL gene ablation.

We will treat the VHL-KO mice with specific HIF-1 inhibitor in order to confirm that the effects of VHL gene ablation on DKD are through the induction of HIF-1. The results from this study will provide evidence for tubular VHL as a potential therapeutic target for DKD.

P44 - Utilizing an immunocompetent 3D skin ulcer model to characterize macrophages in the diabetic foot

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Background: Current treatments for diabetic foot ulcers (DFU) do not guarantee a permanent solution, highlighting the necessity for more effective interventions. Immune cells involved in the healing process have been previously considered an attractive target. However, the complexity of the DFU, the difficulties in obtaining human material and the lack of animal models, have prevented a clear understanding of the molecular mechanism leading to dysfunctional monocytes/macrophages. Here we have developed a human skin organoid to study DFUs' pathophysiology and dissect the molecular pathway directly involved in the impaired immune response.

Methods: Based on a previously published method, we have established a 3D immunocompetent skin ulcer model, consisting of fibroblasts, monocytes, and keratinocytes layered step-by-step on trans-well inserts in six-well plates. This way, the skin models can be air exposed from the apical side leading to keratinization of the upper layer, imitating real skin. To study the DFU, we perform an incision on the keratinocytes layer, through which acute stimuli (such as LPS or fluids collected from patients) can diffuse into the model.

Results: Primary human monocytes are cultured in the 3D skin model and successfully differentiate into macrophages, migrating to the location of the artificial wound. Using glucan-encapsulated siRNA particles (GeRPs), a patented technology which is specifically targeting macrophages, we can track their movement and manipulate their gene expression.

Conclusions: Functional studies in human organoids using gene manipulation in macrophages will provide a novel tool to understand and validate the molecular target aiming to treat patients with non-healing ulcers and significantly decrease the amputation rate.

P45 - Elucidating the role of Circular RNA cGlis3 in skin wound healing

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Skin wound healing is a delicate process that goes through several phases including inflammation, proliferation, and remodeling. Patients with abnormal wound healing, such as delayed healing or excessive scarring due to poor circulation, diabetes, or other immunodeficiency disorders, are becoming an increasing obstacle to human well-being and an economic burden. Circular RNAs (CircRNAs) are a class of RNA transcripts with combined 3' and 5' ends, forming a back-splicing structure, which gives them more stable properties comparing to their linear counterparts. CircRNAs have been discovered in many human diseases, however, wound healing related CircRNAs are not well characterized and elucidated. Here we discover a circRNA derived from human Glis3, naming cGlis3, showing upregulation in wound fibroblast comparing to normal skin, it's upregulation is also found in keloid comparing to healthy donor. cGlis3 mainly locates in dermal fibroblast cytoplasm, inducing fibroblast activation and ECM production by interacting with PCPE1 to activate TGF-β signaling pathway. Human ex vivo assay shows the role of cGlis3 in re-epithelialization and skin contraction during the wound healing. Due to existence of the active IRES in cGlis3 sequence, small protein peptide translated from cGlis3 is identified and then confirmed by Mass spectrometry analysis. Further investigations on the biological role of cGLIS3 in wound healing will focus on its role on RNA level as well as its protein level.

Cardiovascular disease

P46 - Calcification and coagulation related pathways are enriched in atherosclerotic plaques of diabetic patients

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Background – The pathophysiology behind aggravated atherosclerosis in diabetes is still incompletely understood. We hypothesized that transcriptomic analysis of plaques from type 2 diabetic patients can reveal underlying molecular mechanisms common between diabetes and atherosclerosis.

Methods – The Biobank of Karolinska Endarterectomies (BiKE) comprises plaques and clinical data from patients undergoing endarterectomy for carotid stenosis, profiled with whole-genome transcriptomic arrays. Multilevel bioinformatic analyses of differentially expressed genes were performed comparing plaques from patients with type 2 diabetes (n=30) vs. non-diabetics (n=39) and within the S and AS groups. The stratification of patients in the two groups was performed according to HbA1c levels (HbA1c <4.9% for the control group) and medication for already existing diabetes diagnosis (for the diabetic group).

Results – In microarrays from diabetic vs. non-diabetic atherosclerotic patients, the most affected pathways were related to metabolic , coagulation, calcification and cell trans-differentiation processes. CHIT1 protease was repressed specifically in plaques from diabetic patients ($p<0.0001$). CHIT1 expression was associated with macrophage, extracellular matrix and markers related to ossification processes both in transcriptomic and proteomic level. Immunohistochemical staining revealed co-localization of CHIT1 and CD68 in multinucleated cells resembling osteoclasts. Whole transcriptome Nanostring analysis revealed a 10-fold increase in CHIT1 expression in multinucleated cells in the plaques compared to other regions of interest e.g. fibrous cap.

Conclusions – Our findings reveal induction of stabilization processes related to ossification in plaques from all diabetic patients, while inflammation was relatively more enriched in diabetic atherosclerotic patients without symptoms. CHIT1 was identified as one of the novel genes that appear to distinguish atherosclerotic plaques from type 2 diabetic patients that are currently being investigated mechanistically.

P47 - Erythrocyte-derived extracellular vesicles from type 2 diabetes patients induce endothelial dysfunction through arginase 1

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Background: We recently demonstrated that red blood cells (RBCs) cause endothelial dysfunction in type 2 diabetes (T2D) via the upregulation of arginase 1 in RBCs. However, the mechanism by which RBCs induce endothelial dysfunction is not fully understood. It is increasingly clear that extracellular vesicles (EVs) are actively secreted by all cell types, including RBCs, and represent a novel mechanism of intercellular communication.

