30th Annual Scandinavian Atherosclerosis Conference

March 13–16, 2024 at Krogerup Højskole, Humlebæk, Denmark

2024 Program

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Anna-Kaisa Ruotsalainen (Finland)
Tuva Dahl (Norway)
Takahito Doi (Denmark)
Jeanine Roeters van Lennep (Netherlands)
Liv Nordestgaard (Denmmark)
Matteo Pedrelli (Sweden)
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Organized by

SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH

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Wednesday, March 13, 2024

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 2024 Nikkilä Lecturer **Anna-Kaisa Ruotsalainen** *(Finland)*
19.40 – 20.25	*2024 Nikkilä Lecture* Role of SOAT2 and cholesterol esterification in lipoprotein metabolism … and for much more **Paolo Parini** *(Sweden)*
20.25 – 20.45	Discussion
20.45 –	Pub will be open

Thursday, March 14, 2024

07.30 – 08.30	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Chaired by **Anna-Kaisa Ruotsalainen** *(Finland)* and **Tuva Dahl** (*Norway*)
08.30 – 09.00	*Invited speaker* Neural Control of Inflammation and Cardiovascular Disease **Peder Olofsson** *(Sweden)*
09.00 – 09.15	Hypoxia drives the progression of a pro-atherogenic arterial extracellular matrix that may be attenuated by heparin **Christine Chuang** *(Denmark)*
09.15 – 09.30	Autoimmune diseases, coronary atherosclerosis severity and cardiovascular disease events: The Western Denmark Heart Registry **Martin Bødtker Mortensen** *(Denmark)*
09.30 – 09.45	The Polycomb Repressive Complex 2 (PRC2) in Macrophages and Atherosclerosis **Annette Neele** *(Netherlands)*
09.45 – 10.00	First genome-wide association study of plasma transthyretin concentration **Mette Christoffersen** *(Denmark)*
10.00 – 10.45	**Poster Walk (Session I)** Coffee and tea
10.45 – 11.15	*Invited speaker* Fine-tuning the macrophage's inflammatory potential **Marten Hoeksema** *(Netherlands)*
11.15 – 11.30	Spatial proteomics of the human atherosclerotic microenvironment **Luke Gamon** *(Denmark) - YIA*
11.30 – 11.45	Genetic variation in SLC39A8 increases the risk of hepatocellular carcinoma in the Danish general population **Anne-Sofie Seidelin Helweg Rasmussen** *(Denmark) - YIA*
11.45 – 12.00	Extracellular lipoproteins from carotid endarterectomy samples activate different pathways in human macrophages than native and in-vitro modified LDL and VLDL **Martina Lorey** *(Finland)*
12.00 – 13.00	Lunch

SESSION II	**CARDIOVASCULAR DISEASE** Chaired by **Takahito Doi** (*Denmark*) and **Jeanine Roeters van Lennep** (*Netherlands*)
13.00 – 13.30	*Invited speaker* Cardiovascular disease in women; Lessons learned from epidemiological studies **Maryam Kavousi** *(Netherlands)*
13.30 – 13.45	Remnant cholesterol reduction of 2 mmol/L likely reduces absolute 10-year risk of atherosclerotic cardiovascular disease by 6-17% in primary prevention **Karen Hvid** *(Denmark) - YIA*
13.45 – 14.00	Age-associated B cells are clonally expanded, aggravate murine plaque development and are associated with coronary events in humans **Pernilla Katra** *(Sweden) - YIA*
14.00 – 14.15	Testosterone and male sex exacerbate neutrophilia and cardiac injury in acute myocardial infarction **Åsa Tivesten** *(Sweden)*
14.15 – 14.30	Brown seaweed extracts in the prevention of aortic valve stenosis **Dieter Lütjohann** *(Germany)*
14.30 – 15.30	**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)
16.00 – 17.00	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
18.00 – 19.00	Dinner
SESSION II	**CARDIOVASCULAR DISEASE – continued** Chaired by **Takahito Doi** (*Denmark*) and **Jeanine Roeters van Lennep** (*Netherlands*)
19.00 – 19.30	*Invited speaker* Immunopathogenesis of atherosclerosis and its adaptation to cardiovascular medicine **Göran K. Hansson** *(Sweden)*
19.30 – 20.30	**Poster Walk (Session II)** Coffee and tea
20.30 – 20.45	Low Density Lipoprotein Cholesterol and Cardiovascular Disease Risk in Patients with Absence of Coronary Artery Calcification: The Western Denmark Heart Registry **Malene Højgaard Andersen** *(Denmark) - YIA*
20.45 – 21.00	Designer cytokine IC7Fc attenuates atherosclerosis development by targeting hyperlipidemia and bile acid metabolism in mice **Milena Schönke** *(Netherlands) - YIA*

