29th Annual Scandinavian Atherosclerosis Conference

March 22–25, 2023 at Krogerup Højskole, Humlebæk, Denmark

2023 Program

SCIENTIFIC COMMITEE

Maaike Schilperoort (Netherlands)
Camilla Huse (Norway)
Stefan Stender (Denmark)
Marit Westerterp (Netherlands)
Simon Pfisterer (Finland)
Knut Tomas Dalen (Norway)
Carolina Hagberg (Sweden)
Kirsten Holven (Norway)

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Organized by

SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH

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HOMEPAGE

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Wednesday, March 22, 2023

16.00 – 18.00	Arrival, registration, and coffee (dining room until 17.45)
18.00 – 19.30	Dinner
19.30 – 19.35	Welcome **Anne Langsted** *(Denmark)*
THE NIKKILÄ MEMORIAL LECTURE	
19.35 – 19.40	Introduction of the 2023 Nikkilä Lecturer **Minna Kaikkonen-Määttä** *(Finland)*
19.40 – 20.25	*2023 Nikkilä Lecture* **Marianne Benn** *(Denmark)*
20.25 – 20.45	Discussion
20.45 –	Pub will be open

Thursday, March 23, 2023

07.45 – 08.45	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Chaired by **Maaike Schilperoort** (*Netherlands*) and **Camilla Huse** (*Norway*)
08.45 – 09.10	*Invited speaker* IL-6 signaling in myocardial infarction **Tuva Dahl** (*Norway*)
09.10 – 09.15	Discussion
09.15 – 09.30	Characterisation of extracellular traps released by macrophages: a contributing factor to lesion formation in atherosclerosis? **Clare Hawkins** *(Denmark)*
09.30 – 09.45	Dual elevated remnant cholesterol and low-grade inflammation in atherosclerotic cardiovascular disease and mortality **Takahito Doi** *(Denmark)*
09.45 – 10.00	The effect of colchicine on neutrophil function in patients with coronary artery disease: a double-blind, randomized placebo-controlled cross-over study **Helin Tercan** *(The Netherlands) - YIA*
10.00 – 10.15	Imaging macrophages in their natural habitat: Integrated multiplex immunofluorescent and mass spectrometry imaging to dissect atherosclerotic plaque myeloid heterogeneity in its metabolic and cellular context **Pieter Goossens** *(The Netherlands)*
10.15 – 11.15	**Poster Walk (Session I)** Coffee and tea
11.15 – 11.40	*Invited speaker* Innate immune reprogramming in cardiovascular diseases **Siroon Bekkering** *(Netherlands)*
11.40 – 11.45	Discussion
11.45 – 12.00	Dietary quality and associations to plasma fatty acids and inflammatory markers among Norwegian patients with familial hypercholesterolemia **Kirsten Holven** *(Norway)*
12.00 – 12.15	PCSK6 ablation in vascular smooth muscle cells promotes abdominal aortic aneurysm development and rupture **Hong Jin** *(Sweden)*
12.15 – 12.30	Somatic mutations reveal clonal cell populations in atherosclerotic plaques **Lasse Steffensen** *(Denmark)*

12.30 – 13.30	Lunch
SESSION II	**CARDIOVASCULAR DISEASE** Chaired by **Stefan Stender** (*Denmark*) and **Marit Westerterp** (*Netherlands*)
13.30 – 13.55	*Invited speaker* The fundamental principles of Mendelian randomization **George Davey Smith** *(UK)*
13.55 – 14.00	Discussion
14.00 – 14.15	Statin use and reduced STEMI relative to non-STEMI: a nationwide study in Denmark **Sofie Simony** *(Denmark) - YIA*
14.15 – 14.30	Plasma levels of CCL21, but not CCL19, independently predict future coronary events in a prospective population-based cohort **Pernilla Katra** *(Sweden) - YIA*
14.30 – 14.45	Integration of genetic and clinical data, with single-cell transcriptomics of atherosclerotic plaques identifies novel smooth muscle cell genes **Sampath Narayanan** *(Sweden) - YIA*
14.45 – 15.45	**General meeting of the *Scandinavian Society for Atherosclerosis Research*** Open for all participants Afternoon free for the Louisiana Museum of Modern Art (5 min walk), beach (5 min walk), Kronborg, the castle of Hamlet (12 min by train) or downtown Copenhagen (50 min by train)
16.30 – 17.30	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
18.15 – 19.15	Dinner
SESSION II	**CARDIOVASCULAR DISEASE – continued** Chaired by **Stefan Stender** (*Denmark*) and **Marit Westerterp** *(The Netherlands)*
19.15 – 19.40	*Invited speaker* Regulation of smooth muscle cell plasticity in vascular disease **Helle Jørgensen** *(UK)*
19.40 – 19.45	Discussion
19.45 – 20.45	**Poster Walk (Session II)** Coffee and tea
20.45 – 21.00	Normalization for age of death but not coronary heart disease in familial hypercholesterolemia - nationwide study in Denmark from 1978 through 2018 **Børge G Nordestgaard** *(Denmark)*

21.00 – 21.15	Adipocyte-Nfe2l1 protects from cholesterol-induced lipoatrophy and atherosclerosis **Carolin Muley** *(Germany) - YIA*
21.15 – 21.30	Metabolomic biomarkers compared for different types of cardiovascular events in large general population biobanks **Peter Würtz** *(Finland)*
21.30 – 21.45	Inverse effects of APOC2 and ANGPTL4 on the conformational dynamics of lid-anchoring structures in lipoprotein lipase **Anni Kumari** *(Denmark) - YIA*
21.45 –	Pub will be open

Friday, March 24, 2023

07.45 – 08.45	Breakfast
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Chaired by **Simon Pfisterer** *(Finland)* and **Knut Tomas Dalen** *(Norway)*
08.45 – 09.10	*Invited speaker* The ER:lysosome bridge and the saga of cholesterol transport **Emily Eden** *(UK)*
09.10 – 09.15	Discussion
09.15 – 09.30	High lipoprotein(a) increases risk of peripheral arterial disease, abdominal aortic aneurysms, and major adverse limb events **Peter Engel Thomas** *(Denmark) - YIA*
09.30 – 09.45	Increasing insight in the VLDL secretory pathway; a role for small leucin-rich protein 1 (SMLR1) **Ankia Visser** *(The Netherlands) - YIA*
09.45 – 10.00	Effect of breastfeeding on lipid profile and cardiovascular risk markers in women with familial hypercholesterolemia: The FH-FEMINA study protocol **Marianne Klevmoen** *(Norway) - YIA*
10.00 – 10.15	Liver-targeted Angptl3 and Angptl4 silencing by antisense oligonucleotide treatment attenuates hyperlipidemia and atherosclerosis development in APOE*3-Leiden.CETP mice **Melanie Modder** *(The Netherlands) - YIA*
10.15 – 11.15	**Poster Walk (Session III)** Coffee and tea
11.15 – 11.40	*Invited speaker* Pharmacogenetics of lipid-lowering drugs **Mikko Niemi** *(Finland)*
11.40 – 11.45	Discussion
11.45 – 12.00	Lipid profile during the first year of life **Sofie Taageby Nielsen** *(Denmark) - YIA*
12.00 – 12.15	Functional analysis of LDLR variants using automated systems to improve rare-variant association studies and risk assessment in hypercholesterolemia **Mohammad Majharul Islam** *(Finland) - YIA*
12.15 – 12.30	ApoM and S1P – markers for incident heart failure and death in patients with chronic kidney disease? Sarunja Vijayakumar (Denmark) - YIA
12.30 – 13.30	Lunch

SESSION IV	**OTHER TOPICS** Chaired by **Carolina Hagberg** *(Sweden)* and **Kirsten Holven** *(Norway)*
13.30 – 13.55	*Invited speaker* Of women and men: lipids during the life course **Jeanine Roeters van Lennep** *(The Netherlands)*
13.55 – 14.00	Discussion
14.00 – 14.15	Stimulation of the beta-2-adrenergic receptor activates human brown adipose tissue in vivo **Patrick Rensen** *(The Netherlands)*
14.15 – 14.30	Proprotein Convertase Subtilisin/Kexin 6 is involved in lipid metabolism in liver **Bianca Suur** *(Sweden) - YIA*
14.30 – 14.45	New evidence of cardiovascular risk factors for Alzheimer's disease **Jiao Luo** *(Denmark) - YIA*
14.45-15.00	Purinergic crosstalk between adipocyte-macrophage promotes degeneration of thermogenic brown adipose tissue **Michelle Jaeckstein** *(Germany) - YIA*
15.00 – 16.00	**Poster Walk (Session IV)** Coffee and tea
16.00 – 16.25	*Invited speaker* Novel mechanisms of adipocyte function and metabolism **Alexander Bartelt** *(Germany)*
16.25 – 16.30	Discussion
16.30 – 16.45	Risks associated with use of statins and other lipid-modifying agents across pregnancy – a nationwide drug safety study in Norway in 2005-2018 **Jacob Juel Christensen** *(Norway) - YIA*
16.45 – 17.00	The STEROL ELEMENT BINDING PROTEIN 1C (SREBP1c) is an immunometabolic checkpoint of regulatory T lymphocytes **Fabrizia Bonacina** *(Italy)*
17.00 – 17.15	Genome-Wide Sleep-SNP interactions on lipid traits to identify biomolecular pathways underpinning sleep-associated lipid disturbances **Raymond Noordam** *(The Netherlands) - YIA*
17:15 – 17:30	Health economic costs generated by an FH mutation **Kjetil Retterstøl** *(Norway)*
17.30 - 17.35	Concluding remarks **Anne Langsted** *(Denmark)*
18.30 – 19.00	**Cocktail**
19.00 –	**Banquet and dancing**

Saturday, March 25, 2023

08.30 – 10.00	Breakfast and departure

Safe travels and see you next year

29th Annual Scandinavian Atherosclerosis Conference

March 22–25, 2023 at Krogerup Højskole, Humlebæk, Denmark

2023 Posters

Thursday, March 23, 2023

Posters are displayed in "Lille Sal". Posters should be in place before 9.00 and removed after the last poster session of the day. You should be present at your poster during all poster sessions of the day. Your poster should be placed on the board with your number on.

SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY**
No. 07	Blood Milieu in Acute Myocardial Infarction Reprograms Macrophages for Trauma Repair **Lieve Temmerman** *(The Netherlands)*
No. 17	Hypoxia induces a proatherogenic arterial extracellular matrix **Christine Chuang** *(Denmark)*
YIA Poster walk I 10.15 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 02	HOCl-induced modification of NET-derived histones **Els Alletta Hartsema** *(Denmark)*
No. 03	EZH2 inhibition reduces macrophage inflammatory responses in atherosclerosis **Rosalie Kempkes** *(The Netherlands)*
No. 04	Role of inflammatory signaling pathways involving the CD40-CD40L-TRAF cascade in animal models of diabetes and hypertension as well as coronary artery disease patients with one or two of these comorbidities **Lea Sophie Strohm** *(Germany)*
No. 08	Sequestering inflammation in macrophages by modulation of 2-hydroxyglutarte **Laszlo Groh** *(The Netherlands)*
No. 12	Iodide as a potential therapeutic in atherosclerosis **Kathrine Væver Jokumsen** *(Denmark)*
No. 15	Modulation of smooth muscle cells to treat atherosclerosis **Laura Alonso Herranz** *(Denmark)*
No. 22	The role of Nrf2 activity in cell-cell interaction in atherosclerosis **Katarzyna Sarad** *(Poland)*
SESSION II	**CARDIOVASCULAR DISEASE**
No.38	Low plasma transthyretin is associated with all-cause and cardiovascular mortality in the general population **Mette Christoffersen** *(Denmark)*
No.53	Amyloidosis-related orthopedic events, low plasma transthyretin, and risk of cardiac events **Anders Greve** *(Denmark)*

YIA Poster walk II 19.45 – 20.45	Selected abstracts (3 min presentation + 2 min discussion)
No. 32	Transcriptomic and physiological analyses reveal temporal changes contributing to the delayed healing response to arterial injury in diabetic rats **Sampath Narayanan** *(Sweden)*
No. 35	Carbamylated Proteins Accumulate with Atherosclerotic Plaque Progression and Associate With Foam Cells **Valeria Saar-Kovrov** *(The Netherlands)*
No. 36	Calcification and coagulation related pathways are enriched in atherosclerotic plaques of diabetic patients **Glykeria Karadimou** *(Sweden)*
No. 37	Control of heart calcium homeostasis by adipocyte-derived microRNAs **Henver Brunetta** *(Germany)*
No. 42	Sex-specific influence of visceral and subcutaneous adipose tissue volumes on systemic inflammation and innate immune activation in obese subjects **Harsh Bahrar** *(The Netherlands)*
No. 50	Myeloid CD40 deficiency reduces atherosclerosis by impairing macrophages' transition into a pro-inflammatory state **Laura Bosmans** *(The Netherlands)*
No. 51	Genetic variation in SLC5A2 mimicking SGLT2-inhibition and risk of cardiovascular disease and all-cause mortality: reduced risk not explained by lower plasma glucose **Louise Ellegaard Bechmann** *(Denmark)*
No. 52	Difference in PBMC gene expression between elderly event-free FH patients and FH patients with CHD **Torunn Melnes** *(Norway)*
No. 56	Smoothe muscle cell-specific translatome profiling of mouse atherosclerosis uncovers Itih4 with sQTL variant rs77347777, as a novel gene of SMC-derived microenvironmental factor in atherosclerosis **Aarthi Ravindran** *(Finland)*

Friday, March 24, 2023

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SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT**
No. 25	Small dense LDL cholesterol and ischemic stroke **Mie Balling** *(Denmark)*
No. 44	Elevated plasma adiponectin in risk of heart failure, atrial fibrillation, aortic valve stenosis, and myocardial infarction:observational and Mendelian randomization studies **Maria Booth Nielsen** *(Denmark)*
No. 73	Loss-of-function mutations in APOC3, thrombocytopenia, and incidence of bleeding events **Anders Wulff** *(Denmark)*
YIA Poster walk 10.15 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 60	Genetic variants in ABCA1 and risk of age-related macular degeneration **Liv Tybjærg Nordestgaard** *(Denmark)*
No. 61	Lipids, the Achilles' heel of inflammatory macrophages? **Nico Hahn** *(The Netherlands)*
No. 62	Icosapent ethyl supplementation rapidly reduces cardiovascular disease risk markers and improves plasma and lipoprotein lipidome reducing their atherogenicity in humans **Lauri Äikäs** *(Finland)*
No. 64	Adipose tissue exposed to high fat diet affects extracellular matrix genes in the mesenchymal stem cell population **Uma Thanigai Arasu** *(Finland)*
No. 68	MECR is essential for coordinated energy transformation **Ioannis Evangelakos** *(Germany)*
No. 74	Charge neutralization of the acidic domain residues in GPIHBP1 attenuates its effects towards Lipoprotein Lipase **Kristian Kølby Kristensen** *(Denmark)*
No. 20	A possible role of ApoB in degenerative ascending aortic aneurysm formation and progression **David Freiholtz** *(Sweden)*
SESSION IV	**OTHER TOPICS**
No. 90	Identification of Side Chain Oxidized Sterols as Novel Liver X Receptor Agonists with Therapeutic Potential in the Treatment of Cardiovascular and Neurodegenerative Diseases

	Monique Mulder *(The Netherlands)*
No. 94	Cardiovascular risk factors and risk of non-Alzheimer's dementia **Ida Juul Rasmussen** *(Denmark)*
No. 97	Effect of krill oil intervention in vivo on energy metabolism in human skeletal muscle cells **Parmeshwar Katare** *(Norway)*
YIA Poster walk IV **15.00 – 16.00**	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 77	Narcolepsy drug γ-hydroxybutyric acid improves hepatic mitochondrial function to attenuate obesity **Milena Schönke** *(The Netherlands)*
No. 78	Maternal overweight alters cord blood but not maternal plasma bile acid pool hydrophobicity **Sebastian Graute** *(Germany)*
No. 82	SHB is a novel regulator of insulin signaling and adipocyte function **Anna Jung** *(Germany)*
No. 84	The oxidative stress response in obesity is mediated by adipocyte Nfe2l2 **Stefan Kotschi** *(Germany)*
No. 91	MALRD1 variant associated with increased bile acid synthesis and hepatic cholestasis **Valentin Reichenbach** *(Germany)*
No. 93	Patients with familial hypercholesterolemia have shorter telomeres than controls in older, but not in young subjects: a cross-sectional study **Amanda Rundblad** *(Norway)*
No. 99	Development of liver-on-a-chip to study coronary artery disease -related processes **Siiri Suominen** *(Finland)*
No. 102	Telomere length and liver disease in the Danish General Population **Helene Gellert-Kristensen** *(Denmark)*

Oral Presentations – Abstracts –

Inflammation and Vascular Biology

SESSION I

Characterisation of extracellular traps released by macrophages: a contributing factor to lesion formation in atherosclerosis?

Mathias Jensen, Nicoline W. Thorsen, Line A.E. Hallberg, Per M. Hägglund and Clare L. Hawkins

Department of Biomedical Sciences, University of Copenhagen, Copenhagen N, DK-2200, Denmark.

Neutrophil extracellular trap (NET) release plays a key role in many chronic disease settings, including atherosclerosis. NETs are critical to innate immune defence, but also contribute to disease by promoting thrombosis and inflammation. Macrophages are a key driver of lesion formation in atherosclerosis. These cells also release extracellular traps or "METs", but their composition and role in pathological processes is poorly understood. In this study, we examined MET release from human THP-1 macrophages exposed to inflammatory and pathogenic stimuli, including tumour necrosis factor α (TNFα) and nigericin. Each treatment resulted in the release of DNA from the macrophages, as visualised by fluorescence microscopy with the cell impermeable DNA binding dye SYTOX Green. With TNFα and nigericin, release of DNA occurred in the absence of cell lysis, and the extracellular DNA was of both nuclear and mitochondrial origin. Proteomic analysis of these METs revealed some similarities but also key differences compared to NETs. METs contained a high amount of linker and core histones (H1.5, H2A, H2B, H3 and H4), together with other cytosolic and mitochondrial proteins, including actin, annexin (A4 and A5), HMG-1, S100 proteins (A7, A8, A9), lysozyme C and calreticulin analogous to NETs. Quinone oxidoreductase and a succinyltransferase enzyme (DLST) were also highly abundant in METs but have not been reported in NETs. In addition, there was an absence of proteases in METs, which are key components of NETs. The MET proteins also contain a range of post translational modifications, including acetylation, citrullination, and methylation. Interestingly, there was evidence of chlorination and nitrile formation on the MET proteins consistent with oxidative modification by the myeloperoxidase oxidant hypochlorous acid (HOCl). These data provide new insight into the potential implications of MET formation in vivo and possibly pathways by which they could contribute to atherosclerosis.