Purpose: This study aimed to determine whether RBC-derived EVs from T2D patients are involved as mediators in endothelial dysfunction through arginase 1.

Methods: RBCs from T2D patients and age-matched healthy controls were isolated and incubated with Krebs-Henseleit buffer at 20% hematocrit. Following 18h incubation, the conditioned medium was collected for EV isolation by a membrane affinity column. EV concentration was measured by nanoparticle tracking analysis. Aortas from wild-type mice were incubated with EVs for 18h. Endothelium-dependent relaxation (EDR) was evaluated in a wire myograph. The uptake of the EVs and the involvement of arginase were investigated by the addition of heparin (0.3 mg/mL) or the arginase inhibitor 2(S)-amino-6-boronohexanoic acid (ABH, 10 mM), respectively, during the co-incubation.

Results: The concentration of RBC-derived EVs from T2D patients (T2D-RBCs EVs) was ten times lower than those from healthy controls. T2D-RBCs EVs significantly impaired EDR (Fig.1A). This effect was observed irrespective of if the same volume or concentration of EVs were administered. Inhibition of the uptake by heparin or arginase with ABH during the co-incubation completely prevented the impairment of EDR induced by T2D-RBCs EVs (Fig.1B-C). Immunohistochemical staining revealed upregulation of arginase 1 (Fig.1D-E) but not arginase 2 (Fig.1F-G) in the vasculature following incubation with T2D-RBCs EVs.

Conclusion: T2D-RBCs EVs induce endothelial dysfunction, and arginase 1 derived from these EVs mediates this endothelial dysfunction. These results shed new important light on the mechanism underlying vascular injury mediated by RBCs in T2D.

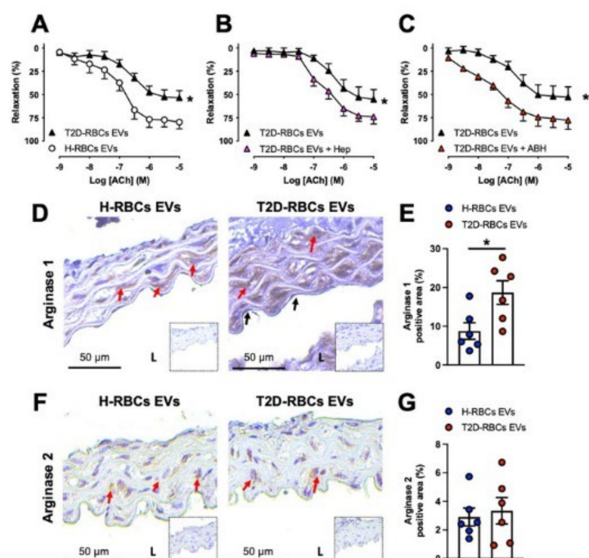

Figure 1. EVs released from T2D-RBCs induce impairment in endothelial function. Endothelium-dependent relaxation evoked by acetylcholine (ACh) in mouse aorta following 18h of incubation with EVs released from either healthy subjects (H-RBCs EVs) or patients with T2D (T2D-RBCs EVs; A, n=9-11 in each group). Co-incubation of T2D-RBCs EVs with heparin (B, n=7 in each group) or the arginase inhibitor ABH (T2D-RBCs EVs + ABH; C, n=6 in each group) rescued the endothelial function. Representative immunohistochemical images depicting arginase 1 (D) and arginase 2 (F) in aortic rings following incubation with H-RBCs EVs and T2D-RBCs EVs. Immunoglobulin G controls are presented in inserts for each experimental condition. L indicates the luminal side of the vessel, black arrows indicate endothelial cells, and red arrows indicate smooth muscle cells. Quantitative analyses of positivity of the total area in mice aortas for arginase 1 (E) and arginase 2 (G). Values are mean ± SEM. *$p<0.05$ with repeated two-way ANOVA in A-C and with the Mann-Whitney test in E.

P48 - Hepatocyte-specific depletion of the corepressor GPS2 alleviates atherosclerosis and dyslipidemia in mice

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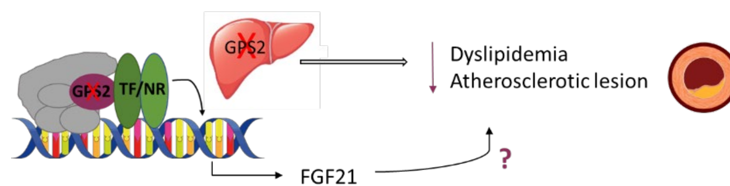
Background: Alterations of hepatic lipid metabolism and liver disorders have been implicated in the pathogenesis of atherosclerosis, a chronic cardiovascular inflammatory disease. Non-alcoholic fatty liver disease, for instance, has been linked with insulin resistance, obesity, and metabolic syndrome, conditions known to be associated with cardiovascular disease and subclinical atherosclerosis. Our previous work has demonstrated that hepatocyte-specific GPS2 knockout in mice alleviates diet-induced steatosis and fibrosis and causes activation of lipid catabolic genes by de-repression of PPARα.

Methods: To analyse how this liver GPS2 pathway affects atherogenesis, we have generated hepatocyte-specific GPS2 KO mice in the ApoE KO background (ApoE-LKO).