21.00 – 21.15	Impaired renal function with elevated remnant cholesterol related to risk of atherosclerotic cardiovascular disease: The Copenhagen General Population Study **Daniel Elías-López** *(Denmark)*
21.15 – 21.30	Inhibition of NNMT protects against dyslipidemia dependent on LDL receptor and attenuates atherosclerosis in mice **Kaiming Yue** *(Netherlands) - YIA*
21.30 –	Pub will be open

Friday, March 15, 2024

07.30 – 08.30	Breakfast
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Chaired by **Liv Nordestgaard** *(Denmark)* and **Matteo Pedrelli** *(Sweden)*
08.30 – 09.00	*Invited speaker* Lipoprotein(a) in cardiovascular disease **Børge Nordestgaard** *(Denmark)*
09.00 – 09.15	Hepatic disruption of Abca6 increases circulating non-HDL-cholesterol by decreasing hepatic uptake of VLDL remnants **Xiaoke Ge** *(Netherlands) - YIA*
09.15 – 09.30	Lipoprotein(a) cardiovascular disease risk not captured by low density lipoprotein cholesterol and apolipoprotein B **Peter Engel Thomas** *(Denmark) - YIA*
09.30 – 09.45	Pharmacological induction of mitochondrial uncoupling outside brown adipose tissue attenuates atherosclerosis development in APOE*3-Leiden.CETP mice **Jamie van der Vaart** *(Netherlands) - YIA*
09.45 – 10.00	Polygenic risk of high LDL cholesterol and ischemic heart disease in the general population **Tim Eyrich** *(Denmark) - YIA*
10.00 – 11.00	**Poster Walk (Session III)** Coffee and tea
11.00 – 11.30	*Invited speaker* Cytosolic and lysosomal lipid hydrolysis in atherosclerosis: insights from lipase-deficient mouse models **Dagmar Kratky** *(Austria)*
11.30 – 11.45	A rare gain-of-function variant of hepatic lipase attenuates hypercholesterolemia and atherosclerosis in mice independent of the LDL receptor **Patrick Rensen** *(Netherlands)*
11.45 – 12.00	High lipoprotein(a) as a cause of chronic kidney disease: a population-based Mendelian randomization study **Børge Nordestgaard** *(Denmark)*
12.00 – 12.15	Mitochondrial fatty acid synthesis is essential for coordinated energy transformation **Ioannis Evangelakos** *(Germany) - YIA*
12.15 – 13.15	Lunch

SESSION IV	**OTHER TOPICS** Chaired by **Jiao Luo** *(Denmark)* and **Peter Saliba Gustafsson** *(Sweden)*
13.15 – 13.45	*Invited speaker* Inhibition of VEGF-B signaling prevents non-alcoholic fatty liver disease development by targeting lipolysis in the white adipose tissue **Annelie Falkevall** *(Sweden)*
13.45 – 14.00	Repurposing statin treatment for the prevention of gallstone disease **Søren Nicolaj Rønborg** *(Denmark) - YIA*
14.00 – 14.15	Ube2h regulates insulin signaling in adipocytes via Akt phosphorylation **Lukas Blaas** *(Germany) - YIA*
14.15 – 14.30	Efficacy and safety of PCSK9 monoclonal antibodies in older adults: a real-world single center cohort study **Janneke Mulder** *(Netherlands) - YIA*
14.30 – 14.45	Adipokines mediating stress-dependent adipose-liver endocrine crosstalk **Christoph Gibis** *(Germany) - YIA*
14.45 – 15.45	**Poster Walk (Session IV)** Coffee and tea
15.45 – 16.15	*Invited speaker* The genetics of obesity **Ruth Loos** *(Denmark)*
16.15 – 16.30	Integrative common and rare variant analyses provide insights into the genetic architecture of liver cirrhosis **Stefan Stender** *(Denmark)*
16.30 – 16.45	Deletion of the murine ortholog of human 9p21.3 CAD risk locus leads to insulin resistance and obesity in mice **Sanna Kettunen** *(Finland) - YIA*
16.45 – 17.00	Circulatory hsa-miR-339-5p is associated with later development of metabolic dysfunction-associated steatotic liver disease (MASLD) in the prospective Young Finns Study **Daria Kostiniuk** *(Finland) - YIA*
17.00 – 17.15	Remnant cholesterol, not LDL cholesterol, explains peripheral artery disease risk conferred by apolipoprotein B: a cohort study **Benjamin Wadström** *(Denmark) - YIA*
17.15 - 17.20	Concluding remarks **Anne Langsted** *(Denmark)*
18.30 – 19.00	**Cocktail**
19.00 –	**Banquet and dancing**