Dual elevated remnant cholesterol and low-grade inflammation in atherosclerotic cardiovascular disease and mortality

Takahito Doi, MD, PhD, Anne Langsted, MD, PhD, Børge G. Nordestgaard, MD, DMSc

All from Department of Clinical Biochemistry, Copenhagen University Hospital, Herlev and Gentofte

Aims: Elevated remnant cholesterol and low-grade inflammation each cause atherosclerotic cardiovascular disease (ASCVD); however, it is unknown whether joint elevation of both factors confers the highest risk. We tested the hypothesis that dual elevated remnant cholesterol and low-grade inflammation marked by elevated C-reactive protein is associated with the highest risk of myocardial infarction, ASCVD, and all-cause mortality.
Methods and Results: The Copenhagen General Population Study randomly recruited white Danish individuals aged 20-100 years in 2003-2015 and followed them for a median 9.5 years. ASCVD was cardiovascular mortality, myocardial infarction, stroke, and coronary revascularization. In 103,221 individuals, we observed 2,454 (2.4%) myocardial infarctions, 5,437 (5.3%) ASCVD events, and 10,521 (10.2%) deaths. The hazard ratios increased with each of stepwise higher remnant cholesterol and stepwise higher C-reactive protein. In individuals with the highest tertile of both remnant cholesterol and C-reactive protein compared to individuals with the lowest tertile of both, the multivariable adjusted hazard ratios were 2.2 (95%CI: 1.9-2.7) for myocardial infarction, 1.9 (1.7-2.2) for ASCVD, and 1.4 (1.3-1.5) for all-cause mortality. Corresponding values for only the highest tertile of remnant cholesterol were 1.4 (1.2-1.8), 1.2 (1.0-1.4), and 1.1 (1.0-1.2), and those for only the highest tertile of C-reactive protein were 1.6 (1.3-2.0), 1.5 (1.3-1.7), and 1.3 (1.2-1.5), respectively. There was no statistical evidence for interaction between elevated remnant cholesterol and elevated C-reactive protein on risk of myocardial infarction (P=0.10), ASCVD (P=0.40), or all-cause mortality (P=0.74).
Conclusions: Dual elevated remnant cholesterol and C-reactive protein confers the highest risk of myocardial infarction, ASCVD, and all-cause mortality.

The effect of colchicine on neutrophil function in patients with coronary artery disease: a double-blind, randomized placebo-controlled cross-over study

Tjerk SJ Opstal*,2, Helin Tercan*,1, Harsh Bahrar1, Amber van Broekhoven2, Benjamin Cossins PhD1, Saloua El Messaoudi2, Niels P Riksen MD PhD1, Siroon Bekkering PhD1, Jan H Cornel**2,**

1. Dept of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands
2. Dept of Cardiology, Radboud University Medical Centre, Nijmegen, The Netherlands
**Shared first authorship, ** Shared senior authorship*

Introduction – Chronic low-grade inflammation plays a key role in coronary artery disease (CAD). Treatment with low-dose colchicine, an anti-inflammatory drug, significantly lowers the occurrence of secondary cardiovascular events. Targeted proteomics analysis showed that treatment with colchicine lowered the plasma levels of neutrophil specific granule proteins. We speculate that the beneficial cardiovascular effects of colchicine are, at least in part, due to downregulating of neutrophil activity. Therefore, we aimed to investigate in detail the effects of colchicine treatment on neutrophil phenotype and function.
Methods – In a double-blinded, placebo-controlled, randomized cross-over design, we recruited 20 patients with a history of myocardial infarction. 90% of the patients were male and had a median age of 64 and BMI>27. They were treated with colchicine for one month in a dose of 0.5 mg per day, or matching placebo, with a wash out period of at least 2 weeks. Blood was collected by venous puncture 4 times, before and after each treatment. We characterized the effects of colchicine on neutrophils by flow cytometry, ex vivo stimulation of neutrophils and granule protein secretion, NOX-dependent and -independent NETosis assays, ROS production and bulk RNA sequencing.
Results – Flow cytometry results showed that after a month of colchicine treatment the expression of CD62L on mature neutrophils was significantly decreased. Secretion of neutrophil granulocytic proteins myeloperoxidase, lipocalin-2 and s100A8/9 upon TLR2 stimulation was significantly decreased after treatment colchicine compared to placebo.
Conclusions – Our results demonstrate that colchicine treatment impaired the mobility and activation of neutrophils. Functional analyses of NETosis pathways, ROS production and transcriptomic profiling of neutrophils will enable us to understand the exact mechanisms of colchicine action.

Imaging macrophages in their natural habitat: Integrated multiplex immunofluorescent and mass spectrometry imaging to dissect atherosclerotic plaque myeloid heterogeneity in its metabolic and cellular context

Pieter Goossens 1; Chang Lu 1; Jianhua Cao 2; Marion Gijbels 1,3; Joël Karel 4; Erwin Wijnands 5; Britt Claes 2; Gregorio Fazzi 1; Tim Hendriks 2; Kristiaan Wouters 6; Evgueni Smirnov 4; Marc van Zandvoort 7,8; Benjamin Balluff 2; Eva Cuypers 2; Marjo Donners 1; Ron Heeren 2; Erik Biessen 1,8

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2 Division of Imaging Mass Spectrometry, Maastricht MultiModal Molecular Imaging institute (M4I), Maastricht University, The Netherlands
3 Department of Medical Biochemistry, Amsterdam AMC, The Netherlands.
4 Department of Data Science and Knowledge Engineering, Maastricht University, The Netherlands.
5 Central Diagnostic Laboratories, Maastricht University Medical Center+, The Netherlands.
6 Department of Internal Medicine, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands.
7 Department of Genetics & Cell Biology - Molecular Cell Biology, Maastricht University, The Netherlands.
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Macrophages play a central role throughout atherogenesis. They can adopt a spectrum of different phenotypes, and a multitude of subsets has indeed already been described in atherosclerotic plaques, reflecting the broad range of functions, some even opposing, exerted by these cells. Macrophage heterogeneity is dictated by local signals such as cell-cell and cell-matrix contacts and by the molecular micro-environment. This "niche" imprint however cannot be adequately studied by applications such as cytometry or single-cell RNAseq as these require cells to be detached from their context. Meanwhile, histology-based assessment lacks the phenotypic depth due to limitations in marker combination.
We therefore developed a novel, integrative approach in which single-shot, 15-plex multispectral immunofluorescent imaging allows comprehensive plaque macrophages' classification based on their multi-marker expression patterns, followed by a downstream analysis pipeline to link these phenotypes to contextual, microenvironmental cues such as their cellular ("community") and metabolic ("local lipidome") niche in this highly complex tissue. This allowed us to identify seventeen distinct myeloid phenotypes in murine aortic root atherosclerotic plaques, including three distinct subsets of the prototypical, but in cytometry or transcriptomics often underrepresented, foam cell phenotype, and to further characterize them based on their phenotypic marker expression, histopathology, intra-plaque (co)localization, proliferative capacity and strongly correlating lipid signatures.

Dietary quality and associations to plasma fatty acids and inflammatory markers among Norwegian patients with familial hypercholesterolemia

Eirin B. Løvheim a, Kjetil Retterstøl b, Ingunn Narverud c, Martin P. Bogsrud d, Bente Halvorsen e, Thor Ueland e, Pål Aukrust e, Stine M Ulven a and Kirsten B Holven a, c

Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, P.O box 1046 Blindern, 0317 Oslo, Norway. b Lipid Clinic, Oslo University Hospital, Norway. C Norwegian National Advisory Unit on Familial Hypercholesterolemia, Oslo University Hospital, Norway. c. d Unit for Cardiac and Cardiovascular Genetics, Oslo University hospital, Oslo, Norway. e Research Institute for Internal Medicine, Oslo University Hospital,

Background and aims: Familial hypercholesterolemia (FH) is an inherited disease associated with hypercholesterolemia from birth and increased risk of premature cardiovascular disease. Dietary treatment is recommended as part of FH treatment, but little is known about the diet of FH patients and their adherence to the dietary advice. Herein we aimed to assess the dietary pattern in a group of Norwegian patients with FH in relation to the healthy Nordic food index (HNFI) and examine the associations between dietary quality and biomarkers related to cardiovascular disease.

Methods: We included 205 adults (⩾above 18 years) with FH who received regular follow-up at the lipid clinic in Oslo and compared them to healthy Norwegian adults (n=228). Dietary intake was assessed using a food frequency questionnaire and dietary quality was assessed using the HNFI. Non-fasting blood sample was analyzed for levels of blood lipids, plasma fatty acids (FA) and markers of inflammation and platelet activation.

Results: Adult FH patients (median 60 years; 50.2% female) have lower intake of total and saturated fat compared to controls (32.6 Energy% (E%) vs 34.9E% and 9.6 E% vs 12.0 E%, respectively; $p<0.001$ for both). Increasing dietary quality was associated with increased plasma levels of the n-3 polyunsaturated FA (PUFA); EPA and DHA and the n-6 PUFA LA and lower plasma levels of the inflammatory cytokines; tumor necrosis factor ··interleukin-6, and interferon ··g and of the platelet-derived inflammatory chemokines CXCL4 and CXCL7.

Conclusion: Norwegian patients with FH receiving treatment at the lipid clinic in Oslo eat healthier than controls. Adherence to a healthy dietary pattern is associated with higher plasma levels of n-3 and n-6 PUFA and lower levels of inflammatory markers, including markers of platelet-driven inflammation. This suggests that adherence to an overall healthy dietary pattern might be beneficial for FH patients independent of the cholesterol-lowering effect of the diet.

PCSK6 ablation in vascular smooth muscle cells promotes abdominal aortic aneurysm development and rupture

Hong Jin1, Moritz Lindquist Liljeqvist1, Zhiyaun Wu2, Linda Renata Micali1, Bianca Suur1, Mariette Lengquist1, Xiang Zhang1, Malin Kronqvist1, Jessica Pauli2, Ulf Hedin1, Joy Roy1, Lars Magdefessel2, Ljubica Matic1

1 Department of Molecular Medicine and Surgery, Karolinska Institutet, Sweden
2 Department of Vascular and Endovascular Surgery, Technical University of Munich, Germany

Aim: We have previously shown that Proprotein Convertase Subtilisin/Kexin type 6 (Pcsk6) is strongly enriched in atherosclerotic plaques and plays a key role in lesion vulnerability and vessel wall healing by regulating vascular smooth muscle cell (VSMC) migration, proliferation, and extracellular matrix remodeling. Considering the importance of these processes in vascular pathologies, this study was aimed to investigate the expression and function of Pcsk6 in abdominal aortic aneurysm (AAA).
Methods and Results: Bioinformatic analysis of bulk transcriptomic and single cell RNA sequencing data from human AAA biobanks of the Technical University of Munich and Karolinska Institute, have demonstrated a significantly increased level of PCSK6 in fibroblasts, T cells, especially in ruptured AAA tissue, but decreased in VSMCs. To further clarify if PCSK6 dysregulation is a pathological reaction or if it has a causal role in AAA development, we performed pilot animal studies: 1) peri-adventitial Porcine pancreatic elastase (PPE) model on SMC conditional Tagln-Pcsk6 deficient and control mice; 2) AngII infusion model on Tagln-Pcsk6 deficient and control mice with ApoE-/- background. Interestingly, although no difference on baseline aortic dilatation and constriction was found between Tagln-Pcsk6 deficient and control mice, ultrasound imaging and histology analyses showed that mice with Pcsk6 deficiency in SMCs specifically manifested remarkably larger AAA, as well as significantly increased rupture and dissection rate. Mechanistically, RNA sequencing analysis indicated significant inhibition of extracellular matrix components and biosynthesis signaling in aortas from Tagln-Pcsk6 deficient mice.
Conclusion: PCSK6 expressed by SMCs plays a substantial role in AAA progression and pathophysiology, opening new avenues for therapeutic assessment against AAA dissection.

Somatic mutations reveal clonal cell populations in atherosclerotic plaques

Steffensen LB(1,2), Dembic M(3,4,5), Kaven S(3), Jensen PS(2,6), Andersen LvB(3), Lindholt JS(2,7), Houlind KC(8), Thomassen M(3), Rasmussen LM(2,6)

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In recent years, there has been increasing evidence suggesting that clonal cell populations may play a role in the development of atherosclerosis. Studies conducted by independent research groups have demonstrated the presence of clonally expanded smooth muscle cells in experimental models of the disease, and clonal hematopoiesis of indeterminate potential (CHIP) was identified as a novel independent risk factor for atherosclerotic cardiovascular disease. However, whether clonal cell populations contribute to human atherosclerotic lesions remains elusive.

In this study, we performed deep whole-exome sequencing of 31 segments from 14 carotid plaques obtained from patients undergoing carotid endarterectomy. We discovered a landscape of somatic mutations that were confined to the plaque tissue, and not present in patient-matched buffy coats. By analyzing variant allele frequencies, we estimated that individual, locally expanded clones often comprised more than 10% of the cell content in the plaque segments. Furthermore, seven of the patients in our study were CHIP carriers, and in several of these cases, hematopoietic clones comprised over 30% of the cell population of the plaque segments.

In conclusion, our findings provide evidence that somatic mutations and clonal cell populations, both locally expanded and derived from the circulation, are inherent features of atherosclerosis.

Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION II

Statin use and reduced STEMI relative to non-STEMI: a nationwide study in Denmark

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Background and Aims: Myocardial infarction due to atherosclerotic plaque rupture is classified in two subtypes: ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (non-STEMI), with STEMI indicating complete occlusion of a coronary artery, requiring immediate treatment to reduce risk of death or permanent myocardial damage. Lipid-lowering with statin therapy profoundly changes plaque morphology in the coronary arteries, which may affect the relative distribution between STEMI and non-STEMI events. We tested the hypothesis that statin use is associated with reduced STEMI relative to non-STEMI in patients with myocardial infarction.
Methods: This Danish nationwide study included all patients who suffered from a first-time myocardial infarction between 2010 and 2018 (n=72,761) and investigated the odds ratio for STEMI versus non-STEMI events according to prescribed doses of statin treatment.
Results: The odds ratio for STEMI versus non-STEMI was 0.64(0.61-0.68) in current statin users, and 0.95(0.89-1.01) in previous statin compared to never statin users. With higher intensity of daily statin dose, the odds ratio for STEMI versus non-STEMI was 0.81(0.76-0.85) for low statin dose, 0.60(0.57-0.64) for regular statin dose, and 0.47(0.41-0.52) for high statin dose compared to never statin users. The hazard ratio for 60-day mortality after first-time STEMI versus non-STEMI was 1.73(1.64-1.84).
Conclusion: Statin use is associated with reduced STEMI relative to non-STEMI in a dose dependent manner. This indicate that statin therapy, in addition to reducing myocardial infarction event-rates, also results in less severe presentations of myocardial infarction when it occurs.

Plasma levels of CCL21, but not CCL19, independently predict future coronary events in a prospective population-based cohort

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Background and aims
The homeostatic chemokines CCL21 and CCL19 have been explored as biomarkers in cardiovascular disease prediction in patients with established cardiovascular disease, but associations between these chemokines and first-time coronary event incidence have not been investigated before. Here, we explored associations between CCL21 or CCL19 and first-time incident coronary events in the general population-based Malmö Diet and Cancer cohort with two decades of follow-up.
Methods
CCL21 and CCL19 levels in plasma were analysed with ELISA and proximity extension assay and associations with disease incidence were explored with conditional logistic regression in a nested case-control cohort (CCL21; n=676) and with Cox regression in a population-based cohort (CCL19; n=4636).
Results
CCL21 and CCL19 levels were increased in plasma from incident coronary event cases compared to controls, $p<0.001$, $p=0.002$, respectively. High CCL21 levels were associated with incident coronary events independently of traditional risk factors with an odds ratio of 2.64 with 95% confidence interval 1.62-4.31, $p<0.001$, when comparing the highest versus the lowest tertile of CCL21. Also, likelihood ratio tests showed that addition of CCL21 to the regression model containing traditional risk factors significantly improved its predictive ability for incident coronary events, $p<0.001$. CCL19 was, however, not associated with incident coronary events, but was instead associated with incident heart failure, as well as increased all-cause, cardiovascular and cancer mortality independently of age and sex.
Conclusions
Even though CCL21 and CCL19 both signal through CCR7, these chemokines may not be interchangeable as disease predictors and CCL21 could be used for prediction of future coronary events in individuals without any previous coronary heart disease history.