Results: ApoE-LKO mice showed smaller atherosclerotic lesion areas in the aortic root, their body and epididymal fat pad weights were lower, exhibited less atherogenic lipoprotein profiles (lower cholesterol and triglycerides with enrichment in HDL fractions), and their liver total cholesterol content was decreased. In addition, the hepatic expression of inflammatory genes was reduced, along with changes in lipid metabolism gene expression. The expression of fibroblast growth factor 21 (FGF21) was increased in liver, as well as circulating FGF21 levels. Furthermore, we demonstrate that liver-specific FGF21 knockdown using AAV-shRNA seems to corroborate the positive effect of FGF21 induction in ApoE-LKO mice.

Conclusion: Collectively, our study suggests that the loss of the corepressor GPS2 in hepatocytes improves atherosclerosis development and alleviates dyslipidemia, probably in part by the induction of FGF21.



P49 - High Glucose Downregulates miR-210 Resulting in Endothelial Dysfunction

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Background/Aim: Micro (miR)-210 is an important regulator of cardiovascular function and is reduced in several cardiovascular diseases. We recently demonstrated that miR-210 levels are lower in plasma, erythrocytes, and atherosclerotic plaques derived from patients with type 2 diabetes (T2D) compared with healthy subjects. Of further interest, arteries isolated from miR-210 knockout mice undergo endothelial dysfunction. However, the mechanism behind the downregulation of miR-210 in T2D and its association with endothelial dysfunction remains to be elucidated. High glucose, oxidative stress, and high insulin levels are highly associated with the pathogenesis of T2D. In this study, we aim to elucidate whether these factors affect miR-210 expression and function, thereby inducing endothelial dysfunction.

Materials and Methods: Aortic segments isolated from wild-type mice were incubated with and without high glucose (25 mM), high insulin (50 µg/mL), and SIN-1 (25 µM), an oxidative stress stimulator, for 48h. Subsequently, endothelium-dependent relaxation (EDR), a well-established readout for evaluation of endothelial function, was measured by using the Wire Myograph. EDR was also examined in aortas isolated from miR-210 transgenic mice (with or without miR-210 overexpression) under similar conditions. miR-210 levels were measured by qPCR in human carotid endothelial cells treated with and without high glucose.

Results: High glucose and SIN-1, but not high insulin, impaired EDR in the aortas isolated from wild-type mice. Of note, the impairment of EDR induced by high glucose was attenuated in aortas from miR-210 transgenic mice, while the impairment of EDR induced by SIN-1 was unaffected by miR-210 overexpression. Furthermore, high glucose treatment decreased the expression levels of miR-210 in human carotid endothelial cells.

Conclusions: High glucose downregulates miR-210 expression levels, resulting in endothelial dysfunction. Future studies are needed to identify key molecules that are affected by high glucose and can act as upstream regulators of miR-210 for the induction of endothelial dysfunction.

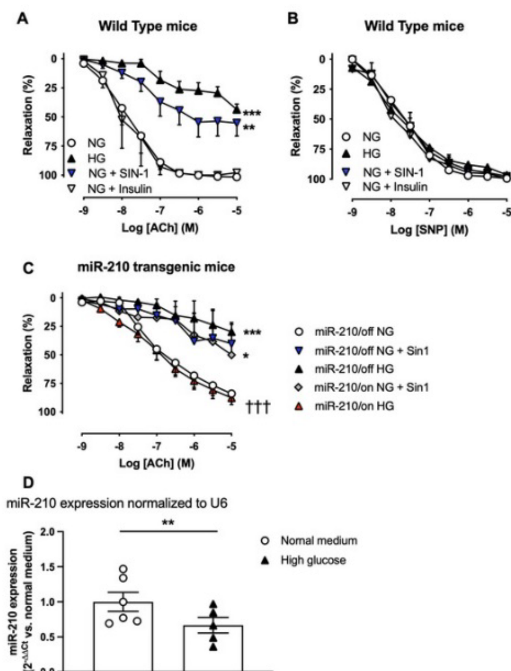

Fig. High glucose mediates the downregulation of miR-210 resulting in endothelial dysfunction in type 2 diabetes. ***A-B:*** Effects of HG, oxidative stress, and high insulin levels on endothelial function. Wild-type mice aortic segments were incubated in NG, NG + SIN-1 or NG + Insulin, and in HG medium for 48h. Following incubation, endothelium-dependent and -independent relaxations induced by Ach and SNP, respectively were evaluated. ***C:*** miR-210 overexpression attenuates HG-induced endothelial dysfunction. Aortic segments from miR-210 transgenic mice (with or without overexpressing miR-210) were incubated in NG, NG + SIN-1, and HG medium for 48h. Following incubation, endothelium-dependent relaxation was evaluated. ***D:*** miR-210 expression levels in HCtAECs. HCtAECs were cultured either in NG or HG medium for 24h. miR-210 expression levels were measured with qPCR and normalized according to U6. Values are presented as mean ± SEM (n=4-7). *$^{**}P<0.01$, $^{***}P<0.001$ vs. NG* in *A-B*. *$^{*}P<0.05$ vs. miR-210/off NG, $^{***}P<0.001$ vs. miR-210/off NG, †††$P<0.001$ vs. miR-210/off HG* in *C*. *$^{**}P<0.01$ vs. NG* in *D*.