Saturday, March 16, 2024

08.30 – 10.00	Breakfast and departure

Safe travels and see you next year

30th Annual Scandinavian Atherosclerosis Conference

March 13–16, 2024 at Krogerup Højskole, Humlebæk, Denmark

2024 Posters

Thursday, March 14, 2024

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. 11	Dysregulation of micro-RNA 143-3p as a biomarker of carotid atherosclerosis and the associated immune reactions during disease progression **Anton Gisterå** *(Sweden)*
No. 02	Blockage of endoglin prevents endothelial dysfunction development in type 2 diabetic coronary artery endothelial cells **Martina Vasinova** *(Czechia)*
No. 06	Oxidative modification of histones in "NETs" – a new mechanism of cellular dysfunction in atherosclerosis? **Clare Hawkins** *(Denmark)*
No. 14	Degradomics analysis of human atherosclerotic plaques **Lasse Lorentzen** *(Denmark)*
YIA Poster walk I **10.00 – 10.45**	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 01	The Immunometabolic Role of Pyruvate Dehydrogenase Kinase-1 in Smooth Muscle Cells in Atherosclerosis **Andrietta Grentzmann** *(Denmark)*
No. 10	Adipose Tissue's Eosinophils and Macrophages - The Untold Story Exposed **Karel Paukner** *(Czechia)*
No. 13	Intensive periodontal treatment reduces serum-induced activation of macrophages **Anni Niemelä** *(Finland)*
No. 09	Human atherosclerotic plaques contain oxidant-modified proteins **Karen Yang-Jensen** *(Denmark)*
No. 05	Role of selenocyanate to modulate cellular damage resulting from chronic inflammation in atherosclerosis **Els Hartsema** *(Denmark)*
SESSION II	**CARDIOVASCULAR DISEASE**
No. 24	Ex vivo culture of atherosclerotic plaques provides a new model for the study of plaque stability **Sara Marthedal Jørgensen** *(Denmark)*
No. 16	Lipoprotein(a) during early life course in girls and boys with genetically verified familial hypercholesterolemia **Kirsten Holven** *(Norway)*

YIA Poster walk II 19.30 – 20.30	Selected abstracts (3 min presentation + 2 min discussion)
No. 17	Saturated fatty acids and total and cardiovascular disease mortality in Norway: A prospective cohort study with up to 45 years of follow-up **Erik Arnesen** *(Norway)*
No. 19	Excess apoB in risk of cardiovascular disease and mortality in women and men **Camilla Johannesen** *(Denmark)*
No. 21	LDL-cholesterol burden in elderly patients with familial hypercholesterolemia: insights from real-world data **Torunn Melnes** *(Norway)*
No. 22	A Comprehensive Analysis of Women's Participation in Cardiovascular Trials **Marte van der Bijl** *(Netherlands)*
No. 25	DNA methylation differences between Eastern- and Western-originating Finns **Joanna Ciantar** *(Finland)*
No. 28	Long-term prognosis of unrecognised myocardial infarction in women and men from the general population: The Rotterdam Study **Julianne Van Oortmerssen** *(Netherlands)*
No. 31	High intake of omega-3 fatty acids reduces the odds to subclinical atherosclerosis in German descendants living in Brazil: SHIP-Brazil Study **Ribanna Braga** *(Brazil)*