Integration of genetic and clinical data, with single-cell transcriptomics of atherosclerotic plaques identifies novel smooth muscle cell genes

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Background and Aim: Vascular smooth muscle cells (SMCs) have been recently implicated with causal genetic links to disease processes in atherosclerosis. Single cell sequencing (scRNAseq) studies of atherosclerotic plaques have identified various transitional mesenchymal cell (MC) populations within the plaques. Here, we correlated cell fractions from plaques to patient clinical parameters and coronary artery disease (CAD) related gene polymorphisms to identify MC-specific and symptom-specific molecular targets.
Methods: Deconvolution analysis was performed on bulk microarray data from carotid plaques in the Biobank of Karolinska Endarterectomies (BiKE, n=127) using scRNAseq data from coronary plaques (n=5). Cell fraction QTL (cfQTL), and BiKE expression QTL (BiKE eQTL) for CAD-associated GWAS loci highly associated with MC fractions (mesen-SNPs) were calculated sequentially to obtain MC-specific and symptom-specific SNPs. The function of the gene affected by the top SNP was studied in vitro using primary human carotid SMCs (n = 4).
Results: Deconvolution analyses revealed that SMC cell fractions were significantly reduced in symptomatic plaques. Sequential cfQTL and BiKE eQTL analyses of mesen-SNPs generated 84 eQTLs highly relevant for SMC function. The most significant BiKE eQTL was identified as a SNP located at the regulatory region of gene ARNTL. This SNP increased the expression levels of ARNTL specifically in symptomatic plaques. Silencing of ARNTL in vitro led to inhibition of proliferation (2-way ANOVA, $p < 0.01$), increase in contractility (student t-test, $p < 0.05$), induction of senescence measured by β-galactosidase assay (student t-test, $p < 0.05$) and gene expression of senescence markers (student t-test, $p < 0.05$).
Conclusions: This study has identified several SNPs that may influence SMC function and may provide insights into novel SMC-specific genetic links to the disease in symptomatic patients.

Normalization for age of death but not coronary heart disease in familial hypercholesterolemia - nationwide study in Denmark from 1978 through 2018

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Aim: Familial hypercholesterolemia leads to lifelong elevated LDL cholesterol and increased risk of coronary heart disease and premature death. We lack information on whether morbidity and mortality have improved for individuals with familial hypercholesterolemia simply because prevention and acute therapy for coronary heart disease have improved over the past 40 years. In individuals diagnosed with familial hypercholesterolemia, we tested the hypothesis that age of death and coronary heart disease have improved in Denmark over the past 40 years.
Methods: From nationwide health registries, we included all Danish residents from 1978 through 2018 and identified individuals diagnosed with familial hypercholesterolemia and coronary heart disease.
Results: For 8,037 individuals diagnosed with familial hypercholesterolemia compared to 8,694,858 other individuals from the general population, the age of death was approximately 20 years lower at the end of the 1970s; however, in 2018 the age of death for individuals with familial hypercholesterolemia had increased to the same level as seen in the general population. Unlike age of death, age at acute coronary syndrome, stable angina pectoris, and revascularization for people with familial hypercholesterolemia did not increase over time to the same level as seen in the general population.
Conclusion: Nationwide in Denmark from 1978 through 2018 we observed normalization for age of death but not age at coronary heart disease for individuals diagnosed with familial hypercholesterolemia. This suggests that even patients with familial hypercholesterolemia have benefitted from improved prevention and acute therapy implemented during this period, while further efforts are needed to improve early diagnosis and aggressive LDL cholesterol-lowering therapy to also reduce occurrence of coronary heart disease to levels seen in the population at large.

Adipocyte-Nfe2l1 protects from cholesterol-induced lipoatrophy and atherosclerosis

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Healthy adipocytes are essential for cardiometabolic health, since they buffer lipids and regulate insulin sensitivity. Adipocyte dysfunction in obesity as well as lipodystrophy are both linked to inflammation and insulin resistance, which are major risk factors for atherosclerosis. Here we investigate the role of Nfe2l1, a cholesterol-sensing transcription factor that regulates proteasome function, in adipocyte health and cardiometabolic disease. Nfe2l1 was deleted in adipocytes or specifically in thermogenic adipocytes with Adipoq-Cre and Ucp1-Cre respectively as well as in apoE-deficient background. We fed high-fat diet (HFD), Western diet (WD) as well as cholesterol-enriched HFD. Mechanistic studies were performed in primary and 3T3-L1 adipocytes. Adipose NFE2L1 expression was inversely correlated with measures of obesity in a human cohort over a broad range of BMI. Adipoq-Cre Nfe2l1 knockout (KO) mice on HFD displayed adipose tissue inflammation and insulin resistance compared to their wild-type (WT) littermates. On a cholesterol-containing WD, however, Adipoq-Cre KO mice developed a wasting-phenotype with severe lipoatrophy, which was not observed on HFD or in Ucp1-Cre KO mice. Notably, ApoE-deficient Nfe2l1 KO mice on WD had 40% more aortic plaques than their WT controls. Mechanistically, loss of Nfe2l1 in primary white and 3T3-L1 adipocytes resulted in lower proteasomal activity, aggravated ER stress, and inflammation. Cholesterol treatment of 3T3-L1 combined with proteasomal inhibition exacerbated ER stress and inflammation, which was mediated by Atf3, a stress-induced transcription factor. Our results demonstrate that Nfe2l1 protects adipocytes from lipotoxic cholesterol through improved proteasomal protein quality control. This highlights a novel link between adipocytes and atherosclerosis based on the interaction of Nfe2l1 with dietary cholesterol.

Metabolomic biomarkers compared for different types of cardiovascular events in large general population biobanks

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Background: Many blood biomarkers are predictors of future cardiovascular events, but their association magnitudes may differ for different type of cardiovascular diseases. Detailed metabolic biomarker profiling by Nuclear Magnetic Resonance (NMR) is a powerful technique to clarify pathophysiological differences for atherosclerosis in different vascular beds.

Methods: We measured blood biomarkers by NMR metabolomics using the Nightingale Health platform in 300,000 samples from the UK Biobank. We assessed common and discordant biomarkers for different types of major adverse cardiovascular events. During ten years of follow-up from blood sampling, 5-10.000 incident events occurred for myocardial infarction, ischemic stroke, peripheral artery disease and heart failure. Findings were replicated in other large biobanks.

Results: The overall pattern of biomarker associations differed for myocardial infarction, angina , chronic ischemic heart disease, and different types of stroke (Julkunen et al Nature Communications, in press). Further, the biomarkers for risk of heart failure hospitalisation are substantially deviating from those of myocardial infarction, even though these endpoints are combined in the five-point major adverse cardiovascular event definition.

These results extend recent studies with the same metabolomics platform that have compared the biomarker signatures for coronary heart disease and peripheral artery disease in FINRISK cohorts (Tikkanen et al JAHA 2021) and different types of stroke in the Chinese population (Holmes et al JACC 2018).

Conclusions: Different cardiovascular endpoints have partly different metabolic biomarkers signatures, which may represent pathophysiological differences and have relevance for cardiovascular risk prediction.

Inverse effects of APOC2 and ANGPTL4 on the conformational dynamics of lid-anchoring structures in lipoprotein lipase

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The lipolytic processing of triglyceride-rich lipoproteins (TRLs) by lipoprotein lipase (LPL) is crucial for the delivery of dietary lipids to the heart, skeletal muscle, and adipose tissue. The processing of TRLs by LPL is regulated in a tissue-specific manner by a complex interplay between activators and inhibitors. Angiopoietin-like protein 4 (ANGPTL4) inhibits LPL by reducing its thermal stability and catalyzing the irreversible unfolding of LPL's α/β-hydrolase domain. We previously mapped the ANGPTL4 binding site on LPL and defined the downstream unfolding events resulting in LPL inactivation. The binding of LPL to its endothelial cell transporter, GPIHBP1, protects against LPL unfolding. The binding site on LPL for an activating cofactor, apolipoprotein C2 (APOC2), and the mechanisms by which APOC2 activates LPL have been unclear and controversial. Using hydrogen–deuterium exchange/mass spectrometry, we now show that APOC2's C-terminal α-helix binds to regions of LPL surrounding the catalytic pocket. Remarkably, APOC2's binding site on LPL overlaps with that for ANGPTL4, but their effects on LPL conformation are distinct. In contrast to ANGPTL4, APOC2 increases the thermal stability of LPL and protects it from unfolding. Also, the regions of LPL that anchor the lid are stabilized by APOC2 but destabilized by ANGPTL4, providing a plausible explanation for why APOC2 is an activator of LPL, while ANGPTL4 is an inhibitor. Our studies provide fresh insights into the molecular mechanisms by which APOC2 binds and stabilizes LPL—and properties that we suspect are relevant to the conformational gating of LPL's active site.

Oral Presentations – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

High lipoprotein(a) increases risk of peripheral arterial disease, abdominal aortic aneurysms, and major adverse limb events

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Background: Lipoprotein(a) may be implicated in peripheral arterial disease (PAD) and abdominal aortic aneurysms (AAA), yet data from general population studies are limited, and large studies are needed to determine risk in individuals with the highest levels and associated risk of major adverse limb events (MALE).
Aims: To test whether high lipoprotein(a) levels associate with increased risk of PAD, AAA, and MALE, and to provide genetic evidence of causality using LPA genotypes.
Methods: We included 108,146 individuals from the contemporary Copenhagen General Population Study (CGPS). During follow-up, 2,450 developed PAD, 1,251 AAA, and 489 MALE. We used the historic Copenhagen City Heart Study (CCHS, N=10,960) to replicate findings for MALE (N=116).
Results: High lipoprotein(a) levels and corresponding LPA risk genotypes associated with increased risk of PAD and AAA in the CGPS. For individuals with lipoprotein(a) levels >99th percentile (⩾143mg/dL, ⩾ 306nmol/L), multivariable adjusted hazard ratios were 2.99 (95% confidence interval: 2.09-4.30) for PAD and 2.22 (1.21-4.07) for AAA compared to individuals with levels <50th percentile (⩽9mg/dL, ⩽17nmol/L). Corresponding hazard ratios for MALE were 2.70 (1.67-4.37) in the CGPS and 8.12 (3.56-18.55) in the CCHS. Absolute 10-year risks of PAD and/or AAA were 11% and 29% in smoking women aged 70-79 years with lipoprotein(a) <50th vs. >99th percentile. Equivalent values in men were 19% and 47%. The percentage of events attributable to lipoprotein(a) >50th percentile was 9.5% and 12.3% for PAD and AAA, respectively.
Conclusion: High lipoprotein(a) levels increased risk of PAD, AAA, and MALE in the general population, opening opportunities for prevention given future lipoprotein(a) lowering therapies.

Increasing insight in the VLDL secretory pathway; a role for small leucin-rich protein 1 (SMLR1)

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Background. The intracellular assembly, trafficking, and secretion pathway of very low-density lipoproteins (VLDL) is not fully understood. Increasing insight in this pathway will help us finding tools to modify hepatic as well as plasma lipid metabolism to reduce fatty liver disease and atherosclerosis, respectively. One of the latest novel players in VLDL metabolism and the associated pathologies, is SMLR1. We previously showed that hepatic SMLR1 ablation in mice attenuates VLDL secretion which is associated with hepatic steatosis and protection against atherosclerosis [PMID: 36053190].

Aim. To increase insight in the molecular mechanism by which SMLR1 affects (intracellular) VLDL metabolism.

Results. In pulse-chase labelling ApoB100 in primary hepatocytes of mice lacking (hepatic) SMLR1, we now show similar intracellular ApoB100 levels but four times lower ApoB100 in medium compared to controls. To study which mechanisms are underlying this phenotype, we set out to study SMLR1 in various in vitro and in vivo models. Since human SMLR1 is predicted to be a small (107aa) transmembrane protein, membrane topology studies were performed which indicate that the C-terminal domain faces the cytosol. Based on immunofluorescence studies in hepatocarcinoma cells as well as sucrose gradients of mouse liver, we have shown that SMLR1 resides in the ER and Cis-Golgi. Following immunoprecipitation with SMLR1, most interacting partners were found to play a role in ER-Golgi transport and lipid metabolism. This suggests a potential role of SMLR1 in enabling the assembly and/or transport of VLDL transport vesicles from the ER to the Golgi. We are currently studying the intracellular fate of ApoB100 after SMLR1 ablation with the use of a novel mouse model in which the ApoB gene is endogenously tagged (apoB-Flag).

Conclusions. Our studies thus far lead us to suggest that SMLR1 is enabling the generation and/or trafficking of VLDL transport vesicles.

Effect of breastfeeding on lipid profile and cardiovascular risk markers in women with familial hypercholesterolemia: The FH-FEMINA study protocol

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Background: Patients with familial hypercholesterolemia (FH) have genetically elevated cholesterol levels from the first years of life. The cholesterol burden throughout life defines the risk of cardiovascular disease (CVD). Early and lifelong treatment is therefore crucial. However, in female FH patients, treatment periods are interrupted due to contraindication of lipid lowering medication during planning of pregnancy, the pregnancy and breastfeeding. Due to these off-treatment periods, women with FH may accumulate a higher cholesterol burden at young age. It is less known how breastfeeding affects lipid profile and other CVD risk markers in women with FH and to what extent statins transfer to breast milk.

Aims: 1) Investigate the effect of breastfeeding on serum lipid profile in women with FH. 2) Investigate the effects of breastfeeding on other CVD risk markers, measured in circulation and at gene expression level, in FH women. 3) Investigate transfer of statins in breast milk samples.

Methods: We designed a prospective study aiming to include 50 women with FH in Norway and the Netherlands. The women are included at gestational week 36 and followed at study visits 1, 3, 6, 9, 12 months after delivery. At all visits, a non-fasting blood sample, breast milk sample and information on diet, weight, height and blood pressure are collected. At the first visit, information on lifestyle factors, treatment history, current and previous pregnancies are collected. At start of statin treatment, we will measure transfer of statins to breast milk. We will also measure lipid profile (lipidome) and the metabolome in breast milk throughout the breastfeeding period.

Discussion: There is a need for more information on the effects of breastfeeding on lipid profile and other CVD risk markers in women with FH and transfer of statins to breast milk, both among the women and general physicians treating the women. Our aim is that these data will improve information and treatment during childbearing age for women at high risk of CVD.

Liver-targeted Angptl3 and Angptl4 silencing by antisense oligonucleotide treatment attenuates hyperlipidemia and atherosclerosis development in APOE*3-Leiden.CETP mice

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Background
Angiopoietin-like 3 (ANGPTL3) and ANGPTL4 inhibit lipoprotein lipase (LPL) to regulate tissue fatty acid uptake from triglyceride (TG)-rich lipoproteins (TRLs). While pharmacological inhibition of ANGPTL3 is under clinical investigation as lipid-lowering strategy, systemic ANGPTL4 inhibition is not pursued due to expected adverse effects.
Aim
To compare the therapeutic potential of liver-specific silencing of Angptl4 to that of Angptl3 to attenuate hyperlipidemia and atherosclerosis development in APOE*3-Leiden.CETP mice, a well-established model for humanized lipoprotein metabolism.
Methods
Mice were subcutaneously injected twice-weekly with saline, triantennary N-acetyl galactosamine (GalNAc) conjugated antisense oligonucleotides (ASOs) targeting murine Angptl3, Angptl4, both, or negative (scrambled) ASO. Plasma lipid levels were assessed, hepatic TRL production was studied by accumulation of TG and ApoB after blocking of LPL, TRL clearance was studied using glycerol tri[3H]oleate and [14C]cholesteryl oleate-labelled TRL-mimicking particles, and atherosclerosis development was assessed in a separate study.
Results
In mice, liver-targeted Angptl4 silencing reduced plasma TG (-48%) and total cholesterol (TC; -56%) levels, explained by higher [3H]oleate uptake by brown adipose tissue and lower hepatic TG and ApoB production. Accordingly, Angptl4 silencing reduced the atherosclerotic lesion size (-86%) and number of severe lesions (-63%) and improved the lesion stability index compared with negative ASO treatment. Hepatic Angptl3 silencing similarly attenuated hyperlipidemia and atherosclerosis development, albeit less effectively. Interestingly, combined Angptl3/4 silencing tended to further improve the lesion stability index.
Conclusion
Our data suggests that liver-specific ANGPTL4 inhibition holds therapeutic promise for the treatment of hyperlipidemia and atherosclerotic cardiovascular disease without causing the adverse effects observed with systemic Angptl4 deficiency.

Lipid profile during the first year of life

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Aim: Risk factors developed during childhood influence cardiovascular health in adulthood. The aim of the present study was to investigate atherogenic lipid traits during the first year of life and to identify influential factors for lipid concentrations in early childhood.
Methods: For this purpose, we used the Copenhagen Baby Heart Study comprising more than 13,000 umbilical cord blood samples and parallel venous blood samples from children and parents at birth (n=444), at two months (n=363), and at 14-16 months (n=158). Lipid traits were determined in all samples.
Results: Concentrations of low-density lipoprotein (LDL) cholesterol, non-high-density lipoprotein (HDL) cholesterol, and apolipoprotein B increased during the first year of life. A linear mixed model showed that high concentrations at birth predicted high concentrations at two months and at 14-16 months. Girls had higher concentrations at birth and at two months compared with boys. Children born preterm had higher cord blood concentrations than children born at term. Multivariable adjusted odds ratios (95% CI) for having high concentrations at two months when children had high concentrations at birth were 1.95 (1.01-3.79) for LDL cholesterol, 1.36 (0.69-2.67) for non-HDL cholesterol and 1.90 (1.02-3.53) for apolipoprotein B.
Conclusion: The lipid profile change during the first year of life and sex and gestational age influence concentrations. Children with high concentrations of LDL cholesterol, non-HDL cholesterol and apolipoprotein B at birth had higher levels at two and at 14-16 months. Concentrations at birth might thus be used to identify children at risk of dyslipidemia in later life.

Functional analysis of LDLR variants using automated systems to improve rare-variant association studies and risk assessment in hypercholesterolemia

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Background and aim

Lack of functional information for most low-density lipoprotein receptor (LDLR) mutations limits the use of genetic tools for early diagnosis of familial hypercholesterolemia (FH) and risk assessment in cardiovascular disease (CVD). The goal of this study was an in-depth functional characterization of LDLR variants at large-scale, to improve rare-variant association studies and decision-making in the treatment of FH.

Methods

We combined open-source robotics with multiplexed high-content imaging and python-based image and data analysis to establish a semi-automated analysis pipeline, enabling large-scale functional characterization of LDLR variants regarding LDL uptake, LDLR expression and subcellular localization. LDLR variants were expressed in a LDLR deficient liver cell line using CRISPR technology. The multiparametric functional data was then integrated with genetic and health data from UK Biobank and FinnGen.