P50 - Transcriptomic and physiological analyses reveal temporal changes contributing to the delayed healing response to arterial injury in diabetic rats

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Aim

Atherosclerosis is a major complication of diabetes mellitus and a leading cause of mortality in diabetic patients. Vascular interventions in diabetic patients can lead to complications attributed to defective vascular remodeling and impaired healing response. In this study, we aim to elucidate the physiological and molecular differences in the vascular healing response over time using a rat model of arterial injury applied in healthy and diabetic conditions.

Methods

Wistar (healthy) and Goto-Kakizaki (GK, diabetic) rats (n = 40 per strain) were subjected to left common carotid artery (CCA) balloon injury and euthanized at different timepoints: 0 and 24 hours, 5 days, 2, 4 and 6 weeks. Non-invasive morphological and physiological assessment of the CCA was performed with Ultrasound Biomicroscopy (US). Total RNA was isolated from the injured CCA at each timepoint and microarray profiling was performed (n=3 rats per timepoint). Bioinformatic analyses were conducted using R software, DAVID bioinformatic tool, online STRING database and Cytoscape software.

Results

Significant increase in the neointimal thickness ($p<0.01$; 2-way ANOVA) was observed after 2 weeks of injury in diabetic compared to heathy rats, which was confirmed by histological analyses.

Bioinformatic analyses showed that expression of contractile SMC and extrcellular matrix genes were increased in diabetic rats, coupled with the dysregulation of immune pathways. TF-PPI analysis provided mechanistic evidence wherein an array of transcription factors was dysregulated in diabetic rats specifically from 2 weeks after injury.

Conclusions

In this study, we have demonstrated that diabetic rats exhibit impaired arterial remodeling characterized by a delayed healing response. These results further corroborate the higher prevalence of restenosis in diabetic patients and provide molecular insights into the mechanisms contributing to the impaired arterial healing response in diabetes.

P51 - Transvascular Interstitial Fluid-to-Serum ratios of lipoproteins in T2D

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AIM (Background and objectives) : Interstitial fluid (IF) represents the immediate environment for most cells in the body and is the major arena for the interaction between lipoproteins and peripheral cells. The levels and functional properties of IF lipoproteins should therefore be of high relevance for the study of lipoprotein metabolism and susceptibility to atherosclerosis. In this context, we have explored possible pathophysiological mechanisms related to the transvascular interstitial fluid to serum ratios (IF:S) of lipoproteins in T2D, a patient group disproportionately affected by atherosclerosis.

Methods: Fasting serum and IF properties were characterized in 75 T2D patients and matched controls of varying age. IF collected from abdominal skin blisters was compared with serum obtained from peripheral blood.

Results: IF:S was 0.2 for total cholesterol in controls and the relative abundance of IF lipoproteins were inversely correlated to particle size. In T2D, the atherogenic lipoproteins were sparser in IF with a significantly reduced IF:S for ApoB ($p<0.001$). Assessing serum LDL's proteoglycan binding susceptibility (LPBS) revealed increased binding in T2D ($p<0.001$) and linked enhanced LPBS to a reduced IF:S for ApoB ($p<0.01$ for controls and T2D). Both increased LPBS and reduced IF:S for ApoB were closely related to aging in controls ($p<0.001$ IF:S, $p<0.01$ LPBS) which was not observed in T2D. Lowest IF:S and highest LPBS were observed in patients with a record of cardiovascular events ($p<0.05$).

Conclusions

Transvascular IF-to-serum ratios of ApoB reflect serum LDL binding affinity to arterial proteoglycans. Serum LDL from T2D patients have an increased binding to proteoglycans in association with a reduced transvascular ratio of LDL. In accordance with the "response to retention" hypothesis of atherosclerosis, this indicates an increased peripheral accumulation of LDL cholesterol in T2D, which can be seen as a process of "premature ageing".

Diabetes care and therapies

P52 - Identification and characterization of small molecules increasing enteroendocrine-cell density across species

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Increasing the action of the incretin hormones Gip and/or Glp-1 is a widely exploited strategy to treat type-2 diabetes and obesity; however, affecting several hormones simultaneously is more effective in experimental diabetes models. To identify small molecules that increase the number of incretin-expressing cells, we previously established a high-throughput *in vivo* chemical screen by using the *gip* promoter to drive the expression of luciferase in zebrafish. Many hits increased the numbers of neurogenin 3-expressing enteroendocrine progenitors, Gip-expressing K-cells, and Glp-1-expressing L-cells. We have now screened an additional chemical library containing natural products and have a few tentative hits: in the prospective characterization phase, we plan to treat mice/human organoids with these compounds, for confirmation of a conserved and direct effect. From previous experiments we know that hits from other libraries also increased the number of incretin-expressing cells in diabetic mice, paving the way for testing the most promising hits in murine models.

P53 - Identification of reno-protective molecular signatures in renal glomerular tissue using single cell transcriptomics

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Chronic kidney disease (CKD) affects >10% of the population. Yet, the therapeutical options are unspecific and often not efficient. Today, drugs targeting renin angiotensin system (mostly angiotensin converting enzyme inhibitors = ACEi) are the standard therapy for CKD patients due to their ability to regulate the glomerular filtration pressure.

We aimed to define the RNA fingerprints of ACEi at single cell level in glomerulus tissue to identify underlying mechanisms that slow the progression of glomerulopathy. In long run, we aim to develop a toolbox for clinicians to guide choice of therapy using non-invasive biomarkers, and pinpoint novel therapeutical targets.