Friday, March 15, 2024

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SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT**
No. 32	Empowering In-depth definition of Simvastatin and Ezetimibe Effects in Humans by Intelligible Heterogeneous Networks **Paolo Parini** *(Sweden)*
No. 38	What happens to Lp(a) when stored in a freezer for many years? Longitudinal analyses of the HUNT Study **Sigrid Blom** *(Norway)*
No. 39	Leukocyte lipid uptake and storage profiles along the lipid-lowering treatment journey: an observational study **Iryna Hlushchenko** *(Finland)*
No. 46	Apolipoprotein M and incident cardiovascular events in patients with chronic kidney disease **Julia Stadler** *(Austria)*
No. 48	Lipoprotein metabolism and inflammation in healthy young subjects – exploring the postprandial and postabsorptive phases following intake of a standardized meal **Jacob J Christensen** *(Norway)*
No. 49	Altered Functionality of Lipoprotein(a) Impacts on Angiogenesis in Diabetic Retinopathy erative Diseases **Monique Mulder** *(Netherlands)*
No. 73	A Novel Flag Epitope Tag Knock-In ApoB Mouse Model to Investigate Intracellular ApoB/VLDL Biology **Jan Albert Kuivenhoven** *(Netherlands)*
YIA Poster walk III **10.00 – 11.00**	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 36	SMLR1 is a novel player in chylomicron metabolism **Ankia Visser** *(Netherlands)*
No. 37	Vacular H+-ATPase as a novel target to lower low density lipoprotein cholesterol **Na Wang** *(Netherlands)*
No. 41	Sex differences of Lp(a) in individuals aged 18-50 years – a nationwide study of 185,000 individuals **Janeni Jeevanathan** *(Norway)*

No. 45	Effects of Intermittent Fasting on HDL Function in Individuals with Type 2 Diabetes Mellitus **Anja Pammer** *(Austria)*
No. 50	Deficiency for VEGF-D leads to delayed intestinal chylomicron release and lipid build-up to enterocytes in hyperlipidaemic mice **Krista Hokkanen** *(Finland)*
No. 44	The choice of lipoprotein(a) immunoassay can affect clinical decisions **Janeni Jeevanathan** *(Norway)*
SESSION IV	**OTHER TOPICS**
No. 54	N-acyl taurine as a regulator of adipose tissue function in obesity-related metabolic disease **Katharina Kuentzel** *(Denmark)*
No. 53	Polyunsaturated fatty acid metabolites in cardiometabolic disease **Trisha Grevengoed** *(Denmark)*
No. 60	Quantification of Hospitalization or Outpatient Consultations Caused by Pre-Selected Diagnostic Codes in Individuals with Familial Hypercholesterolemia (FH) **Kjetil Retterstøl** *(Norway)*
No. 61	Cardiovascular risk factors and risk of vascular-related dementia in high-risk and low-risk prospective cohorts **Ida Juul Rasmussun** *(Denmark)*
No. 68	Identifying biomarkers of fat-specific dietary patterns using a multi-omics approach **Jacob J Christensen** *(Norway)*
YIA Poster walk IV **14.45 – 15.45**	**Selected abstracts (3 min presentation + 2 min discussion)**
No. 51	Regulation of visceral adipose tissue inflammation and metabolism by signals in the vagus nerve **Ting Liu** *(Sweden)*
No. 55	Lifestyle characteristics and plasma biomarkers for risk of NAFLD differ by sex in the general population **Lærke Kristine Kyhl** *(Denmark)*
No. 58	Large-scale gene-age interaction analyses of cardiometabolic risk factors in 270,276 Europeans **Linjun Ao** *(Netherlands)*
No. 59	Obesity and risk of delirium during hospitalization: 109,117 individuals from the general population **Tina Segerberg** *(Denmark)*
No. 63	Expression and characterization of modified recombinant human acid sphingomyelinase variants for LDL aggregation assay

Alina Iakubovskaia *(Finland)*

No. 52 The lifestyle and cardiovascular risk factors in Norwegian patients with a severe mental illness
Madeleine Elisabeth Angelsen *(Norway)*