Results

So far, we have analyzed more than 240 LDLR variants, providing more than 1400 data points for LDL uptake, LDLR localization and expression. This allowed us to group LDLR variants based on their functional activity, shedding light on more than 60 variants of unknown significance, 100 likely pathogenic and 30 likely benign LDLR variants. We utilized the functional activity groups in rare-variant association studies with lipoprotein and CVD outcome data using UK Biobank whole-exome sequencing and FinnGen genotype data. This allowed us to highlight the benefits of including in-depth functional data in genetic studies.

Conclusion

Our detailed functional analysis of LDLR variants paves the way for improved characterization of FH patients and can guide new personalized medicine approaches for lipid-lowering therapy.

ApoM and S1P – markers for incident heart failure and death in patients with chronic kidney disease?

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Background: Heart failure (HF) is the most common heart disease associated with chronic kidney disease (CKD). Apolipoprotien M (apoM) carries a small biactive lipid, sphingosine-1-phospate (S1P). The apoM/S1P complex has anti-inflammatory and antiatherogenic effects. Low concentrations of apoM are also associated with increased progression of HF and death in patients without known CKD. We wanted to determine whether reduced levels of apoM and S1P are associated with a higher risk of HF and death in uremic patients.
Methods: This study included 982 patients with chronic kidney disease from the CPH- CKD Cohort. Information on HF and death was obtained through medical records. Plasma apoM was measured in all available samples with an in-house sandwich enzyme-linked immunosorbent assay (ELISA) and plasma S1P were analyzed by high performance liquid chromatography (HPLC).
Results: During a median follow-up period of 3.0 years a total of 37 heart failure events and 111 all-cause deaths occurred. In multivariable logistic regression models, adjusted for enrollment place, age, gender, BMI, hypertension, hypercholesterolemia and eGFR an inverse association between plasma apoM and prevalent HF in patients with CKD was detected (OR 0.46 [95% CI,0.21-0.99]; $p < 0.05$).The association was however not present when comparing apoM with incident HF, but interestingly in a subcohort the odds ratio of HF for S1P < 1.06 μM versus S1P > 1.06 μM was 3.1 [95% CI,1.2-7.9]. Low apoM levels were associated with higher risk of death even after adjusting for enrollment place, age, gender, BMI, hypertension, hypercholesterolemia in cox proportional hazard models (HR 0.43 [95% CI,0.22-0.87]; $p < 0.05$).
Conclusion: In conclusion, we did detect a significant difference between plasma S1P levels in uremic patients with and without HF. In this study, apoM was not able to predict HF among patients with CKD. However, reduced levels of apoM were strongly associated with the risk of death, in patients with CKD.

Oral Presentations – Abstracts –

Other Topics

SESSION IV

Stimulation of the beta-2-adrenergic receptor activates human brown adipose tissue in vivo

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Background: The beta-3-adrenergic receptor (ADRB3) is the most important activating adrenergic receptor on brown adipose tissue (BAT) in rodents and ADRB3 agonism attenuates atherosclerosis development in APOE*3-Leiden.CETP mice (Nat Commun 2015). Nonetheless, we recently revealed that the ADRB2 is dominantly present and responsible for noradrenergic activation of human brown adipocytes in vitro (Cell Metab 2020). Therefore, we now aimed to assess whether ADRB2 agonism using salbutamol activates human BAT in vivo.

Methods: We performed a randomized double-blinded crossover trial in 10 young (age: 24.4±4.3 years old) and lean (body mass index: 23.1±2.3 kg/m2) males over two study days with one week wash-out in between. Subjects received a single intravenous bolus of salbutamol (250 µg) with or without the ADRB1/2 antagonist propranolol (80 mg), followed by a dynamic 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG) PET-CT scan.

Results: Salbutamol, compared to salbutamol with propranolol, increased glucose uptake by BAT (67±87 vs. 16±5 nmol·g-1·min-1, P=0.03), heart rate (+17±11 vs. -3±9 beats/min, P=0.004) and whole-body energy expenditure (EE; +122±168 vs. -192±91 kcal/day, P=0.003). The salbutamol-induced glucose uptake by BAT was positively associated with the increase in energy expenditure (Spearman rho=0.73, P=0.03). Notably, participants with salbutamol-induced glucose uptake by BAT above the median had a lower body fat mass (11.8±1.3 vs. 16.9±2.3 %, P=0.008), waist-hip ratio (0.8±0.02 vs. 0.9±0.1, P=0.03), and serum LDL-C concentration (1.6±0.4 vs. 2.7±0.5 mmol/L, P=0.02) compared to participants with low glucose uptake by BAT below the median.

Conclusion: Pharmacological stimulation of the ADRB2 with salbutamol acutely increases the rate of glucose uptake by human BAT in vivo, which is largely suppressed after blocking ADRB1/2. Notably, individuals with a healthier metabolic profile show a higher salbutamol-induced glucose uptake by BAT.

Proprotein Convertase Subtilisin/Kexin 6 is involved in lipid metabolism in liver

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Introduction: PCSK6 is a protease that activates cytokines and growth factors and strongly enriched in healthy human liver. We have previously shown that PCSK6 is induced in atherosclerotic plaques from patients with symptoms of stroke and important for regulating several cell types in this context. Here, we aimed to investigate the role of PCSK6 in lipid metabolism in liver, particularly in the context of atherosclerosis and steatosis.

Methods: We used publically available datasets and several atherosclerosis biobanks to investigate the expression of PCSK6 in healthy and diseased human tissues. In addition, we used full Pcsk6-/- mice as well as liver specific conditional Pcsk6-/- knockout mice to investigate the effects of PCSK6 ablation on lipid metabolism.

Results: Genetic analyses of the PCSK6 locus identified a variant, rs7181043, that was significantly associated with PCSK6 mRNA expression in healthy human adipose tissue, liver and in atherosclerotic plaques. The same variant was associated specifically with plaque fat content and atherosclerotic patient's plasma LDL levels. In addition, PCSK6 mRNA expression in plaques was positively correlated with total plasma cholesterol and LDL levels in atherosclerotic patients as well as lipid metabolism associated pathways within the carotid plaque. Microarray comparison of the livers from Pcsk6-/- mice and controls showed that VLDL particle assembly was one of the upregulated processes. In vivo studies showed that Pcsk6-/- mice have higher plasma cholesterol and LPL levels at baseline compared to controls, and lower levels of LDLR in their liver. These findings were further confirmed in liver specific conditional knockouts. Preliminary results show that liver specific knockout mice develop increased liver steatosis and fibrosis on a modified western diet.

Conclusions: Our data suggests that PCSK6 is involved in cholesterol and metabolic control in liver. Breeding of liver specific Pcsk6 knockout mice on an ApoE-/- background is currently ongoing and will provide insight into the role of liver PCSK6 in atherosclerosis development.

New evidence of cardiovascular risk factors for Alzheimer's disease

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Background: Forty percent of dementia is potentially preventable by modifying 12 risk factors throughout the life course, including several cardiovascular risk factors. Robust evidence for most of these risk factors is however lacking. We therefore aimed to comprehensively disentangle the causal associations between 12 modifiable risk factors and risk of Alzheimer's disease (AD).
Methods: We performed two-sample univariable and multivariable Mendelian randomization. Independent genetic variants associated with modifiable risk factors were selected as instrumental variables from large genome-wide association studies. We obtained outcome data for AD from the European Alzheimer's & Dementia Biobank (EADB) including 39,106 clinically diagnosed cases, 46,828 proxy-AD cases and 401,577 controls. Main analyses were conducted using the EADB clinically diagnosed endpoint data.
Results: Genetically determined high HDL cholesterol concentrations were associated with increased risk of AD with an odds ratio (OR) of 1.10 (95% CI: 1.05 to 1.16) per one-SD increase. Genetically determined per 10-mmHg increase of systolic blood pressure was associated with increased risk of AD after adjusting for diastolic blood pressure [OR (95% CI): 1.22 (1.02 to 1.46)]. To minimize bias due to sample overlap, we both excluded the entire UK Biobank from the EADB consortium and used cross-trait linkage disequilibrium-score regression, and the estimates remained similar. No robust genetic associations with AD were identified for other lipid traits, smoking, alcohol consumption, body mass index, and type 2 diabetes.
Conclusions: The results supported novel causal associations between high HDL cholesterol concentrations and high systolic blood with high risk of AD. These findings may inspire new drug targeting and improved prevention implementation.

Purinergic crosstalk between adipocyte-macrophage promotes degeneration of thermogenic brown adipose tissue

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Aim: Conditions like ageing, obesity and living at thermoneutrality are associated with loss of brown adipose tissue (BAT) activity. A phenomenon characterized by lipid accumulation, fibrosis, inflammation and insulin resistance. Next to thermoneutral housing and energy overload, impaired thermogenesis due to loss of uncoupling protein 1 (UCP1) results in pro-inflammatory remodelling of BAT. Here, we aim to assess mechanisms driving BAT degeneration.
Methods: The role of sympathetic input was studied in denervated BAT of Ucp1-/- and control mice. Mechanistic studies were performed in a newly established pharmacological model of inefficient thermogenic activation. Mice were co-treated with the β3-adrenergic agonist CL316,243 and etomoxir, an inhibitor of mitochondrial fatty acid import. Immunohistochemistry, FACS, RNAseq, indirect calorimetry and expression analyses were employed. For mechanistic studies, primary adipocytes and bone marrow-derived macrophages were used.
Results: Sympathetic innervation critically drives BAT degeneration in cold-exposed Ucp1-/- mice. The co-treatment with etomoxir and CL316,243 causes immune cell infiltration, lipid deposition, fibrosis, reduced Ucp1 levels and lower energy expenditure. By GO enrichment analysis of differentially expressed genes, we found pathways involved in purine nucleotide and ATP metabolism to be enriched, suggesting a role of ATP-activated purinergic receptors in BAT degeneration. Mechanistically, ATP is released by brown adipocytes in response to futile thermogenic activation, which in turn activates BAT macrophages via P2RX4/P2RX7. Combined inhibition of these purinergic receptors prevents BAT inflammation and thermogenic dysfunction under conditions of inefficient BAT activation or thermoneutrality.
Conclusion: Inflammatory degeneration of BAT is induced by energetic imbalance in brown adipocytes, a process regulated by a paracrine purinergic axis between adipocytes and macrophages mediated by extracellular ATP.

Risks associated with use of statins and other lipid-modifying agents across pregnancy – a nationwide drug safety study in Norway in 2005-2018

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Background and aims: Statins have traditionally been contraindicated during pregnancy; however, the risks associated with exposure to statins and other lipid-modifying agents (LMAs) in human pregnancies remain unclear. We aimed to examine the associations between exposure to LMAs across pregnancy and health outcomes in mother and offspring.
Methods: We linked registry data for all pregnant women in Norway in 2005-2018 from national registries in Norway. Exposures were pregnancy-related (before, during or after pregnancy) prescription fillings of any LMA, any statin- or non-statin LMA, or subgroups of these. Primary outcomes were major congenital malformations and miscarriage, and secondary outcomes were offspring growth and preeclampsia.
Results: In 2005-2018, 34 778 pregnancies in Norway resulted in an offspring with a congenital malformation (4.3% of all 805 368 pregnancies, omitting multiple births and chromosomal abnormalities). For pregnancies exposed to any type of LMA during first trimester, 22/340 exposed pregnancies developed a malformation (6.5%). For second and third trimester exposure, 3/78 (3.8%) and 2/56 (3.6%) developed a malformation, respectively. For pregnancies exposed 6-12 and 0-6 months before conception, 82/1228 (6.7%) and 70/1217 (5.6%) developed a malformation, respectively; also, for pregnancies exposed 0-6 and 6-12 months after birth, 58/772 (7.5%) and 84/1275 (6.6%) developed a malformation, respectively.
Conclusions: In crude analyses, the prevalence of malformations seemed to be numerically higher for pregnancies exposed to LMAs across pregnancy, although confounding is likely, for example by diabetes mellitus. Drug safety analyses for all outcomes are ongoing and will be presented at the conference, including multivariable models and sensitivity analyses.

The STEROL ELEMENT BINDING PROTEIN 1C (SREBP1c) is an immunometabolic checkpoint of regulatory T lymphocytes

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Aim: It's increasingly recognized how metabolism defines the polarization and activation of immune cells; in the context of adaptive immune system, tolerogenic Tregulatory (Treg) cells rely on fatty acids oxidation (FAO) for their suppressive function while glycolysis is preferred for cell migration. We aimed at studying how SREBP1c, a key protein regulating intracellular fatty acid (FA) metabolism, affects in concert Treg cell metabolism and function.

Material and Methods: A detailed immunophenotyping through flow cytometry and metabolic profiling (metabolomics and seahorse analysis) of isolated Tregulatory (CD4+CD25+) and in vitro induced Treg (iTreg) cells were performed together with in vitro and in vivo assays of Treg function from SREBP1c KO and WT littermates. RNAseq was performed on iTreg.

Results: Srebp1c KO mice presented reduced circulating and tissues' level of Treg compared to WT mice (-66%,$p<0,01$). Functionally, Srebp1c deficiency was associated with a reduced suppressive (-21%,$p<0,01$) and increased migratory function (+40%,$p<0,05$). In Experimental Autoimmune Encephalomyelitis, a model of immune challenge, Treg from KO mice were less effective compared to WT in limiting disease progression. Taking advantage from iTreg we confirmed that the less suppressive and more migratory phenotype was the consequence of Srebp1c deficiency in Treg rather than an effect of reduced cholesterol and triglycerides systemic plasmatic levels in KO vs WT (-56%,-61%,$p<0.01$). Metabolically, KO iTreg showed an increased glycolytic potential with preserved mitochondrial function. Accumulation of glycolytic metabolites and lactate (+20%,$p<0,01$) further confirmed a switch to anaerobic glycolysis in KO Treg. Finally, RNAseq identified a downregulation of pathways associated to lipid metabolism and tolerogenic response but increased migratory pathways, a phenotype associated to a reduced transcription and activation of Foxp3.

Conclusion: By restrain glycolysis and cell migration, our data have identified SREBP1c as a checkpoint crucial for the immunometabolic suppressive response of Tregs.

Genome-Wide Sleep-SNP interactions on lipid traits to identify biomolecular pathways underpinning sleep-associated lipid disturbances

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Aim of study: Disturbances in habitual sleep are (causal) risk factors for cardiometabolic disease, but the biomolecular mechanisms leading to disease are poorly understood. We performed large-scale genome-wide sleep-SNP interactions analysis on lipid levels to identify novel genetic variants underpinning the biomolecular pathways of sleep-associated lipid disturbances.
Methods: We collected summary-level data from 53 ancestry-specific cohorts with a combined sample of 732,564 participants (85.9% European ancestry) with 20% defined as either (age- and sex-standardized) short (STST) or long (LTST) total sleep time and with available data on lipid traits (LDL and HDL cholesterol, triglycerides). Cohort-level summary statistics from genome-wide interaction analyses were collected centrally for quality control, cleaning and joint (2 degrees of freedom) meta-analyses. Ancestry-specific cohorts were divided into 2 similarly-sized samples to perform bidirectional discovery/replication analyses.
Results: Preliminary bidirectional discovery/replication meta-analyses of European-ancestry cohorts (UK Biobank [N = 343,299] versus meta-analysis [N = 286,009]) on triglyceride levels identified 434 and 719 independent ($R2<0.1$) lead SNPs in UK Biobank and in the meta-analysis, respectively, with a minor allele frequency above 0.5%. Of these, 42 independent and replicated lead SNPs were identified through joint meta-analyses with SNP-STST interactions, and 49 SNP independent and replicated lead SNPs were identified through joint meta-analyses with SNP-LTST interactions.
Conclusion: These large-scale efforts identified a significant number of SNPs that could increase out understanding of the mechanisms driving sleep-associated lipid disturbances.

Health economic costs generated by an FH mutation

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Background: The economic costs associated with a pathogenic familial hypercholesterolemia (FH) mutation is not known

Methods: We studied 5,585 individuals with genetically verified FH, and 111,483 age- and sex-matched controls during 2010 to 2019. The total healthcare costs related to cardiovascular disease (CVD) in FH and matched controls were calculated by diagnosis-related groups (DRGs) cost weights and national unit costs for somatic hospital, rehabilitation and contract specialist care activity. CVD-related pharmaceutical costs which included both specialist health care and general practice were based on the actual pharmacies' sales prices for all medications.

Results: During 2010-2019 total healthcare and pharmaceutical CVD costs in FH was 5,364 EUR per person per year as compared to 1,903 in age and sex adjusted controls. Non-pharmaceutical healthcare costs were the major contributor in both FH and controls, EUR 4,679 and 1,778 per person per year in average, respectively. PCI was the treatment with the highest expenditure in FH patients, EUR 561 per person per year, compared to EUR 140 in. Thereafter followed costs due to rehabilitation, dialysis, pacemaker or implantable cardioverter-defibrillator (ICD) procedures, and heart valve surgery. Costs for coronary by-pas surgery was 5.2 times higher in FH as controls, angina 4.7 times higher, 4.0 for PCI, 4.1 times for heart valve surgery/intervention, 3.2 for pacemaker/ICD and 2.6 for major cardiovascular surgery. Average total pharmaceutical costs related to CVD 2010-2019 was 685 EUR per person per year in FH vs. 125 EUR in controls.

Conclusion: Total health expenditure was 2.8 times higher in FH than in controls, mainly driven by somatic hospital, rehabilitation and contract specialist that generated 2.6 times higher costs in FH than in controls. Pharmaceutical costs constituted 13% of total cost in FH and was 5.5 higher in FH compared to controls.