We perform single cell RNA sequencing (scRNAseq) experiments in mouse glomerulopathy models, renal biopsies from DN patients with and without ACEi therapy. scRNA-seq is performed using drop-based 10XGenomics platform with glomerulus single cell suspension.

Previously, we performed scRNAseq in a mouse model of kidney disease treated with ACEi. Treatment significantly ameliorated albuminuria and histological damage. We captured main glomerular cells, cells from afferent and efferent arterioles, and immune cells. Majority of differentially expressed genes were in mesangial population. Renin, a key regulator of blood pressure, was the most up-regulated gene in mesangial cells upon ACEi treatment. Most downregulated genes were related to extracellular matrix organization, insinuating ACEi’s role in mesangial cell contractility. Only minor molecular changes were observed in podocytes, endothelial cells and parietal epithelial cells. Afferent and efferent arterioles, which are believed to be the main target for ACEi, revealed only minor differences in molecular responses. Furthermore, we identified a subcluster of parietal epithelial cells with molecular features of podocytes, supporting the idea that these cells can be podocyte progenitors.

Using scRNA seq, we identified mesangial cells as the main responders in the early stage of ACEi treatment, and scRNAseq is a suitable platform for profiling glomeruloprotective mechanisms of reno-protective drugs.

P54 - Undiagnosed type 2 diabetes and associated factors in Stockholm County

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Aim: To estimate the proportion of individuals with undiagnosed type 2 diabetes (T2D) and factors associated with T2D being diagnosed by healthcare.

Methods: We used data from a prospective cohort and registers. We followed individuals without T2D for two periods of ten years each. We gathered information on T2D diagnosis from registers and from oral glucose tolerance tests at the end of each period and calculated the cumulative incidence. With logistic regression, we estimated factors associated with the probability of T2D being detected by health care compared to being undiagnosed.

Results: In the two ten-year periods the cumulative incidence of diagnosed T2D was 2.3% and 6.5%, and the cumulative incidence for undiagnosed T2D was 2.8% and 5.2%. In period 1, 54.5% of individuals with T2D were undiagnosed and 44.4% in period 2. Factors associated with being diagnosed compared to undiagnosed in a crude model were age (OR 1.04, 95%CI 1.02-1.07), overweight (OR 1.77, 95%CI 1.17-2.65), obesity (OR 2.53, 95%CI 1.66-3.87), and fasting glucose (OR 2.13, 95%CI 1.70-2.68). After adjusting for age and sex, those with alcohol risk consumption were less likely to be diagnosed (OR 0.69 95%CI 0.49-0.97), and those with self-reported poor general health were more likely to be diagnosed (OR 2.33, 95%CI 1.04-5.24). After adjusting for age, sex, and lifestyle factors, the association of being overweight, obese, have high fasting glucose or poor general health remained. Socioeconomic factors were not associated with being diagnosed.

Conclusion: Many individuals with T2D are undiagnosed. Healthcare follows recommendations on testing those at higher ages and who are overweight or obese, but there is a need for improved detection of T2D. We found no association with socioeconomic factors, suggesting no inequality in the diagnosis of T2D in Stockholm County. We found that self-estimated poor general health is associated with being diagnosed by healthcare.

Inflammation and metabolism

P55 - Dissecting the functionality and disease relevance of Ccl2 enhancers across human/mouse tissues

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Over-nutritional and inflammatory signals in the microenvironment drive chromatin remodelling activities linked with various metabolic disorders, such as obesity, diabetes, atherosclerosis, and non-alcoholic fatty liver disease. Epigenetic mechanisms play major roles in this process by modulating tissue-specific gene expression. Enhancers are located in the non-coding genomic regions and are major binding sites for transcription factors and coregulators, whose remodelling events present unique features and disease relevance. We aim to profile the tissue- and signal-specificity of enhancer remodelling events initially at CCL2 loci (a major chemokine involved in metabolic disorder progression) with future plans to extend to other single gene sites or genome-widely. Our preliminary analysis of the public data confirmed the tissue-specificity of Ccl2 enhancer clusters by tracking the H3K27ac distribution in mice and humans. This specificity appeared consistent among the same cell type and irrelevant to gender. Using two novel Ccl2 enhancers KO mice models, we plan to evaluate the tissue-specific enhancer functionality by comparing Ccl2 transcription in different tissues. We further plan to investigate their involvement in metabolic disorders during diet-induced obesity development, providing information on their signal (obesity)-specificity. Moreover, the therapeutic potential of LNA-mediated Ccl2 enhancers manipulation could be further tested in the metabolic disorder mouse models. These analyses will be repeated in human primary cells, on account of the highly conserved core sequences of Ccl2 enhancers. Combining with the publicly available eQTLs dataset, we could estimate the causal effects of functional Ccl2 enhancers on metabolic disorders.

P56 - Epigenetics in adipocyte biology

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Cellular senescence is a state of irreversible replicative arrest that is often accompanied by secretion of pro-inflammatory factors. An increase in senescent cell burden is observed in the adipose tissue (AT) of obese individuals. Amongst the various cell types in AT, adipocytes are generally not considered in the study of senescence because of their postmitotic nature. The Spalding lab recently challenged this dogma and showed that mature adipocytes have an endoreplicative cell cycle, and that senescent adipocytes accumulate in obese and hyperinsulinemic individuals.