No. 69 Tspan18 regulates preadipocyte expansion during adipogenesis
Theresa Auer *(Germany)*

Oral Presentations – Abstracts –

Inflammation and Vascular Biology

SESSION I

Hypoxia drives the progression of a pro-atherogenic arterial extracellular matrix that may be attenuated by heparin

Sara M. Jørgensen1, Song Huang1, Lasse G. Lorentzen1,2, Laura Gonzalez Requeson1, John R. Harkness1, Michael J. Davies1, Christine Y. Chuang1

1. Department of Biomedical Science, University of Copenhagen, Copenhagen, Denmark

2. Vascular Research Unit, Department of Vascular Surgery, Rigshospitalet, Copenhagen, Denmark.

Atherosclerosis is the major underlying cause of cardiovascular disease, with this characterized by the accumulation of lipids and activated leukocytes. These cells release myeloperoxidase (MPO), resulting in localised damage to the arterial extracellular matrix (ECM), endothelial cell dysfunction, inflammation and hypoxia. Hypoxia has been shown to drive further ECM modifications which increases the risk of lesion rupture and stroke. Pilot data indicate that infusion of heparin, into humans with cardiovascular disease, results in higher plasma MPO levels, probably via release of MPO from the artery wall, and improved endothelial function. Thus, the aim of this study was to examine whether exposure of human coronary artery endothelial cells (HCAEC) to 1% versus 20% O2 gives rise to an altered ECM composition, which modulates HCAEC behaviour, increases hyaluronan and MPO binding, and whether heparin can promote MPO removal, thereby minimising damage.

Exposure of HCAECs to 1% compared to 20% O2 altered mRNA expression of genes involved in endothelial dysfunction (eNOS) and inflamation (ICAM-1 and IL-6) and the synthesis of ECM components, in particularly the proteoglycan versican and associated matrix enzymes. This versican-rich ECM resulted in reduced HCAEC adhesion, enhanced proliferation, increased binding of both hyaluronan (an ECM component that interacts with versican in lesions) and MPO (which generates damaging oxidants and whose concentration correlates with disease severity). However, MPO binding and its generated damage was attenuated upon treatment with heparin.

These data indicate that exposure of HCAEC to 1% versus 20% O2, alters ECM composition. The increased synthesis of versican and its subsequent binding of MPO may exacerbate the progression of atherosclerosis, as this proteoglycan is a well-established binding site for lipoproteins. However, heparin treatment may be a therapeutic approach to disrupt MPO binding and subsequent damage.

Autoimmune diseases, coronary atherosclerosis severity and cardiovascular disease events: The Western Denmark Heart Registry

Martin Bødtker Mortensen MD PhD1,2, Jesper Møller Jensen MD PhD1, Niels Peter Rønnow Sand MD PhD3, Kristian Kragholm MD PhD4, Michael J. Blaha MD MPH2, Erik Grove MD PhD1, Henrik Toft Sørensen MD DMSc5 Kevin Olesen MD PhD1, Michael Maeng MD PhD1,6, Brian Løgstrup MD PhD1, Martin Busk MD PhD7, Ellen Margrethe Hauge MD PhD6,8, Ann Marie Navar MD PhD9, Hans Erik Bøtker MD DMSc1, Bjarne Linde Nørgaard MD PhD1

1Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark 2Johns Hopkins Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States. 3Department of Cardiology, University Hospital of Southwest Jutland and Institute of Regional Health Research, University of Southern Denmark, Denmark. 4Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark. 5Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark. 6Department of Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark. 7Department of Cardiology, Lillebaelt hospital, Vejle, Denmark. 8Department of Rheumatology, Aarhus University Hospital, Aarhus, Denmark. 9 Division of Cardiology, University of Texas Southwestern Medical Center, Dallas.