Posters – Abstracts –

Inflammation and Vascular Biology

SESSION I

No 07

Blood Milieu in Acute Myocardial Infarction Reprograms Macrophages for Trauma Repair

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Acute myocardial infarction (AMI) is accompanied by a systemic trauma response that impacts on the whole body, including blood. In this study, we addressed whether macrophages, key players in trauma repair, sense and respond to these changes. For this, healthy human monocyte-derived macrophages were exposed to 20% human AMI (n=50) or control (n=20) serum and analyzed by transcriptional and multiparameter functional screening followed by network-guided data interpretation and drug repurposing. Results were validated in an independent cohort at functional level (n=47 AMI, n=25 control) and in a public dataset. AMI serum exposure resulted in an overt AMI signature, enriched in debris cleaning, mitosis, and immune pathways. Moreover, we identified gene networks associated with AMI and with poor clinical prognosis in AMI. Network-guided drug screening on the latter unveiled Prostaglandin E2 (PGE2) signaling as target for clinical intervention in detrimental macrophage imprinting during AMI trauma healing. Our results demonstrate pronounced context-induced macrophage reprogramming by the AMI systemic environment, to a degree decisive for patient prognosis. This offers new opportunities for targeted intervention and optimized cardiovascular disease risk management.

No 17

Hypoxia induces a proatherogenic arterial extracellular matrix

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Low oxygen (O2) tension, hypoxia, is an important stimulus of atherosclerosis and correlates with intra-plaque angiogenesis in vivo. Increasing evidence suggests that hypoxia may be a driver of further extracellular matrix (ECM) modification, contributing to lesion development and instability. Accumulation of hypoxia-inducible factor-1α is believed to mediate many of the hypoxia-induced processes during lesion initiation and growth. However, the effects of hypoxia on arterial endothelial cells and associated ECM proteins are poorly understood.

The aim of this study was to investigate whether exposure of human coronary artery endothelial cells (HCAEC) to 1% O2 (hypoxic) versus 20% O2 (tissue culture environment) alters synthesis and composition of the ECM and whether this affects HCAEC activity, gene expression of ECM proteins, production of inflammatory cytokines, and generation of reactive oxidants.

Increased versican mRNA and protein were detected in HCAECs exposed to 1% O2 for 7 days via qPCR, and liquid chromatography-mass spectrometry respectively. Increased formation of reactive oxidants was detected by immunocytochemistry. The versican-rich ECM generated at 1% O2 showed reduced HCAEC adhesion, although these cells demonstrated increased metabolic activity as measured by MTS. This versican-rich ECM also bound more hyaluronic acid (HA), a known ECM component that interacts with versican in atherosclerotic lesions.

These data indicate that exposure of HCAEC to 1% (versus 20% O2) promotes the synthesis of a versican-rich ECM. In addition to HA, versican is known to bind low-density lipoproteins (LDL), this may contribute to LDL accumulation within lesions. The concurrent increase in oxidant production may exacerbate LDL modification, and contribute to the unregulated accumulation of lipids within macrophage cells in developing lesions and exacerbate the progression of atherosclerosis.

YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

No 02

HOCl-induced modification of NET-derived histones

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The release of neutrophil extracellular traps (NETs) is a key innate immune defense to combat infection. NETs consist of a mesh of DNA and histones, decorated with neutrophil granule proteins, including myeloperoxidase (MPO). Although NETs have important anti-bactericidal properties, they are also implicated in thrombosis and the development of atherosclerosis. However, the mechanisms involved in the pathological effects of NETs are not well understood. Enzymatically-active MPO is present on the NET backbone and produces the potent oxidant hypochlorous acid (HOCl). This study examines how HOCl-induced modification of NET-associated histones affects their function and reactivity with a vascular smooth muscle cell model. Experiments were performed with a commercial histone preparation containing H1, H2A, H2B, H3, and H4 histones. Cell viability was assessed with MTS assays to determine the cytotoxic effect of histones on human coronary smooth muscle cells. Exposure of primary human coronary artery smooth muscle cells to both unmodified and HOCl-modified histones resulted in a dose-dependent loss of viability. This finding is in line with the known cytotoxicity of extracellular histones. Importantly, HOCl-modified histones had a reduced cytotoxic effect on the cells in comparison to unmodified histones. The cell viability was dependent on the extent of oxidative modification. In addition, cells which received histone treatment showed altered redox balance as reflected by reduced intracellular thiol concentration as well as upregulated inflammatory molecules IL-6, MCP-1, VCAM-1 and oxidative stress gene HO-1 as shown by qPCR. Alterations in redox balance and inflammatory signalling were greater in cells treated with HOCl-modified histones in comparison to cells treated with unmodified histones. Combined, these results suggest that HOCl-induced histone modifications affect cytotoxicity of extracellular histones through increased inflammation. This novel insight into the mechanisms underlying NET-associated inflammation in atherosclerosis might aid the development of therapeutic interventions.

No 03

EZH2 inhibition reduces macrophage inflammatory responses in atherosclerosis

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AIM: Epigenetic processes are essential modulators of macrophage inflammatory responses. We postulate that interference in the epigenetic machinery of macrophages might offer novel approaches to combat atherosclerosis. Here, we investigate the repressive histone modification H3K27Me3 deposited by the polycomb repressor complex 2 (PRC2) with its catalytic component EZH2. We studied the therapeutic potential of macrophage EZH2 inhibition in the context of atherosclerosis.
METHODS: Human monocyte-derived macrophages and murine peritoneal and bone-marrow derived macrophages were treated with EZH2-specific inhibitor GSK126 and subsequently activated with LPS to mimic TLR4-inflammatory responses. The impact of GSK126 on macrophage differentiation and activation compared to vehicle (DMSO) was assessed by RNA-seq, flow cytometry, western blot, ELISA, RT-qPCR, and ChIP-seq.
RESULTS: GSK126 treatment lowered global H3K27Me3 levels without altering macrophage viability and differentiation, showing effective EZH2 inhibition. RNA-seq revealed that more than one-third of the LPS-induced genes were significantly downregulated by GSK126 treatment. Subsequent pathway analysis identified cytokine and interferon signaling, co-stimulation, and cell migration as the top down-regulated pathways (padj<0.05). Indeed, we confirmed that gene expression and cytokine secretion of the inflammatory mediators IL-6, IL-12, and TNF were reduced. Furthermore, membrane marker expression of co-stimulatory CD40, CD80, and CD86 were significantly decreased.
CONCLUSION: Overall, we show that EZH2 inhibition reduces inflammatory responses in human and murine macrophages. We are currently analyzing ChIP-seq data to identify direct targets of EZH2. Furthermore, we are performing ex vivo experiments on human endarterectomy plaques and an in vivo murine atherosclerosis study to assess the therapeutic potential of EZH2 inhibition on atherosclerosis development and progression.

No 04

Role of inflammatory signaling pathways involving the CD40-CD40L-TRAF cascade in animal models of diabetes and hypertension as well as coronary artery disease patients with one or two of these comorbidities I

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CD40L-CD40-TRAF signaling plays a role in the progression of atherosclerosis and influences the pathogenesis of coronary heart diseases. According to our previous data, we tested further the hypothesis that CD40L-CD40-TRAF signaling is a potential therapeutic target in diabetes and hypertension. In a mouse model of diabetes, TRAF6 inhibitor treatment led to a significant decrease in cardiac CD40L, p-47Phox, NOX2, and eNOS protein expression compared to untreated db/db mice. In a second mouse model of arterial hypertension, TRAF6 inhibitor treatment caused a significant reduction of MDA and 3NT protein expression in plasma compared to TRAF6 inhibitor untreated hypertensive animals. Our results indicate that TRAF6 inhibition could be a therapeutic target in diabetes and hypertension by reducing the expression of pro-inflammatory and oxidative stress markers. The translational aspect of our study is based on the characterization of the CD40L-CD40-TRAF cascade and major markers of thrombosis and inflammation in vascular bypass material and sera from patients with coronary artery disease (CAD) with diabetes and/or hypertension. Olink targeted plasma proteomic analysis showed a stepwise increase in markers of atherothrombosis and endothelial cell activation with each additional comorbidity for 24 protein targets. In addition, specific gene clusters that correlate with the comorbidities were identified in isolated aortic mRNA of CAD patients through next-generation sequencing. Key markers like NOX2, CD40L, CD68, and 3NT were validated quantitatively using qRT-PCR and immunoblotting analysis. A stepwise increase of these markers was observed with each additional comorbidity in CAD patients' plasma. Analysis of the patient's sera and vascular bypass material for pro-oxidative, pro-inflammatory, and pro-thrombotic parameters were employed to understand the function of the CD40-CD40L-TRAF axis in diabetes and hypertension-associated cardiovascular disease.

No 08

Sequestering inflammation in macrophages by modulation of 2-hydroxyglutarte

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Macrophages are critical drivers of atherosclerosis development and progression, and indeed inflammation lowering-strategies have been shown to be an effective target for atherosclerosis treatment. We have shown previously that stimulated macrophages produce high levels of the immunometabolite 2-hydroxyglutarate (2HG) during the resolution phase of an inflammatory response. 2-HG is a chiral metabolite with a D- and an L- variant. We have shown that when D-2HG is added to macrophages inflammation is lowered in response to LPS stimulation. While L-2HG has been demonstrated to be pro-inflammatory by others. Therefore we hypothesize that through the use of D-2HG induction, and L-2HG depletion, we can lower the pro-inflammatory response of macrophages in atherosclerosis. In order to investigate the mechanisms by which D-2HG is synthesized in stimulated macrophages, we investigated the transcription of ADHFE1 and D2HGDH which synthesize and degrade D-2HG respectively. ADHFE1 shows a transient increase in mRNA expression while D2HGDH shows a lowered transcription, hinting at D-2HG accumulation. We aim to further investigate this route to D-2HG accumulation by knocking out of ADHFE1, D2HGDH, as well as L2HGDH, which is responsible for the degradation of L-2HG, respectively through CRISPR/Cas and determining levels of D-2HG and L-2HG accumulation via mass-spectrometry in stimulated macrophages, as well as the capacity of these knock-out cells to produce pro-inflammatory cytokine upon immune stimulation. We expect that with D-2HG accumulation the levels of pro-inflammatory cytokines TNF and IL-6 will be lowered, while with accumulation of L-2HG will lead to greater production of pro-inflammatory cytokines. With greater insights into the enzymatic dynamics leading suppression of inflammation by D-2HG over-production we hope to determine whether this is an effective means to lower inflammation in macrophages.

No 12

Iodide as a potential therapeutic in atherosclerosis

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Background: Myeloperoxidase (MPO) is an enzyme released at sites of inflammation which generates powerful oxidants. It is strongly associated with atherosclerosis with both MPO, and products from its reactions, present at elevated levels in human atherosclerotic plaques. Furthermore, MPO is an independent prognostic marker in both patients with coronary artery disease and healthy individuals. Inhibition of oxidant formation by MPO may therefore have therapeutic potential in preventing atherosclerosis. MPO generates the oxidant hypochlorous acid (HOCl) from Cl-, but can also use alternative substrates, including I- and SCN-, to form less damaging species. We therefore hypothesized that I- alone, or in combination with SCN-, might reduce atherosclerotic plaque development by decreasing oxidative damage and inflammation.
Methods: Apolipoprotein E-deficient mice were fed a western-type diet for 16 weeks with concomitant supplementation with I- (18 μM) and/or SCN- (10 mM) in drinking water. Plaque burden was evaluated in the aortic arch by en face analysis. Plaque size and lipid and collagen composition in the aortic valves were examined and quantified by histological staining.
Results: Treatment with I- decreased atherosclerotic plaque burden in the aortic arch but not the aortic valves. Both I- and SCN-, alone or in combination, significantly reduced plasma cholesterol levels. Quantification of plasma thyroid hormone levels indicated that neither treatment affected basic metabolism.
Conclusions: Both I- and SCN- appear to have positive effects in reducing disease. Whether the reduction in atherosclerotic plaque burden seen with I- is due to changes in lipid metabolism or a decrease in MPO-induced oxidative damage remains to be clarified. However, I- supplementation appears to have potential as a cheap, safe, and readily applicable primary prevention strategy to address the increasing global burden of atherosclerosis.

No 15

Modulation of smooth muscle cells to treat atherosclerosis

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Aim: We aim to investigate if SMAD7 can be used to force contractile phenotype in smooth muscle cells (SMCs).
Background: In atherogenesis, SMCs lose their contractile phenotype, proliferate, and modulate to alternative mesenchymal phenotypes that drive plaque growth. Thus, inhibition of SMC modulation is a potential target for therapy, however, the best means to achieve that is unknown. Recent evidence suggests that SMAD7, an inhibitory member of the SMAD family, suppresses the formation of contractile vascular SMCs, but the mechanism is not explored.
Methods: We perfomed in vitro functional assays and gene expression analysis in siRNA-mediated SMAD7-knockdown rat aortic SMCs. In addition, we generated an inducible SMC-specific SMAD7 KO mouse (Smad7SMC-KO) to investigate the impact of SMAD7 on functional and structural properties of the arteries using wire myography, histological examination, and RNA sequencing.
Results: Data obtained in cultured aortic SMCs showed that genetic inhibition of SMAD7 reduced migration and proliferation capacities and enhanced the expression of contractile genes while decreasing the levels of markers for modulated SMCs. Wire myography of the mesenteric artery of Smad7SMC-KO mice revealed that Smad7-deficient arteries are more compliant and constrict better in response to noradrenaline and potassium. In addition, Smad7-deficient arteries expressed increased levels of genes with anti-proliferative and anti-migratory effects in SMCs (i.e. Kit, Rgs5).
Conclusion: Our data supports that contractile SMC function can be achieved through SMAD7 suppression and fundament our strategy to promote plaque-stabilizing cell types in atherosclerotic lesions in the next steps of the project. If forcing changes in the balance of modulated SMC types by targeting SMAD7 (i.e. increasing the proportion of cap ACTA2+ cells versus other SMC phenotypes) proves beneficial in our experimental model, it may open for a new type of anti-atherosclerotic therapies.

No 22

The role of Nrf2 activity in cell-cell interaction in atherosclerosis

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Endothelial dysfunction and inflammation are the key factors in atherosclerosis development. These and other proatherogenic changes are believed to be induced via increased oxidative stress. As Nrf2 (nuclear factor erythroid 2-like 2) is a master transcriptional regulator of antioxidant response, its lowered (for e.g. with age) activity may impair the arterial wall homeostasis. Here, we hypothesized that a decreased Nrf2 activity in endothelial cells (EC) will result in their dysfunction, dysregulated cellular cross-talk in the arterial wall and proatherogenic phenotype.

To verify our hypothesis, we implemented the animal model of atherosclerosis based on the AAV-mediated overexpression of proprotein convertase subtilisin/kexin type 9 (Pcsk9) and high-fat diet in mice lacking Nrf2 transcriptional activity in EC (Nrf2flox;Cdh5-Cre, further called Nrf2 EC tKO). After 14 weeks the mice were sacrificed and the aortas were used for histological and transcriptomic analysis using scRNA-seq.

The atherosclerosis phenotype was successfully developed in both control and Nrf2 EC tKO groups and revealed increased plaque size in Nrf2 EC tKO mice. This indicates the atheroprotective role of Nrf2 in aortic endothelial cells in dyslipidemic stress conditions. The scRNA-seq analysis revealed 11 clusters covering all the cell populations within the aortic wall. In the EC cluster of atherosclerotic Nrf2 EC tKO mice, as compared to the control, we found 68 up- and 59 downregulated genes involved in cell adhesion, extracellular matrix (ECM)-receptor interaction, IL-17 signaling, complement and coagulation cascade, cytokine-cytokine receptor interaction. Next, we performed cell interaction analysis with CellChat package. Receptor-ligand analysis revealed a changed interplay between cell sub-population in Nrf2 EC tKO mice as well as new signaling pathways and ligand-receptor pairs that may be implicated in more advanced atherosclerosis progression.

To sum up, the impact of the activity of endothelial Nrf2 on plaque size and cell-cell interaction makes this protein a potential target in atherosclerosis prevention.

Posters – Abstracts –

Cardiovascular Disease

SESSION II

No 38

Low plasma transthyretin is associated with all-cause and cardiovascular mortality in the general population

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Background:
Transthyretin tetramer destabilization is the rate-limiting step in transthyretin cardiac amyloidosis, which is an underrecognized contributor to heart failure and mortality in older adults. Evidence suggests that low plasma transthyretin is an in vivo marker of transthyretin tetramer instability.
We tested the hypothesis that low plasma transthyretin genetically and observationally associates with all-cause and cardiovascular mortality.
Methods:
We genotyped 102,204 individuals and measured plasma transthyretin concentrations in 19,619 individuals from two studies of the Danish general population, the Copenhagen City Heart Study and the Copenhagen General Population Study. We first tested whether genetic variants in TTR, which associated with increasing transthyretin tetramer instability, also associated with lower plasma transthyretin and higher risk of all-cause and cardiovascular mortality. Second, we tested whether low plasma transthyretin associated with higher risk of all-cause and cardiovascular mortality.
Results:
Compared to p.T139M, a well-known transthyretin stabilizing mutation, TTR genotype was associated with stepwise lower transthyretin concentrations of -20% for wild-type and -30% for heterozygotes for “Other mutations” (p.V142I, p.H110N, p.D119N). The corresponding hazard ratios (HR) for all-cause and cardiovascular mortality were 1.37(95% CI: 1.06-1.77) and 1.63(0.92-2.89) in wild-types, and 1.66(0.95-2.88) and 2.23(0.78-6.34) in carriers of “Other mutations”, respectively. In adjusted observational analysis individuals with plasma transthyretin $\leqslant$5th percentile (<19.1 mg/dL) versus 6-95th percentile had HRs of 1.39(1.17-1.64) and 1.66(1.17-2.38) for all-cause and cardiovascular mortality.
Conclusions:
Genetically and observationally low transthyretin concentrations are associated with higher risk of all-cause and cardiovascular mortality.