In fibroblasts, cellular senescence is accompanied by changes in chromatin landscape, an aspect of adipocyte senescence that remains unexplored. Therefore, the primary aim of this proposed work is to characterize the chromatin landscape of senescent human adipocytes. Since epigenome profiling by classic ChIP-seq might be impractical when working with freshly isolated adipocytes, we propose to perform epigenome profiling by CUT&RUN.

Adipocytes play pivotal roles in lipid and glucose homeostasis, it is hence important to understand the gene regulatory network ofadipocytes. However, the epigenomes of primary human adipocytes are yet to be mapped. Our plan to map the histone epigenome of primary human adipocytes will not only help us better understand adipocyte senescence; but will also expand our general knowledge of human adipocyte biology. Given the world-wide obesity epidemic and related co-morbidities, research in this area is much needed.

P57 - Molecular Assessment of Skeletal Muscle Mitochondrial Alterations Induced by Chronic Inflammation

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BACKGROUND: Chronic inflammatory disorders including rheumatoid arthritis are associated with a higher risk of developing type 2 diabetes (T2D). In addition, low-grade inflammation has been implicated in metabolic syndrome and related disorders, contributing to the pathogenesis of insulin resistance and T2D. The metabolic syndrome and T2D have been associated with mitochondrial alterations contributing to muscle dysfunction with altered metabolism. Here, we aimed to study inflammation-induced mitochondrial alterations using a Complete Freund's Adjuvant (CFA) as a mouse model of arthritis and whether these alterations could be ameliorated by PGC-1α1 overexpression.

METHODS: Chronic inflammation was induced in two months old wildtype (WT) mice (C57BL/6J, n=3) and PGC-1α1 skeletal muscle-specific transgenic mice (MCK-PGC1α1, n=4). Intra-articular injection of CFA into the right ankle joint was used to induce chronic inflammation. Cytoplasmic Ca^{2+} levels and mitochondrial Ca^{2+} uptake was measured in isolated muscle fibers using Fluo-4 and Rhod-2, respectively, in response to two electrical stimulation protocols. Changes in the mitochondrial membrane potential (Ψ) were measured with JC-1 using the same stimulation protocols. Finally, mRNA expression of genes involved in mitochondrial Ca^{2+} handling was measured.

RESULTS: Repeated tetanic stimulations to mimic fatigue of muscle fibers from flexor digitorum brevis revealed no significant difference in mitochondrial Ca^{2+} uptake between the groups. The Ca^{2+} uptake was drastically reduced and Ψ was less polarized during recovery in muscle fibers from CFA, as compared with controls. However, the Ca^{2+} uptake was rescued during the recovery in PGC1α1-CFA. Enhanced PGC-1α1 expression was also able to rescue the inflammation-induced reduction of mRNA levels of several genes involved in mitochondrial Ca^{2+} handling, including mitochondrial calcium uptake 1 (Micu1).

CONCLUSION: Chronic inflammation induces altered mitochondrial function with reduced Ca^{2+} uptake and PGC-1α1 overexpression appears to ameliorate these inflammation-induced alterations.

Gender related metabolism and (patho)physiology

P58 - Cell-type-specific disease signatures and mechanisms regulating the endometrium of women with polycystic ovary syndrome

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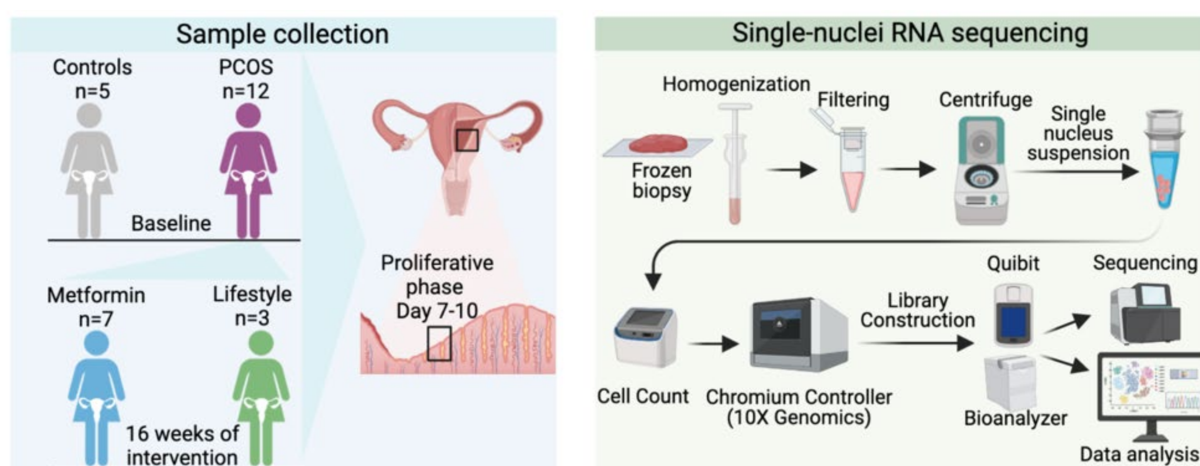
Polycystic ovary syndrome (PCOS) is associated with type 2 diabetes and reduced fertility, linked to endometrial dysfunctions. We hypothesize that cell-type-specific endometrial dysfunctions in insulin-resistant and hyperandrogenic women with PCOS contribute to observed endometrial dysfunctions and that treatment to improve insulin sensitivity and decrease androgen excess can reverse identified alterations.
To reveal cell-type-specific disease signatures and molecular pathways of PCOS-specific endometrial dysfunctions, we extracted single-nuclei (sn) from frozen endometrial biopsies collected in the proliferative phase (day 7-10) of controls (n=5) and women with PCOS (n=12) at baseline and in PCOS after 16 weeks of metformin (n=7) or lifestyle management (n=3) for snRNA-sequencing. We sequenced ≈10,000 nuclei/sample and ≈20,000 reads/nuclei, capturing a total of 247,791 nuclei and 6 major cell types. The three largest cell clusters were stromal with uterine smooth muscle cells, epithelium and immune cells whilst endothelial and lymphatic clusters consisted of too few nuclei for further analysis.
Subsetting epithelial cells revealed functional lumenal, glandular, and ciliated cell types, as well as proliferative cells. In the immune cell cluster, myeloid and lymphoid lineage cells were identified, of which uterine NK cells (uNK) and macrophages (uM) were the largest populations. Several differentially expressed genes (DEGs) of epithelial subtypes, uNKs, uMs, and stromal cells were identified in PCOS compared with controls using the MAST hurdle model. DEGs in cell types between PCOS and controls were enriched in pathways related to cilium organization in the ciliated epithelium, extracellular matrix structure in stromal cells, and cysteine-type endopeptidase activity in uM type 1. Moreover, 16 weeks of treatment with metformin and/or lifestyle management restored several DEGs in each subtype.
This rigorous mapping increases our understanding of the cellular complexity in the PCOS endometrium, providing new mechanistic insights into disease-specific endometrial dysfunction(s) and if identified dysfunctions are reversed by current first-line interventions.