Background: Some autoimmune diseases carry elevated risk for atherosclerotic cardiovascular disease (ASCVD), yet the underlying mechanism and the influence of traditional risk factors remain unclear.
Objectives: To determine whether autoimmune diseases independently correlate with coronary atherosclerosis and ASCVD risk, and whether traditional cardiovascular modulate the risk.
Methods: 85.512 patients from The Western Denmark Heart Registry undergoing coronary computed tomography angiography. A diagnosis of one of 18 autoimmune diseases were assessed. Adjusted odds ratios (aOR) for any plaque, any coronary artery calcification (CAC), CAC>90th percentile, and obstructive coronary artery disease (CAD) and adjusted hazard ratios (aHR) for ASCVD were calculated.
Results: During 5.3 years of follow-up, 3832 ASCVD events occurred. A total of 4.064 patients had a diagnosis of autoimmune disease which was associated with both presence of any plaque (aOR 1.29 [1.20-1.40]), any CAC (aOR 1.28 [1.19-1.37])) and severe CAC>90th percentile (aOR 1.53 [1.39-1.68]), but not with having obstructive CAD (1.04 [0.91-1.17]). Patients with autoimmune diseases had a 46% higher risk (aHR 1.46 [1.29-1.65]) for future ASCVD events. Traditional cardiovascular risk factors were strongly associated with future ASCVD events, and a favorable cardiovascular risk factor profile in autoimmune patients was associated with ≈54% lower risk (aHR 0.46 [0.27-0.81].
Conclusions: Autoimmune diseases were independently associated with higher burden of coronary atherosclerosis and higher risk for future ASCVD events, with risk accentuated by traditional cardiovascular risk factors. These findings suggest that autoimmune diseases increase risk through accelerated atherogenesis, and that cardiovascular risk factor control is key for improving prognosis in patients with autoimmune diseases.

The Polycomb Repressive Complex 2 (PRC2) in Macrophages and Atherosclerosis

Rosalie W.M. Kempkes (1), Lea Rief (1), Cindy P.A.A. van Roomen (1), Marion J. Gijbels (1,2,3), Guillermo Griffith (1), Winnie G. Vos (1), Laura A. Bosmans (1), Hung-Jen Chen (1), Marten A. Hoeksema (1), Lisa Willemsen (1), Koen H.M. Prange (1), Menno P.J. de Winther (1), Annette E. Neele (1)

1. Department of Medical Biochemistry, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Center, University of Amsterdam, the Netherlands.

2. Department of Pathology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, the Netherlands.

3. Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center, Maastricht, the Netherlands.

Aim

Since epigenetic processes are crucial in controlling macrophage inflammatory responses, interfering with epigenetic pathways might be a novel approach to combat atherosclerosis. Histone H3K27 trimethylation is a repressive histone mark catalyzed by the Polycomb Repressive Complex 2 (PRC2). We here studied macrophage EZH2 & JARID2, both part of the PRC2 complex, in macrophage activation and atherosclerosis.

Methods

Human monocyte-derived macrophages were treated with the EZH2 inhibitor GSK126 and were subsequently activated with LPS. The impact of EZH2i on macrophage activation was assessed by RNA-seq, ChIP-seq, FACS and ELISA. Additionally, female Ldlr-/- mice on a HCD were treated with GSK126 or vehicle to assess the impact on atherosclerosis. A Myeloid specific Jarid2 deficient mouse strain was generated (LysM-Cre+ Jarid2-fl/fl) to study Jarid2.

Results

EZH2 inhibition lowered global H3K27Me3 levels without altering macrophage viability and differentiation, showing effective EZH2 inhibition. RNA-seq revealed that of more than one-third of the LPS-induced genes were significantly downregulated by EZH2i. Subsequent pathway analysis identified cytokine, interferon signaling and co-stimulation as the top down-regulated pathways. Indeed, cytokine secretion of the inflammatory mediators IL-6, IL-12, and TNF were reduced and membrane expression of the co-stimulatory CD40, CD80, and CD86 were also decreased. We are currently assessing lesion size and composition of GSK126 treated Ldlr-/- mice. To assess the effects of JARID2 as important co-factor of the PRC2 complex, we study Jarid2-del BMDMs and use JARID2 siRNA's in human macrophages.

Conclusions

EZH2 inhibition significantly reduces inflammatory responses in both human and mouse macrophages, indicating its potential as a therapeutic approach targeting inflammation in atherosclerosis. We are currently assessing the effects of Ezh2 inhibition on atherosclerotic lesion size and phenotype in Ldlr-/- mice.