No 53

Amyloidosis-related orthopedic events, low plasma transthyretin, and risk of cardiac events

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Background and Aims: Carpal tunnel syndrome, spinal stenosis, and biceps tendon rupture may precede cardiac transthyretinamyloidosis (ATTR-CA). We tested the hypothesis that amyloidosis-related orthopedic events herald amyloidosis and cardiac events consistent with ATTR-CA through transthyretin destabilization.
Methods: In observational analysis in the Copenhagen General Population Study (CGPS; n=93,637), we first testedwhether amyloidosis-related orthopedic events at baseline were associated with amyloidosis and incident cardiac events consistent with ATTR-CA (heart failure, atrial fibrillation, myocardial infarction, or death), and whether a low plasma transthyretin was associated with a higher risk. In genetic analysis, in CGPS and the Copenhagen City Heart Study(CCHS) combined (n=102,496), we tested whether TTR genotypes associated with stepwise lower plasma transthyretin, marking lower transthyretin tetramer stability and higher amyloidogenicpotential, was associated with both orthopedic and incident cardiac events, implying a common mechanisticbackground through transthyretin destabilization.
Results: In individuals with versus without orthopedic events at baseline, hazard ratios (HRs) were 10.7 (95% CI: 3.9-29.3) for amyloidosis, and 1.3(1.1-1.4) for cardiac events. Furthermore, in individuals with orthopedic events at baseline, HRs for cardiac events were 3.8(1.9-7.6) in those with transthyretin <20 mg/dL versus ⩾ 20 mg/dL(Figure). Finally, HRs as a function of TTR genotype increased with lower transthyretin and lower transthyretintetramer stability up to 3.0(1.4-6.6) for orthopedic events and 1.6(95% CI: 1.0-2.6) for cardiac events.
Conclusions: Amyloidosis-related orthopedic events are associated with increased risk of incident cardiac events, with the highest risk in those with low transthyretin levels. Orthopedic and cardiac events are linked throughtransthyretin tetramer destabilization.

YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION II

No 32

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 coagulation 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.

No 35

Carbamylated Proteins Accumulate with Atherosclerotic Plaque Progression and Associate With Foam Cells

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Carbamylation is a non-enzymatic post-translational protein modification common in patients with uremia that causes pro-atherogenic alterations in plasma proteins. Interestingly, it is abundantly present in late-stage atherosclerotic plaques in non-uremic patients, however the mechanisms or consequences of this accumulation are not known. Human atherosclerotic plaque tissue samples (n= 27) were analyzed by immunohistochemistry (IHC) for extent of carbamylated lysine (carb-lys). Cellular carbamylation was studied by IHC of a parallel cohort of stable vs unstable plaques (n= 19). Functional effects of carbamylation on THP-1 macrophages were studied in vitro by assessing their morphological, functional, and inflammatory status on a microscale multi-assay platform. Advanced plaques showed significantly higher carb-lys positive area (relative to plaque area) compared to early-stage plaques (160% increase, $p< 0.01$) and a significant correlation with the total plaque area ($r= 0.794$; $p< 0.0001$). Cellular carb-lys signal did not differ between plaque stages but significantly correlated with CD68 ($r= 0.468$; $p= 0.05$) signal, and with PLIN2 ($r= 0.532$; $p< 0.05$) and LGALS3 ($r= 0.578$; $p< 0.05$) foam cell marker signals. In vitro experiments showed similar uptake of carbLDL and foam cell formation compared to oxLDL by THP-1 cells, as defined by Oil RedO staining. However, in contrast to oxLDL, carbLDL did not induce PLIN2 expression ($p< 0.0001$), suggesting a different lipid processing mechanism. Moreover, unlike oxLDL, carbLDL did not induce apoptosis or ROS production and inhibition of phagocytosis was significantly reduced. Taken together, our findings show accumulation of carbamylated protein with plaque progression which can be at least partially explained by ingestion of carbLDL particles by the macrophages. CarbLDL uptake, in turn, induces foam cell formation but seems to be less toxic to the cells than oxLDL. The consequences of this accumulation are yet to be investigated.

No 36

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

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The pathophysiology behind aggravated atherosclerosis in diabetes is still incompletely understood. We performed transcriptomic analysis of plaques from type 2 diabetic (T2D) patients with an aim to revealunderlying molecular mechanisms common between diabetes and atherosclerosis.

The Biobank of Karolinska Endarterectomies comprises plaques and clinical data from patients undergoingendarterectomy for carotid stenosis, profiled with whole-genome transcriptomic arrays. Multilevel bioinformatic analyses of differentially expressed genes were performed comparing plaques from patients with T2D (n=30, HbA1c>4.9) vs. non-diabetics (n=39, HbA1c<4.9) and further stratified according to stroke symptoms.

In microarrays from diabetic vs. non-diabetic patients, the most affected pathways were related to metabolic, coagulation, calcification and cell trans-differentiation processes. In the asymptomatic group, cholesterol storage genes were supressed, while proinflammatory, calcification and cell phenotypic transition genes were upregulated. In the symptomatic group, the dominating overexpressed genes were related to cell transdifferentiation. CHIT1 protease was strongly and specifically repressed in plaques from diabetic patients ($p<0.0001$). CHIT1 expression was associated with macrophages and markers of ossification. Immunohistochemical staining revealed co-localization of CHIT1 and CD68 in multinucleated cells resembling

osteoclasts. Whole transcriptome Nanostring analysis revealed 10-fold increase in CHIT1 expression in multinucleated cells in the plaques compared to other regions of interest.

Our findings reveal induction of stabilization processes related to ossification in plaques from all diabetic patients, but also cell trans-differentiation was relatively enriched in diabetic plaques from both symptomatic and asymptomatic patients. CHIT1 was identified as a novel gene inversely associated with atherosclerotic plaques from T2D patients that is currently being investigated mechanistically.

No 37

Control of heart calcium homeostasis by adipocyte-derived microRNAs

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Adipose tissue dysfunction, as observed in obesity or lipodystrophy, is associated with the development of cardiovascular diseases (CVD). It is now recognized that adipocyte-derived microRNAs act as endocrine molecules in distant organs. However, very little is known regarding the involvement of adipocyte-derived microRNAs in the development of CVD. Here, we used an adipocyte-specific deletion model of Dicer (Adicer), which is a key protein involved in microRNA processing, to determine the impact of microRNAs depletion on heart function.

First, we isolated cardiomyocytes from adult AdicerKO mice and their control littermates. Interestingly, we observed greater cytosolic calcium levels in AdicerKO-derived cardiomyocytes compared to WT. Notably, high cytosolic calcium levels were associated with longer time to relaxation following contraction-induced by electrical field stimulation in AdicerKO-derived cardiomyocytes. Next, we aimed to determine the mechanism by which AdicerKO present imbalance in calcium homeostasis and observed a marked reduction on SERCA2a levels in the hearts of AdicerKO mice with no compensatory changes in phospholambam or NCX proteins. Notably, specificity protein 1 (Sp1) mRNA levels, a transcriptional factor controlling SERCA2a expression, was reduced by ~80% in hearts of AdicerKO mice. Finally, to determine the causal effect of Sp1 in calcium homeostasis, we used small interfering RNA (SiRNA) to knock down Sp1 in H9c2 cells in vitro. Silencing of Sp1 led to a ~50% reduction in SERCA2a mRNA levels and, as a result, greater accumulation of cytosolic calcium.

In summary, depletion of adipocyte-derived microRNAs leads to calcium imbalance in cardiomyocytes through reduction of Sp1/SERCA2a axis. Exploiting this mechanistic link through targeting specific miRNAs might help to support cardiac function in people with diabetes and CVD.

No 42

Sex-specific influence of visceral and subcutaneous adipose tissue volumes on systemic inflammation and innate immune activation in obese subjects

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Introduction: Obesity predisposes to cardiometabolic/vascular diseases. Adipose tissue can actively contribute to the development of these complications. Women have more subcutaneous adipose tissue and less visceral adipose tissue than men. The aim of our study was to explore the sex differences in the association between the different adipose tissue compartments on circulating leukocytes and inflammatory proteins that are important in the pathophysiology of cardiometabolic disease.
Methods: We recruited 288 individuals (160 males, 128 females) with a BMI⩾27 kg/m2, aged 55-81 years. Abdominal magnetic resonance imaging was performed to measure visceral adipose tissue (VAT), deep subcutaneous adipose tissue (dSAT) and superficial SAT (sSAT) volumes. Leukocyte count and differentiation and ex vivo cytokine production capacity of peripheral blood mononuclear cells (PBMCs) were determined. In addition, targeted proteomics was performed of peripheral blood samples. Biopsies were taken from the abdominal sSAT and analysed for histology and gene expression of inflammatory genes.
Results: In women, but not in men, (s)SAT volume was associated with circulating leukocytes, monocytes, and neutrophils. Circulating IL-6 and IL-18BP was positively associated with SAT in women and VAT in men. Targeted proteomics revealed several proteins, including M-CSF1 and HGF, associated with sSAT in women and VAT in men. Only in women, SAT volume was positively associated with SAT expression levels of various inflammatory proteins, such as leptin, CD68, TNFα and IL-1α.
Conclusion: In our obese cohort of 288 subjects abdominal SAT volume, especially sSAT, is associated with circulating leukocytes and inflammatory proteins only in women. In men, these parameters mainly show associations with VAT volume. In women sSAT volume was associated with the sSAT expression of inflammatory proteins. These findings underscore that future research on adipose tissue in relation to cardiometabolic/ vascular disease should always take into account sex.

No 50

Myeloid CD40 deficiency reduces atherosclerosis by impairing macrophages' transition into a pro-inflammatory state

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Aims: CD40 and its ligand, CD40L, play a critical role in driving atherosclerotic plaque development. Disrupted CD40-signaling reduces experimental atherosclerosis and induces a favourable stable plaque phenotype. We recently showed that small molecule-based inhibition of CD40-TNF Receptor Associated Factor-6 interactions attenuates atherosclerosis in hyperlipidaemic mice via macrophage-driven mechanisms. The present study aims to detail the function of myeloid CD40 in atherosclerosis using myeloid-specific CD40-deficient mice.

Method and results: Cd40flox/flox and LysM-cre Cd40flox/flox mice on an Apoe-/- background were generated (CD40wt and CD40mac-/-, respectively). Atherosclerotic lesion size, as well as plaque macrophage content, were reduced in CD40mac-/- compared to CD40wt mice and their plaques displayed a reduction in necrotic core size. Transcriptomics analysis of the CD40mac-/- atherosclerotic aorta revealed downregulated pathways of immune pathways and inflammatory responses.Loss of CD40 in macrophages changed the representation of aortic macrophage subsets. Mass cytometry analysis revealed a higher content of a subset of alternative or resident-like CD206 + CD209b- macrophages in the atherosclerotic aorta of CD40mac-/- compared to CD40wt mice. RNA-sequencing of bone marrow-derived macrophages (BMDMs) of CD40mac-/- mice demonstrated upregulation of genes associated with alternatively activated macrophages (including Folr2, Thbs1, Sdc1 and Tns1).

Conclusions: We here show that absence of CD40 signalling in myeloid cells reduces atherosclerosis and limits systemic inflammation by preventing a shift in macrophage polarization towards pro-inflammatory states. Our study confirms the merit of macrophage-targeted inhibition of CD40 as a valuable therapeutic strategy to combat atherosclerosis

No 51

Genetic variation in SLC5A2 mimicking SGLT2-inhibition and risk of cardiovascular disease and all-cause mortality: reduced risk not explained by lower plasma glucose

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Background and aim: Treatment with sodium-glucose co-transporter 2(SGLT2)-inhibitors reduces risk of cardiovascular disease and all-cause mortality, but the mechanism is unclear. We hypothesized that a functional genetic variant in SLC5A2, known to be associated with familial renal glucosuria, would mimic pharmacological SGLT2-inhibition and thus provide an opportunity to examine potential mediators of the effects on lower risk of cardiovascular disease and all-cause mortality.

Methods: We examined 112,745 individuals from the Copenhagen City Heart Study and Copenhagen General Population Study (CCHS+CGPS), 488,682 from the UK Biobank, and 309,154 from FinnGen, all genotyped for SLC5A2 rs61742739,c.1961A>G; p.(Asn654Ser). First, we examined risk of cardiovascular disease and all-cause mortality; second, whether carrying the variant was associated with potential mediators of the effect; and third, whether identified potential mediators could explain the observed reduced risk of cardiovascular disease and all-cause mortality.

Results: In the CCHS+CGPS, carriers vs. non-carries had 31% lower risk of heart failure, 21% lower risk of myocardial infarction, 16% lower risk of ischemic heart disease, and 22% lower risk of all-cause mortality. Corresponding values in meta-analyses of the three studies combined were lower risk by 10%, 9%, 7%, and 9%, respectively. Of the lower risks observed in CCHS+CGPS, lower plasma glucose mediated 2.0%(P=0.004) on heart failure, 3.1%(P=0.09) on myocardial infarction, 4.1%(P=0.02) on ischemic heart disease, and 6.0%(P=0.39) on all-cause mortality; corresponding values in the UK Biobank were 2.9%(P=0.70), 1.5%(P=0.77), 4.1%(P=0.23), and 3.1%(P=0.21), respectively.

Conclusion: A functional genetic variant in SLC5A2, encoding SGLT2, was associated with lower risk of heart failure, myocardial infarction, ischemic heart disease, and all-cause mortality. These effects were at most minimally mediated through lower plasma glucose.

No 52

Difference in PBMC gene expression between elderly event-free FH patients and FH patients with CHD

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Background and aim: Familial hypercholesterolemia (FH) is a genetic disorder characterized by lifelong elevated low-density lipoprotein cholesterol (LDL-C) and increased risk of premature coronary heart disease (CHD). In elderly FH patients having lived most of their life prior to available effective cholesterol-lowering therapy, the CHD risk is extremely high. However, some individuals still remain free of any CHD event, suggesting the presence of protective features. Identifying possible cardioprotective genetic profiles could contribute to our understanding of CHD prevention and potentially future preventive treatment. Therefore, our aim was to investigate gene expression in elderly event-free FH patients.

Methods: We analyzed the expression of 774 genes related to different metabolic pathways, e.g. amino acid synthesis, cytokine signaling and lipid metabolism, using Nanostring, in peripheral blood mononuclear cells (PBMCs) from FH patients > 65 years with CHD (FH CHD, n=39) and without CHD (FH event-free, n=44), and from controls⩾ > 65 years (n=39). The controls were non-FH and non-CHD. In addition, we correlated our data to serum metabolites measured with nuclear magnetic resonance (NMR) spectroscopy.

Preliminary results: Thirty-six genes were differentially expressed between FH event-free and FH CHD ($p<0.05$), adjusted for age, gender, BMI and use of PCSK9 inhibitors. Among these genes, 15 were related to lipid metabolism. The FH event-free group had higher expression of ABCA1 ($p=0.004$) and ABCG1 ($p=0.03$) compared to FH CHD. The expression of both ABCA1 and ABCG1 were positively correlated to serum concentration of large and extra-large HDL particles and correlated negatively to small HDL particles.

Conclusions: Elderly event-free FH patients displayed different expression of lipid related genes than FH patients with CHD. The cholesterol efflux mediating genes ABCA1 and ABCG1 were significantly higher expressed in event-free FH patients, and further associated with larger HDL particles potentially contributing to an event-free phenotype.

No 56

Smooth muscle cell-specific translatome profiling of mouse atherosclerosis uncovers Itih4 as a novel candidate gene for atherosclerosis.

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Vascular smooth muscle cells (SMCs) are a major source of plaque of atherosclerosis, with recent advancement in scRNA we were able to identify the subsets of SMCs. Still, single cell studies are limited by the high cost and tissue dissociation artefacts. To address these limitations, we developed a mouse model using Translating Ribosome Affinity Purification (TRAP-Seq) to study the translational dynamics of vascular smooth muscle cells (SMCs) in each tissue. By expressing EGFP-tagged RL10a under the SMC-specific aSMA promoter, we were able to extract and sequence RNA from the aortas of 15-month-old control and atherosclerotic mice. Our results showed a high enrichment of SMC-specific genes in the pulldown fraction, supporting the success of the TRAP-Seq approach. Further composite analysis of the SMC-specific genes and atherosclerosis-induced genes highlighted the contribution of SMCs to the disease and identified novel genes that warrant further study. One such gene, ITIH4, was found to be involved in extracellular matrix stabilization (Bost F, et.al., 2001). The scRNA data from aorta shows the expression of Itih4 in the de-differentiated smooth muscle cell and immunofluorescence staining shows the expression in the atherosclerotic plaque. We identified a splice variant, rs77347777, associated with coronary artery disease within the Itih4 gene further implicating this disease in genetic predisposition to atherosclerosis. TRAP-Seq is a promising approach to gain in-depth knowledge of the translational dynamics of SMCs in any given tissue and could be used to identify new therapeutic targets for atherosclerosis.

Posters – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

No 25

Small dense LDL cholesterol and ischemic stroke

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Aim: For decades it has been suggested that small dense low-density lipoprotein (sdLDL) may be particularly atherogenic. High levels of sdLDL are associated with an increased risk of ischemic heart disease; however, the association of sdLDL with ischemic stroke has not been explored in a large prospective study on the general population. We tested the hypothesis that high sdLDL cholesterol levels are associated with an increased risk of ischemic stroke.

Methods: This prospective study included 38,319 individuals from the Copenhagen General Population Study with fresh sample measurements of sdLDL cholesterol. Median follow-up time was 3.1 years. We observed 302 and 74 ischemic and haemorrhagic strokes from baseline in 2013-2017 to end of follow-up in 2018. For comparison, we included estimates for large buoyant LDL cholesterol and total LDL cholesterol.

Results: Higher levels of sdLDL cholesterol were log-linearly associated with increased risk of ischemic stroke. Compared to individuals with sdLDL cholesterol in the lowest tertile ($\leqslant$0.60 mmol/L; $\leqslant$23 mg/dL) the multivariable adjusted hazard ratio for ischemic stroke was 1.79 (95%confidence interval: 1.31-2.43) for the highest tertile ($\geqslant$0.86 mmol/L; $\geqslant$33 mg/dL). Multivariable adjusted hazard ratios for ischemic stroke per 1 mmol/L (38.7 mg/dL) higher levels were 1.69 (1.28-2.22) for sdLDL cholesterol, 0.95 (0.78-1.16) for large buoyant LDL cholesterol, and 1.08 (0.93-1.25) for total LDL cholesterol. Hazard ratios were similar when further adjusting for BMI and diabetes mellitus in the biological pathway in combination with related lipids and lipoproteins.