P59 - Developmental programming by maternal androgen excess is mediated by androgen receptor pathways

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Objectives

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.

Methods

We used a PCOS-like mouse model induced by continuous exposure of dihydrotestosterone (DHT) 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. Trophoblast organoid culture system were applied to further evaluate the detrimental effects of maternal hyperandrogenism in placenta trophoblast cell lineage.

Results

We found a lower pregnancy rate and phenotypically impaired placenta and embryonic development, which was partially prevented when co-treated with the androgen receptor blocker, flutamide. RNA sequencing result of the placenta revealed that DHT severely interfere with placental and fetal development, which are prevented by the treatment of Flutamide. In addition, DHT treated placenta showed impaired differentiation capacity of cell types located in the labyrinth. The hyperandrogenic maternal environment led to the development of anxiety like behaviour and impaired metabolism in adult male offspring and partial disturbance in the metabolism of female offspring later in life.

Conclusion

Our results so far suggest that hyperandrogenism greatly compromise the PCOS-pregnancy and embryo development due to placenta dysfunction, which has led to subsequent development of PCOS-like phenotypes in adult male and female offspring. Such effects are mainly mediated by the androgen receptor pathway as administration of flutamide partially prevented the compromised placenta and fetal development.

P60 - Effect of physical exercise, trough miRNA profile modulation, on the metabolic risk of gestational diabetes women and their offspring.

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Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes before pregnancy. It currently affects one in five pregnancies, so early diagnosis of the GDM risk is essential to avoid perinatal morbidity, adverse neonatal deterioration and the high risk of chronic metabolic outcomes in both mothers, such as type 2 diabetes (T2D) and in the offspring. MiRNAs are intracellular regulators of gene expression that have been detected in different body fluids, in a stable manner, constituting the so-called circulating miRNAs (c-miRNAs). Lifestyle modifications, such as diet and exercise, have been described as the first-line treatment for GDM. Furthermore, these lifestyle changes can modify the profile of c-miRNAs in healthy and diseased individuals. Therefore, the aim is to propose an approach based on the implementation of a physical exercise intervention at the time of GDM diagnosis and the evaluation of its effect on the progression of the disease and the metabolic risk both in mothers as in the offspring in the following years, through miRNA regulation. To do this, we will report on the glycemic status, clinical characteristics, assessment of nutritional status, and plasma miRNA profile at the time of GDM diagnosis, 24-28 weeks (A), comparing the exercise intervention and sedentary women and 15 years after pregnancy (B), in the same women and their offspring. The few previous results showed that the Body Mass Index was one of the clinical characteristics that best predicted the development of T2D 15 years after the GDM diagnosis, since women with a BMI greater than 26 kg/m^2 were more likely to develop T2D. In conclusion, this study is an opportunity to develop new research on how physical exercise can have a beneficial effect on the metabolic risk of GDM women and their offspring.

KI core facility

P61 - Bioinformatics and Expression Analysis core facility (BEA)

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Abstract text: BEA – the core facility for Bioinformatics and Expression Analysis (www.bea.ki.se) located in NEO, Karolinska Institutet, Campus Flemingsberg offers access to genomic technologies to help scientists, investigators and the biotech community to conduct and explore their research in the most efficient and economical way. Specifically, BEA provides services and consultation for genomic analyses based on the Illumina, 10X Genomics, Affymetrix, Agilent, and ABI platforms for Next Generation Sequencing (NGS), Microarray analysis and qPCR. This includes differential gene expression and transcriptome analyses in standard and custom formats, single cell expression analysis, miRNA expression analysis, ChIP sequencing of protein-DNA interactions, DNA methylation and SNP profiling, metagenomic profiling and targeted amplicon sequencing. BEA also performs RNA/DNA extractions and quality control from different samples and sources. BEA aims to provide high quality state-of-the-art infrastructure and service in genomic technologies and our goal is to provide internationally competitive services, data analysis and education. Importantly, BEA offers comprehensive solutions at all stages of the analysis from experimental design and integrated wet lab service to data analysis and full bioinformatics support. BEA also provides education and information, including advanced courses, workshops and seminars.