First genome-wide association study of plasma transthyretin concentration

Mette Christoffersen (1), Anders Møller Greve (1), Anne Tybjærg-Hansen (1)

(1)Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

Background:
In plasma, transthyretin circulates as a tetramer with the main function to transport retinol-binding protein and a fraction of thyroxine. Older age, female
sex and inflammation are associated with lower concentrations of plasma transthyretin, while high alcohol consumption and elevated triglyceride levels
are associated with higher concentrations. Aside from rare, structural genetic variants in the TTR gene which affect transthyretin tetramer stability and
thereby have a secondary effect on plasma transthyretin concentration, the genetic determinants of plasma transthyretin concentration are largely
unknown. We aimed to conduct a genome-wide association study (GWAS) to reveal novel genetic determinants of plasma transthyretin concentration.
Methods:
We genotyped and measured plasma transthyretin concentrations in 17,000 individuals from two studies of the Danish general population, the
Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS). We first performed GWAS' on plasma transthyretin
concentrations in the CCHS and CGPS separately. Second, we meta-analyzed results from the two studies.
Results:
The GWAS' from the CCHS and CGPS both confirmed the TTR locus as a genetic determinant of plasma transthyretin concentrations (P=3*10-24). The
meta-analysis of the two studies further revealed two novel loci mapped to the CYP4V2 and MAF genes (P=4*10-9 and P=3*10-12). CYP4V2 is known to
be involved in fatty acid metabolism in the eye and was previously identified in a GWAS of brain amyloid deposition. MAF was previously identified in the
first GWAS of thyroid-stimulating hormone, and has since been associated to thyroid volume, hyperthyroidism, and thyroid function.
Conclusions:
We conducted the first GWAS' of plasma transthyretin concentration. The studies confirmed the TTR locus, and a meta-analysis further revealed two
novel genome-wide significant loci previously associated with ocular lipid metabolism and thyroid function.

Genetic variation in SLC39A8 increases the risk of hepatocellular carcinoma in the Danish general population

Anne-Sofie Seidelin1,3, Børge Grønne Nordestgaard2,3, Anne Tybjærg-Hansen1,3, Stefan Stender1,3

1Department of Clinical Biochemistry, Rigshospitalet; 2Department of Clinical Biochemistry, Herlev and Gentofte Hospital; 3Copenhagen University Hospitals and Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

Background and Aims
Manganese is an important co-factor for numerous biological processes. A common variant in the manganese transporter SLC39A8 (p.Ala391Thr, rs13107325C>T) has been associated with lower blood levels of manganese, and with increases in markers of liver cell damage. We hypothesize that SLC39A8 p.Ala391Thr associates with increased risk of liver disease in the general population.

Methods
We included n=117,074 white participants from the Copenhagen City Heart Study and the Copenhagen General Population Study combined, and n=334,886 white participants from the UK Biobank. We tested associations of SLC39A8, p.Ala391Thr with biochemical and imaging markers of liver disease, risk of liver disease, and liver-related and all-cause mortality.

Results
Biochemically, SLC39A8 p.Ala391Thr was associated with higher levels of alanine transaminase and aspartate transaminase, and with lower levels of albumin and gamma-glutamyl transferase. On imaging, p.Ala391Thr was associated with higher corrected T1 on MRI (P = 1×10^-133), a presumed marker of inflammation and fibrosis, lower hepatic CT attenuation (P=0.04), but not with MRI-determined hepatic fat content (P=0.50). In the Copenhagen cohort, p.Ala391Thr was associated with increased risk of hepatocellular carcinoma (per-allele odds ratio = 1.89, 95 % confidence interval (CI): 1.19-2.98; P = 0.006), and heterozygotes had an increased risk of liver-related mortality (hazard ratio = 1.68, 95 % CI: 1.13-2.48; P = 0.007) compared with noncarriers.

Conclusion
SLC39A8 p.Ala391Thr was biochemically associated with markers of liver cell damage, inflammation, and fibrosis, and with increased risk of developing liver cancer and dying from liver disease in the general population.

Extracellular lipoproteins from carotid endarterectomy samples activate different pathways in human macrophages than native and in-vitro modified LDL and VLDL.