Conclusion: Higher sdLDL cholesterol levels were robustly associated with increased risk of ischemic stroke.

No 44

Elevated plasma adiponectin in risk of heart failure, atrial fibrillation, aortic valve stenosis, and myocardial infarction:observational and Mendelian randomization studies

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Aim: Adiponectin is an adipocyte-secreted hormone with insulin-sensitizing, anti-atherogenic, and anti-inflammatory properties. We tested the hypothesis that plasma adiponectin is associated observationally and causal, genetically with risk of heart failure, atrial fibrillation, aortic valve stenosis, and myocardial infarction.
Method: In the Copenhagen General Population Study, we did observational analyses in 30,045 individuals with plasma adiponectin measurements and genetic one-sample Mendelian randomization analyses in 96,903 individuals using five genetic variants. In the HERMES, UK Biobank, The Nord-Trøndelag Health Study(HUNT), deCODE, the Michigan Genomics Initiative(MGI), DiscovEHR, and the AFGen consortia, we did genetic two-sample Mendelian randomization analyses in up to 1,030,836 individuals using 12 genetic variants.
Results: In observational analyses, a 1 unit log-transformed higher plasma adiponectin was associated with a hazard ratio of 1.51(95% confidence interval:1.37–1.66) for heart failure, 1.63(1.50–1.78) for atrial fibrillation, 1.21(1.03–1.41) for aortic valve stenosis, and 1.03(0.93–1.14) for myocardial infarction; levels above the median was also associated with increased risk of myocardial infarction. Corresponding genetic, causal odds ratios were 0.92(0.65–1.29), 0.87(0.68–1.12), 1.55(0.87–2.76), and 0.93(0.67–1.30) in one-sample Mendelian randomization analyses, while corresponding causal risk ratios were 0.99(0.89–1.09), 1.00(0.92–1.08), 1.01(0.79–1.28), and 0.99(0.86–1.13) in two-sample Mendelian randomization analyses, respectively.
Conclusion: Elevated plasma adiponectin was associated with increased risk of heart failure, atrial fibrillation, aortic valve stenosis, and myocardial infarction. However, genetic evidence did not support causality for those associations.

No 73

Loss-of-function mutations in APOC3, thrombocytopenia, and incidence of bleeding events

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Background: Antisense oligonucleotide(ASO) inhibition of APOC3 lowers plasma triglycerides in patients with hypertriglyceridemia. However, in clinical trials, reported episodes of moderate to severe thrombocytopenia in patients treated with volanesorsen, an ASO inhibitor of APOC3, have raised concern. Using loss-of-function mutations in APOC3 as proxies for APOC3 ASO inhibition, we tested whether these mutations were associated with lower platelet counts and higher risk of bleeding events.
Methods: We performed a naturally randomized trial using Mendelian randomization of genetic APOC3 loss-of-function mutations in 113,423 individuals from two similar general-population studies. We tested whether loss-of-function mutations in APOC3, working as proxies for APOC3 ASO inhibition, and which previously have been associated with lowering of plasma triglyceride levels, were associated with lower platelet counts and with a higher risk of incident bleeding events. During follow-up, bleeding events occurred in 14,580 individuals.
Results: Mean platelet count was 285x10^9/L in APOC3 loss-of-function heterozygotes and 277x10^9/L in noncarriers (P=0.05). Thrombocytopenia (platelet count<140x10^9/L) was observed in 0.5% of heterozygotes and 0.8% of noncarriers (P=0.60) and grade three thrombocytopenia (platelet count <50x10^9) was observed in 0% of heterozygotes and 0.03% of noncarriers (P=0.73). Hazard ratio(HR) for any bleeding event was 0.98 (95% confidence interval: 0.75-1.29) in heterozygotes versus noncarriers, and HRs for cerebrovascular-, airway-, urinary-, or gastrointestinal bleeding were similar (P-values 0.27-0.92).
Conclusions: Loss-of-function mutations in APOC3 were not associated with thrombocytopenia or with increased risk of future bleeding events in the general population, suggesting that the thrombocytopenia reported with volanesorsen treatment is likely due to an off-target effect.

YIA Poster Walk – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

No 60

Genetic variants in ABCA1 and risk of age-related macular degeneration

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Background and Aims
Age-related macular degeneration (AMD) is a disease of the retina characterized by the accumulation of lipid-rich deposits beneath the basal lamina of the retinal pigment epithelium. Genetic variants in the adenosine triphosphate-binding cassette transporter A1(ABCA1) is associated with higher concentrations of high-density lipoprotein (HDL) cholesterol. Higher HDL cholesterol concentrations are observationally and genetically associated with higher risk of AMD. However, whether amino acid changing genetic variants in ABCA1 associated with high HDL cholesterol concentrations confer a higher risk of AMD in the general population is currently unknown.
Methods
We genotyped all amino acid changing ABCA1 variants with a minor allele frequency above 0.002, measured plasma HDL cholesterol, and used Cox regression to assess risk of AMD. We created an HDL cholesterol weighted allele score and tested the association with risk of AMD on a continuous scale and in tertiles. Further,we performed mediation analyses.
Results
We included 90,344 study participants. On a continuous scale higher concentrations of genetically determined HDL cholesterol were associated with higher risk of all-cause AMD, dry AMD, and wet AMD in a multivariable adjusted model. The ABCA1 allele score for the third versus the first tertile was associated with HRs (95% confidence intervals (CIs)) of 1.30 (1.14-1.49) for all-cause AMD, 1.26 (1.06-1.50) for dry AMD, and 1.31 (1.12-1.53) for wet AMD. 6-8% of the effect was mediated through HDL cholesterol. There was no interaction between weighted allele score tertiles and confounding factors on risk of AMD.
Conclusions
Amino acid changing genetic variants in ABCA1 which were associated with higher HDL cholesterol concentrations, were also associated with higher risk of AMD, both on a weighted allele score continuously and when divided into tertiles.

No 61

Lipids, the Achilles' heel of inflammatory macrophages?

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Inflammation plays a central role in the development of cardiovascular disease (CVDs) and is mediated by an invasion of immune cells into arteries that propagate a state of chronic inflammation. Within atherosclerotic plaques, macrophages are skewed towards an inflammatory state by environmental stimuli. Modulating inflammatory macrophage activation has a huge potential for the treatment of CVDs. As a key controller of macrophages, cellular metabolism is the focus in the development of new therapies. Here, we developed a 96-well-plate-based metabolic screening assay to discover new drugs with the potential to treat inflammatory diseases and adjust the balance between inflammatory and anti-inflammatory macrophages. This revealed FATP2, a long chain fatty acid transporter, as a unique therapeutic target in inflammatory macrophages. Inhibition of FATP2 caused a significant downregulation of the metabolic activity and accelerated cell death in LPS-activated macrophages. In addition, depletion of specific fatty acids prevented the metabolic switch towards glycolysis, reduced the generation of ROS and dampened the secretion of inflammatory cytokines triggered by LPS. These metabolic dependencies could be only observed in inflammatory macrophages, and not in naïve or IL-4-stimulated macrophages, highlighting a crucial role for long chain fatty acids in inflammatory macrophages. The aim of this study is to reveal the metabolic pathways explaining the dependency of inflammatory macrophages on lipids and to pinpoint the particular fatty acids underpinning this state. Unraveling this metabolic vulnerability in inflammatory macrophages could provide new strategies to combat CVDs.

No 62

Icosapent ethyl supplementation rapidly reduces cardiovascular disease risk markers and improves plasma and lipoprotein lipidome reducing their atherogenicity in humans

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Background and Aims:
Icosapent ethyl (IPE) -supplementation has postulated benefits in improving cardiovascular health. Here we assessed how IPE-supplementation affects lipoproteins subclass profile, and the lipidome and proatherogenic properties of plasma lipoproteins.
Methods:
39 healthy, normolipidemic volunteers received a 4 g daily dose of IPE for 4 weeks. Plasma samples collected prior, during, and after the supplementation were analysed by NMR spectroscopy.
Results:
Total circulating eicosapentaenoic acid (EPA) increased 4-fold, while omega-6 fatty acids reduced, decreasing the omega-6/omega-3 ratio from 7.9 to 2.8. Plasma triglycerides (TG) (0.89±0.38 mmol/L vs. 0.72±0.34 mmol/L) and apolipoprotein-B (apoB) (0.75±0.15 g/l vs. 0.71±0.16 g/l) were significantly decreased already after 7-days of IPE-supplementation. The concentrations of VLDL-TG, VLDL-C, VLDL particles and total lipid were significantly decreased after IPE-supplementation. The concentration of XXL-VLDL lipids and particles decreased by more than 30%, while XS-VLDL lipids and particles decreased only by 7% and 6%, respectively. IPE-supplementation also decreased the level of lipoprotein lipase inhibitor ANGPTL3 as well as the binding of plasma lipoproteins to human aortic proteoglycans. IPE-supplementation also decreased cardiovascular disease risk, as assessed by lipid-based Coronary Event Risk Test 2 (CERT2).
Conclusions:
IPE-supplementation led to rapid increase in total circulating EPA, reducing the omega-6/omega-3 ratio, improving the plasma lipidome. In addition, TGs and especially TG-rich apoB particles and their probability to bind to proteoglycans were reduced. The CERT2 risk score was improved even after 7-day washout period, suggesting a long-lasting benefit from IPE-supplementation. Our data highlights mechanisms that may explain the previously identified IPE-induced reduction in cardiovascular disease risk.

No 64

Adipose tissue exposed to high fat diet affects extracellular matrix genes in the mesenchymal stem cell population

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Adipose tissue influences the physiological and pathological processes in our body by regulating lipid storage and metabolic homeostasis. Extracellular matrix (ECM) is a dynamic and complex assemblage consisting of polysaccharides, proteogylcans and signaling proteins. Though both adipocytes and non- adipose cells of the stromal fraction contribute to ECM maintenance, role of adipose tissue ECM in the disease remains poorly characterized. High fat diet (HFD) and obesity represent major risk factors for atherosclerosis. Our overall aim in this study was to understand HFD induced changes in the adipose tissue during atherosclerosis progression using single cell RNA sequencing (scRNA-seq) and to identify the ligands and transcription factors responsible for changes in the expression of ECM components that influences the function of adipose using human pre-adipocyte cells (SGBS). We performed scRNA-seq in the adipose tissue of control mice and atherosclerotic LDLR-/- / ApoB100/100 subjected to 1 (early disease) or 3 months (advanced disease) of HFD. This allowed us to identify 13 different cell types in the adipose tissue of the diseased mice. Among them, we identified mesenchymal cells (MSC) undergoing changes from putatively adipogenic to fibrogenic cells. The differentially expressed genes in the MSC population exhibited functions related to ECM development, maintenance and signaling. We predicted the ligand and transcription factor networks using NicheNet and SCENIC tools, respectively and validated them in SGBS cells using RNA sequencing. Based on the top hits from NicheNet and SCENIC analysis, the regulation of ECM genes was validated using the following ligands: TFG-b, SPP1, IL1-b and AGTII. Likewise, the role of MXD4 and CREBs family transcription factors were studied by gene knockdown in SGBS cells. Our results identified that TGF-b acts upstream to transcription factors like CREB3, CREB3L1 and MXD4. Regulation of these transcription factors play a key role in changes related to ECM and adipogenesis. Expanding our knowledge on the key regulators of adipose ECM will lead to a better understanding of the disease process, which is imperative for the future development of therapies. Altogether, our analysis provides the first steps toward understanding the role of MSCs in ECM-related changes during atherosclerosis and HFD stimulation.

No 68

MECR is essential for coordinated energy transformation

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Mitochondria mediate many catabolic processes, but are also capable of anabolic pathways such as fatty acid synthesis (mtFAS). Mitochondrial enoyl-CoA-reductase (MECR) catalyzes the last step of mtFAS, serving as a key mediator for lipoic acid production and acetyl-CoA-dependent cellular metabolic pathways. In humans, genetic mutations in MECR disturb the mtFAS pathway causing MEPAN syndrome, a neurometabolic disorder. However, whether mtFAS affects systemic lipid and glucose metabolism has not been investigated. We identified a patient with compound-heterozygous missense variants in MECR. Variant overexpression in HEK cells led to a specific lipidomic fingerprint especially in membrane lipids, whereas plasma analysis of the patient showed reduced levels. Interestingly, a subset of a cohort of 300 mitochondriopathy patients showed biomarkers of reduced mtFAS. Tissue specific hepatic and adipose tissue knockout mice mitochondria were irregularly shaped with critically affected cristae structures. Hepatic Mecr deletion led to profound insulin hypersensitivity, reduced lipoic acid synthesis and PDH activity, whereas adipose tissue Mecr ablation exacerbated tissue inflammation, attenuated brown fat thermogenic capacity and critically affected survival upon cold exposure. Mechanistically, we show that MECR human variants or deletion in vivo influences energy breakdown by regulating the PDH complex activity, which is important for glucose and lipid use from the circulation. In sum, we introduce mtFAS as a critical regulator of metabolic health, by determining systemic and intracellular glucose and lipid metabolism in liver as well as thermogenic responses in adipose tissues.

No 74

Charge neutralization of the acidic domain residues in GPIHBP1 attenuates its effects towards Lipoprotein Lipase

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GPIHBP1 is the obligatory factor LPL, the rate-limiting enzyme in intravascular lipolysis. Free LPL quickly undergoes spontaneous unfolding, inactivating the enzyme, GPIHBP1 acidic domain can counter-act this inactivation. In this study, we investigated the requirements of the protective effect of the acidic domain of GPIHBP1. We first studied the effect of altering the charge distribution in synthetic acidic domain peptides of GPIHBP1. This revealed that the minimum requirement of charged residue to stabilize LPL is 13 negative charges. This indicated that the positioning of the acidic domain peptides was not random, why we expressed full-length GPIHBP1 proteins containing the same change neutralized residues. Compared to wild-type GPIHBP1, these GPIHBP1 variants had a lower affinity towards LPL and a slightly lowered stabilization effect when investigated with nano-DSF. The GPIHBP1 variants however were able to prevent spontaneous LPL unfolding. When LPL was challenged with ANGPTL4, these variants failed to fully protect LPL, however, one (GPIHBP1 27-35) was better indicating that the C-terminal part of the acidic residues in GPIHBP1 is central for GPIHBP1 protective effects. Combined these results expand our understanding of the protective role of GPIHBP1 acidic domain towards LPL.

No 20

A possible role of ApoB in degenerative ascending aortic aneurysm formation and progression

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BACKGROUND: Aortic valve regurgitation (AR) is associated with degenerative changes in the ascending aorta and the occurrence of ascending aortic aneurysm (AscAA). The molecular mechanism and initiating factors remain largely unknown, but leukocyte infiltration and inflammation are known characteristics. The aim of this study was to investigate the molecular signature of AR-associated degeneration and identify factors associated with degenerative AscAA formation.
MATERIAL AND METHODS: Patients undergoing elective open-heart surgery for AscAA- and/or aortic valve surgery were included. Control samples were attained from organ donors. Histomorphology and protein expression/localization was assessed using immunohistochemistry, and gene expression in aortic intima-media was analyzed for totally n=16 non-dilated AR, n=19 non-dilated aortic stenosis (AS) and 36 dilated patients, as well as in n=6 controls.
RESULTS: Gene expression analysis showed that already prior to aortic dilatation, AR was associated with biological processes related to extracellular matrix turnover, as well as degenerative histological changes. In differential gene expression analysis of non-dilated vs dilated aortic tissue from AR-patients, genes with the greatest fold-change were PRPF40B, FMNL3, OLR1, MS4A7, MS4A14, of which OLR1 has a role in atherosclerosis and vascular inflammation. Apolipoprotein B 100 (ApoB) showed a different expression pattern between AR vs. AS patients with a high degree of medial infiltration in AR-patients. In AS-patients ApoB was almost exclusively localized to the endothelium. Further, the SRBI protein, a protein mediating LDL-transcytosis, was significantly increased in the endothelium of AR-patients compared with AS patients.
CONCLUSIONS: The prevalence of AR-associated AscAA may involve an early infiltration of ApoB in the ascending aortic media, with possible down-stream implications for disease development. Further, this may explain the rarity of AscAA presenting with AS.

Posters – Abstracts –

Other Topics

SESSION IV

No 90

Identification of Side Chain Oxidized Sterols as Novel Liver X Receptor Agonists with Therapeutic Potential in the Treatment of Cardiovascular and Neurodegenerative Diseases

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The nuclear receptors liver X receptors (LXR α and β) are potential therapeutic targets in cardiovascular and neurodegenerative diseases because of their key role in the regulation of lipid homeostasis and inflammatory processes. Specific oxy(phyto)sterols differentially modulate the transcriptional activity of LXRs providing opportunities to develop compounds with improved therapeutic characteristics. We isolated oxyphytosterols from Sargassum fusiforme and synthesized sidechain oxidized sterol derivatives. Five 24-oxidized sterols demonstrated a high potency for LXRα/β activation in luciferase reporter assays and induction of LXR-target genes APOE, ABCA1 and ABCG1 involved in cellular cholesterol turnover in cultured cells: methyl 3β-hydroxychol-5-en-24-oate (S1), methyl (3β)-3-aldehydeoxychol-5-en-24-oate (S2), 24-ketocholesterol (S6), (3β,22E)-3-hydroxycholesta-5,22-dien-24-one (N10) and fucosterol-24,28 epoxide (N12). These compounds induced SREBF1 but not SREBP1c-mediated lipogenic genes such as SCD1, ACACA and FASN in HepG2 cells or astrocytoma cells. Moreover, S2 and S6 enhanced cholesterol efflux from HepG2 cells. All five oxysterols induced production of the endogenous LXR agonists 24(S)-hydroxycholesterol by upregulating the CYP46A1, encoding the enzyme converting cholesterol into 24(S)-hydroxycholesterol; S1 and S6 may also act via the upregulation of desmosterol production. Thus, we identified five novel LXR-activating 24-oxidized sterols with a potential for therapeutic applications in neurodegenerative and cardiovascular diseases.