SRP Diabetes technical platforms

P62 - Beta Cell *in-vivo* Imaging/ Extracellular Flux Analysis core facility.

Noah Moruzzi and Martin Köhler

Techniques for imaging of functional properties in pancreatic islets at cellular resolution are instrumental for diabetes research, both in vitro and in vivo. During the last 30 years we have built a world leading facility with the best tools for such imaging, and our goal is now to maintain and develop this facility further to meet the future needs of diabetes research. Moreover, we recently expanded this facility with the Seahorse Extracellular flux analyzer and a cell culture room adapted for its usage. This instrument allows the measurement of energy utilization in living cells and organoids in vitro, simultaneously quantifying mitochondrial respiration and "lactate" production in real time in a microplate format.
The imaging facility is located at The Rolf Luft Research Center for Diabetes and Endocrinology at Karolinska Institutet, Stockholm, Sweden, where the infrastructure consists of optical microscopes and cytometer for experiments mainly based on optical and electrophysiological parameters. The experiments are ranging from in vitro microscopy on fixed tissues or living cells or tissues, to in vivo experiments where imaging is performed in the living animal. This facility is supported by SRP Diabetes.

P63 - Metabolic Phenotyping Centre for Diabetic Animal Models

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https://ki.se/en/srp-diabetes/metabolic-phenotyping-centre

The Metabolic Phenotyping centre is located in KMB Solna and provides a number of platforms for phenotypic and physiologic analysis of mouse models specifically focused on diabetes and its complications. We also provide guidance for investigators regarding selection of procedures, experimental design and data interpretation.

Available analysis

Feeding, locomotion and calorimetry: Two separate systems for non-invasive assessment of metabolism. 8 cages (Comprehensive Lab Animal Monitoring System, CLAMS) from Columbus Instruments, and 16 cages Phenomaster Home cage system) from TSE Systems. Both systems allows determination of food intake, drink, locomotion and energy expenditure. Additionally, the TSE system allows monitoring in a home cage setting, and temperature control from 0- 60°C. The food and drink system has a more automated access control and the system facilitates pair feeding protocols.

Body composition: The EchoMRI-100™ measures whole body fat mass, lean tissue mass, free water, and total body water in live animals up to 100 grams without the need for anaesthesia or sedation, in less than 2 minutes.

Activity patterns / Exercise interventions: Running capacity and activity patterns are available in the form of voluntary wheel running. The system may also be used as a means of providing voluntary exercise in metabolic-based studies. System of 20 cages with running wheels for mice from STARR Life Sciences, PA, US. Computerized data collection system gives total activity and time of activity. We also have possibilities for treadmill running for mice.

Glucose handling and insulin sensitivity: We have expertise in intraperitoneal (or oral) glucose tolerance test, insulin tolerance tests as well as the Hyperinsulinaemic Euglycaemic Clamp. These latter analyses do require a larger investment from the investigator but advice and training can be discussed.

The user needs to have a valid ethical permit in order to perform any analysis using the Phenotyping Centre*. Please contact us for detailed information of each analysis to help facilitate obtaining ethical permission.*

P64 - The Center for Clinical Metabolic Research in Diabetes (CCMRD)

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Background
The Strategic Research Program in Diabetes (SRP-Diabetes) aims to minimize the bottle necks in performing clinical studies. For this, SRP-Diabetes funds CCMRD-an infrastructure developed to facilitate translational research in cross-sectional and prospective cohorts.

Who and where are we?
CCMRD is headed by Mikael Rydén. We currently engage six M.D/Ph.D and two research nurses. Biopsy samples are phenotyped by two technicians. Facilities are at Karolinska University Hospital, Huddinge and include five rooms for clinical investigations, a fully equipped wet lab, offices and a lounge room for research subjects/patients. We perform most measures relevant to metabolic research including analyses of body composition (bioimpedance, DXA), indirect calorimetry, insulin sensitivity (clamp, OGTT, ITT), ultra sound investigations (e.g., intima-media thickness) and tissue biopsies (fat and skeletal muscle).

How are clinical studies within CCMRD initiated and performed?
Any PI interested in performing clinical studies is responsible for writing the ethical application where the CCMRD is listed as a collaborator. Applications for clinical studies are signed by representatives from both KI and the Stockholm County Council (Region Stockholm). Although this process may differ slightly between departments/clinic, it is important to stress that the process takes time. Power calculations detailing the total number of subjects to be recruited for any specific study is a prerequisite for initiating any clinical investigation. Priority is given to collaborative studies but it is also possible to perform studies as a fee for service.

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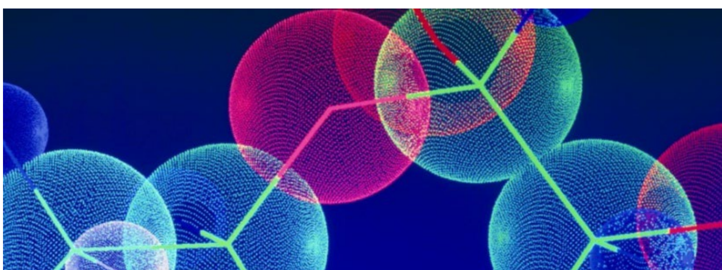

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