No 94

Cardiovascular risk factors and risk of non-Alzheimer's dementia

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Background and aims: Up to 40% of all dementia cases may be preventable, primarily by treating or acting on well-established cardiovascular risk factors such as diabetes, hypertension, smoking, and physical inactivity. The aim was to explore which cardiovascular risk factors are most important for risk of non-Alzheimer's dementia in a general population setting of high-risk individuals.
Methods: Investigating the association between modifiable cardiovascular risk factors and risk of non-Alzheimer's dementia (non-AD), using Cox proportional hazards regression models (with censoring at death) with age as time scale and left truncation (delayed entry), in a prospective cohort study including 14,193 individuals (54% women) from the Copenhagen City Heart Study with up to 43 years of follow-up.
Results: For women diabetes versus no diabetes, low versus high education, hypertension versus no hypertension, 1 SD increase in total cholesterol, and 1 SD increase triglycerides increased risk of non-AD both overall and at midlife with hazard ratios between 1.09-2.21. In midlife 1 SD increase in body mass index also increased risk of non-AD in women. For men diabetes versus no diabetes, low versus high physical activity, low versus high education, and high versus low alcohol intake increased risk of non-AD both overall and at midlife with hazard ratios between 1.31-2.27.
Conclusions: In a high-risk population several modifiable cardiovascular risk factors associate with increased risk of non-AD, the part of dementia enriched in cardiovascular pathology, suggesting that a healthy cardiovascular lifestyle also reduce risk of non-AD.

No 97

Effect of krill oil intervention in vivo on energy metabolism in human skeletal muscle cells

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Intake of omega-3 (n-3) fatty acids (n-3 FA) has many beneficial health effects. Positive effects of n-3 FA have been shown on the body's energy conversion, including improved lipid metabolism and prevention of obesity. The primary objective of this study was to investigate the effects of 7 weeks of krill oil supplementation in vivo (1 g/d Rimfrost Krill oil, containing n-3 FA and the antioxidant astaxanthin) on energy metabolism and substrate turnover in muscle cells in vitro, isolated from biopsies obtained before and after the intervention. Myoblasts (proliferating satellite cells) were isolated from muscle biopsies and grown in 3-4 passages. After the proliferation phase, myoblasts were differentiated into multinuclear myofibrils (myotubes) when protein expression; energy substrate uptake, and oxidation measurement in cells were performed. Protein expression was investigated using proteomic analysis. Moreover, cells isolated before and after the intervention were preincubated with palmitic acid (100 µM, 24 h), eicosapentaenoic acid (n-3 FA) (100 µM, 24 h), high glucose (HG) (20 mM, 96 h), the PPARä agonist GW501516 (100 nM, 96 h), or with insulin (100 nM, 4 h), before uptake and oxidation of [14C] oleic acid (OA), [14C]glucose or [14C]leucine were assessed. The results showed that krill oil intervention in vivo increased glucose oxidation in cultured myotubes. Furthermore, the krill oil intervention also increased OA oxidation after preincubation of muscle cells with HG or GW501516, glucose and leucine oxidation in response of EPA pretreatment, and glucose and leucine uptake after pretreatment with PA. After krill oil intervention protein expressions of insulin-like growth factor 2 mRNA-binding protein 1 and mannose-6-phosphate isomerase were upregulated in myotubes, whereas protein expression of 6-phosphogluconate dehydrogenase was downregulated. In conclusion, our results showed that 7 weeks of krill oil supplementation in healthy subjects may modify energy metabolism and protein turnover in cultured myotubes.

YIA Poster Walk – Abstracts –

Other Topics

SESSION IV

No 77

Narcolepsy drug γ-hydroxybutyric acid improves hepatic mitochondrial function to attenuate obesity

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Introduction & Aim: The narcolepsy drug γ-hydroxybutyric acid (GHB) promotes weight loss via unknown mechanisms. Here, we aimed to unveil the metabolic mechanisms underlying GHB-induced weight reduction in obesity. Furthermore, we aimed to examine whether GHB administration also prevents weight gain during the development of obesity.
Methods & Results: We investigated the role of oral GHB treatment in body weight control in high fat diet (HFD)-induced developing and existing obesity in C57Bl/6J mice. In existing obesity, but not in developing obesity, GHB attenuated HFD-induced fat mass gain, glucose intolerance and insulin resistance. However, in both metabolic conditions, GHB alleviated HFD-induced hepatic steatosis and inflammation without reducing food intake. This was accompanied by improved hepatic mitochondrial function, as evidenced by the upregulated expression of hepatic genes encoding mitochondrial respiratory complexes. In line with this, in developing obesity, GHB alleviated the accumulation of toxic sphingolipids in the liver and the circulation. In existing obesity, GHB prevented hepatic loss of retinoids and increased the level of circulating acyl-carnitine, a liver-derived substrate for brown fat combustion. Consistently, GHB alleviated HFD-induced adipose tissue dysfunction in obese mice, as evidenced by increased UCP-1 abundance in brown fat and decreased white adipocyte size and white fat inflammation.
Conclusion: Collectively, GHB promotes metabolic health in developing and existing obesity through the improvement of hepatic mitochondrial function. These findings uncover previously unknown metabolic effects of GHB related to body weight regulation, and provide novel insights into therapeutic handles for the treatment of obesity and related diseases.

No 78

Maternal overweight alters cord blood but not maternal plasma bile acid pool hydrophobicity

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Maternal nutritional status affects the circulating metabolome and, consequently, placental and fetal metabolic profiles. As the mother eliminates harmful, hydrophobic metabolites through urinary and fecal excretion, mechanisms evolved to protect the growing fetus against such substances. Thus, the fetus can detoxify excess harmful metabolites by increasing their hydrophilicity. Especially hydrophobic bile acid (BA) species possess cytotoxicity and the ability to cross the placenta. As mechanisms of detoxification, BA could be either hydroxylated or sulfated to lower their hydrophobicity. If and how maternal overnutrition affects fetal BA detoxification capacity is unknown. Thus, the current study investigated the impact of maternal overweight on cord blood BA pool composition.

Using liquid chromatography coupled to mass spectrometry, we analyzed the circulating BA pool of longitudinal plasma samples of 170 healthy pregnancies and the respective cord blood plasma. According to pre-pregnancy body mass index (BMI), the cohort was categorized into lean (BMI <25 kg/m2) and overweight (⩾25 kg/m2) females. BA hydrophobicity was calculated using Heuman indices.

Pre-pregnancy BMI had no effect on maternal and cord blood BA pool size as well as conjugated and unconjugated BA levels. Although hydrophilic hyocholic acid (HCA) species and sulfated cholic acid (CA-7S) were mostly absent in maternal plasma, cord blood from overweight women showed decreased levels of those BA species compared to lean controls. Similarly, the hydrophobicity index of the BA pool in maternal blood was also not associated with pre-pregnancy BMI. Contrary, BA determined in cord blood from obese women had a significantly higher hydrophobicity index than that from lean women.

These data imply that maternal overweight impairs fetal BA detoxification rather than increasing transplacental BA transport of hydrophilic BAs towards the fetus. Future studies are required to elucidate how maternal BMI determines fetal and placental BA detoxification capacity.

No 82

SHB is a novel regulator of insulin signaling and adipocyte function

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The ability of adipocytes to respond to insulin is crucial for systemic metabolic health. Obesity is associated with insulin resistance, which is an important risk factor for diabetic dyslipidemia and atherosclerosis. While major key nodes of insulin signaling are established, there is still a gap in understanding its fine-tuning or dysfunction. Here, we determine the role of Src Homology-2 domain containing adapter protein B (SHB), which we have identified through an epigenetic screen, for adipocyte differentiation and function.
In cultured adipocytes, we knocked down or overexpressed tagged SHB. We assessed the effects of manipulating SHB function on insulin signaling by MS-based phosphoproteomics and studied its role in adipogenesis, lipolysis, and respiration. Using insulin pathway inhibitors and pulldown proteomic analysis, we identify potential interaction partners of SHB. We use CRISPR-Cas9 mice injected with gRNA carrying AAVs against SHB to induce in vivo deletion and study energy metabolism.
Using phosphoproteomics we find that SHB depletion markedly altered the phosphoproteome affecting downstream targets of insulin signaling. Immunoblotting revealed an upregulation of the AKT/mTOR pathway and higher basal p38 MAPK signaling. Insulin-related outcomes such as lipolysis and gluconeogenesis inhibition were enhanced, and maximal respiratory capacity was upregulated. SHB depletion in preadipocytes promoted adipocyte differentiation and function in an insulin concentration-dependent manner.
Our results identify SHB as a novel negative feedback regulator of insulin signaling, which potentially implies SHB in the development of insulin resistance. Uncovering the molecular mechanisms of SHB interaction might be pivotal for our understanding of insulin action and consequently for the development of effective treatments for insulin resistance to prevent obesity-linked metabolic disease.

No 84

The oxidative stress response in obesity is mediated by adipocyte Nfe2l2

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Obesity-induced adipose tissue inflammation results in high levels of reactive oxygen species (ROS), which are known to cause cellular damage and insulin resistance. Nuclear factor, erythroid-2, like-2 (Nfe2l2, also known as Nrf2) is a transcription factor that is activated by ROS and serves as a key regulator of the anti-oxidative stress response. However, the regulation and biological significance of Nfe2l2 for oxidative stress in adipose tissue function remain unclear.
We found that adipose tissue Nfe2l2 expression is positively correlated with bodyweight and age in mice. While data suggests a role for Nfe2l2 in adipose tissue plasticity, lean mice with deletion of adipocyte Nfe2l2 by Adipoq-Cre-loxP technology had no discernible adipocyte or energy metabolism phenotype compared to wild-type (WT) controls. However, when fed with a high-fat diet (HFD) for 16 weeks to induce obesity, mice lacking Nfe2l2 in adipocytes showed lower energy expenditure compared to HFD-fed WT controls after adrenergic stimulation. In cultured adipocytes, silencing of Nfe2l2 by RNAi led to increased oxidative stress and higher vulnerability against H2O2-induced cell death. Interestingly, primary adipocytes isolated from Nfe2l2-KO mice showed higher levels of Ucp1, indicating adaptive uncoupling of the electron transport chain to reduce ROS levels and oxidative stress. To further investigate this, we performed RNAseq which showed next to the canonical targets of Nfe2l2, such as Hmox1, Nqo1 and Gsta4, also enriched pathways of fatty acid metabolism and thermogenesis. Complementary results were found after CRISPRa-mediated overexpression of Nfe2l2 or its pharmacological activation by bardoloxone methyl.
Our data highlight the critical role of Nfe2l2 in combating oxidative stress in obesity, which impacts adipocyte health and energy metabolism. Nfe2l2 and its downstream effectors modify adipocyte function and emerge as pharmacological targets for restoring cardiometabolic health in obesity.

No 91

MALRD1 variant associated with increased bile acid synthesis and hepatic cholestasis

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Cholesterol conversion to bile acids is crucial for the sterol's coordinated metabolism and abnormal levels of both are associated with cardiovascular and hepatic disorders. Therefore, bile acid synthesis is tightly regulated via an entero-hepatic crosstalk, where intestinal produced FGF15/19 is mediating a negative feedback mechanism that restrains their hepatic production. MALRD1 (or Diet1) is an intestinal expressed gene that positively regulates FGF15/19 levels. Loss of Diet1 in mice is associated with decreased ileal Fgf15 production and failure to inhibit hepatic bile acid synthesis, resulting in enhanced cholesterol conversion to bile acids. However, bile acid accumulation and impaired bile flow can lead to hepatic injury and cholestasis. To date, how MALRD1 or hypofunctional variants can affect bile acid-related hepatic diseases has not been investigated. By whole exome sequencing of a newborn infant that presented hepatomegaly and severe cholestasis, we identified a putatively pathogenic homozygous missense mutation c.2740A>G [p.(Ile914Val)] in MALRD1. Plasma analysis revealed elevated liver transaminases and hypoalbuminemia, indicating extensive liver damage. Circulating FGF19 levels as measured by ELISA were dramatically reduced in the index patient in comparison to control individuals, pointing towards inhibition of its production and/or secretion. This was further associated with a 20-fold induction of both conjugated (CBA) as well as unconjugated (UBA) bile acids in the plasma, as determined by LC-MS/MS-based approaches. All the above argue towards a dysfunctional FGF19-mediated inhibition of hepatic bile acid synthesis. In conclusion, we describe a putatively pathogenic biallelic MALRD1 variant that causes a cholestatic liver disease by dysregulating FGF19 plasma levels and leading to excess bile acid synthesis. Identification of factors that stimulate MALRD1 expression/function and sustain the FGF19-mediated inhibition of bile acid synthesis could be investigated as potential therapy for cholestatic liver diseases caused by bile acid overload.

No 93

Patients with familial hypercholesterolemia have shorter telomeres than controls in older, but not in young subjects: a cross-sectional study

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Background and aim: Familial hypercholesterolemia (FH) is a genetically inherited disease characterized by elevated LDL cholesterol (LDL-C) and a high risk for cardiovascular disease (CVD). Telomeres are repeated DNA sequences found at chromosome ends that stabilize them. Reduced telomere length is considered a biomarker for chronic disease burden and is associated with biological ageing and age-related diseases such as CVD. The aim of this study was to compare telomere length in FH patients with non-FH controls in young and older subjects.
Methods: Data and sample materials included in this project were from genetically verified FH subjects (n=110) and non-FH controls (n=56). The study population was divided into young subjects (< 30 y, n=31 FH and n=13 controls) and older subjects (> 65 y, n=79 FH and n=43 controls). We isolated whole blood DNA, estimated telomere length with singleplex RT qPCR, and reported telomere length as relative telomere to single-copy gene ratio (T/S ratio). Expression of genes involved in genome stability were measured in RNA from peripheral blood mononuclear cells (PBMC) from only the older subjects using NanoString.
Results: FH subjects had shorter telomeres, adjusted for age and BMI, compared with controls among the older subjects (p=0.001), but not in the young subjects (p=0.6). In the whole population, older subjects had shorter telomeres than young subjects (p=0.04) and there were no sex differences in telomere length (p=0.4). Carriers of an LDL receptor null mutation had similar telomere length as carriers of other LDL receptor mutations (p=0.8, adjusted for age and BMI). The gene expression of PARP1, a gene involved in telomere length regulation, was lower in older FH subjects than controls (p=0.03).
Conclusion: Telomeres were shorter in FH patients compared to controls, which was only observed among older subjects. These findings indicate that a life-long exposure to elevated LDL-C is associated with markers of biological ageing.

No 99

Development of liver-on-a-chip to study coronary artery disease -related processes

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The liver has many essential functions including synthesis of several plasma proteins and producing essential compounds of lipid metabolism such as cholesterol and bile acids. Elevated levels of lipoproteins and blood fats are among the main risk factors of coronary artery disease (CAD), a multifactorial disease commonly linked to atherosclerosis. To better understand the role of liver in atherosclerosis and CAD, our aim is to establish a 3D in vitro hepatic model also known as liver-on-a-chip, which is based on a microfluidic system that recapitulates some of the key features of liver's in vivo microenvironment. In this study, we used novel chip devices developed at the University of Gothenburg (PI Caroline Adiels), that allow for three-dimensional (3D) cell culture and media perfusion while minimizing the shear stress on the growing cells. To overcome challenges associated with primary human hepatocytes and cancer cell lines in modeling human hepatocytes, the main cell type of the liver, induced pluripotent stem cell-derived hepatocyte-like cells (iPSC-HLCs) can be used. iPSC lines are patient-specific, so iPSC- HLCs offer a platform for studying lipoprotein metabolism in, e.g., different CAD patient groups (stable/acute CAD). We differentiate HLCs by a three-step differentiation protocol. After an initial 5-day culture in 2D, the cells are combined with a hydrogel and seeded on the microfluidic chips to continue their differentiation in 3D culture conditions under continuous media flow. Based on the data from immunocytochemistry and albumin secretion assays, we were able to determine that the cells successfully differentiated towards mature hepatocytes even if the differentiation was carried out on-chip. They display liver-specific markers such as AFP, ALB and A1AT. In the future, we aim to establish similar models using iPSC lines produced from CAD patients with different disease phenotypes and healthy individuals with the goal to explore the production of lipids and lipoproteins and the effects of inflammatory factors on these in different CAD patient groups.

No 102

Telomere length and liver disease in the Danish General Population

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Background and aim: Individuals with genetically determined extremely short telomeres have a markedly increased risk of liver cirrhosis. Conversely, longer telomere length has been associated with a higher risk of various cancers. The association between telomere length and liver cancer is unknown. In this study, we tested the association between telomere length and liver cirrhosis as well as liver cancer in the general population.
Method: We combined two studies of the Danish general population: the Copenhagen General Population Study (CGPS) and the Copenhagen City Heart Study (CCHS). Average telomere length was available in a total of 63,324 individuals. We defined cirrhosis and liver cancer cases based on ICD codes in the Danish national registries. We tested whether telomere length was associated with alanine transaminase (a biochemical marker of liver cell damage) using linear regression. The associations between telomere length and cirrhosis and liver cancer were tested with restricted cubic splines and Cox regression.
Results: Longer telomere length was associated with slightly higher alanine transaminase (Beta = 4 x 10-6 log[alanine transaminase] per base pair; P-value = 0.058). During a median follow-up of 11 years, 241 and 76 individuals were diagnosed with cirrhosis and liver cancer, respectively. Telomere length correlated inversely and linearly with the risk of cirrhosis (P-value = 0.004, P for non-linearity = 0.27). For liver cancer, the association was U-shaped, with an elevated risk in individuals either below or above the reference level of 3,397 base pairs (P-value = 0.009, P-value for non-linearity = 0.01). When we compared the quartile with the shortest telomeres to the quartile with the longest, there was a 2.2-fold and 2.3-fold higher risk of cirrhosis and liver cancer, respectively.
Conclusion: Telomere length correlated inversely and linearly with the risk of cirrhosis whereas the association with liver cancer was U-shaped.

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