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Carotid atherosclerosis is driven by the accumulation of extra- and intracellular lipid deposits that are derived from lipoproteins (LPs) modified by various enzymes. Macrophages are key mediators of inflammation in developing plaques, and their number and activation state changes during progression and regression of atherosclerosis. This study aims to better understand how LPs modified during atherogenesis contribute to macrophage activation. We isolated extracellular LPs (ExtLPs) from human carotid endarterectomy samples and characterized them using proteomics and lipidomics. Macrophages were incubated with the ExtLPs and native, oxidized, phospholipase A2 (PLA2)-treated, and PLA2 and cholesterol esterase (CEase)-treated LDL and VLDL. We then isolated the macrophage RNA for transcriptomics and performed pathway analysis. ExtLPs, oxidized LPs, PLA2- and PLA2-Cease-treated LDL induced robust secretion of the pivotal atherogenesis chemokine interleukin-8. Transcriptomics revealed a unique activation of macrophages upon stimulation with ExtLPs akin to exposure to divalent metal ions, as well as ferroptosis, an iron-dependent form of necrosis characterized by oxidative damage to cellular phospholipids, and cardiovascular signaling. This response to metal ion is striking, however, metal ions are known to accumulate in plaques, and they could attach to LPs during purification. The cellular response to modified LPs depended on the modification type: Oxidized LPs induced most differentially expressed genes and a variety of inflammatory responses. PLA2-treated VLDL induced a more pronounced inflammatory response than PLA2-treated LDL despite a similar degree of lipolysis. PLA2-Cease-treated LDL induced a robust cytokine response and increased PPAR signaling. Thus, ExtLPs, which are multiply modified and can contain cholesterol crystals, induce a somewhat different cellular response compared to traditionally used, in-vitro modified LPs.

Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION II

Age-associated B cells are clonally expanded, aggravate murine plaque development and are associated with coronary events in humans

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Aging is a major driver of atherosclerosis and also causes a functional decline of the immune system. However, the effect of age-related changes in B cell immune responses on atherosclerosis development and human cardiovascular disease are not fully elucidated.

In this study we used single cell RNAseq to map out the splenic B cell compartment in aged Ldlr-/- mice, coupled with BCRseq and adoptive transfer experiments. Additionally, we analysed circulating B cells in a cohort consisting of middle-aged and older individuals (N=602) and investigated associations to incident coronary events.

We found an expansion of CD21low age-associated B cells (ABCs) in aged (21 months) Ldlr-/-mice when compared to young Ldlr-/- mice (5 months). This was mirrored by expansion of similar CD21low cells in older ($\geqslant$66 years), compared to middle-aged ($\leqslant$52 years) individuals in the human cohort, P=0.002. Coronary event cases had higher counts of CD21low cells than controls, P=0.006 and regression analysis revealed an association to incident coronary events, independent of cardiovascular risk factors, odds ratio 1.80 (95% CI 1.05-3.07), P=0.032, comparing the 4th vs 1st quartile. Additionally, transferred ABCs increased atherosclerosis development in recipient Rag1-/-Ldlr-/- mice, P<0.05, coupled with increased necrotic core size, P<0.05. BCRseq revealed that the ABCs were clonally expanded in aged Ldlr-/- mice with a high degree of IgG class switching and also shared clonotypes with plasma cells. Adoptive transfer showed that ABCs differentiated into antibody secreting plasma cells, which were capable of homing to the bone marrow.

In conclusion, our study highlights ABCs as important players in the disease pathology of murine atherosclerosis, while also showing that high counts of CD21low cells are associated with incident coronary events in humans.

Brown seaweed extracts in the prevention of aortic valve stenosis

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Aim: The pathogenesis of aortic valve stenosis (AS) features initial endothelial dysfunction and inflammation followed by myofibroblastic and osteoblastic differentiation of valvular interstitial cells (VIC) leading to calcification and degeneration of the aortic valve. Brown seaweed Sargassum fusiforme (S. fusiforme) contains 24S-saringosterol, an agonist of the liver-X-receptor β (LXRβ). LXRβ is a transcription factor that plays a crucial role in lipid metabolism and in a variety of chronic inflammatory diseases.
Methods: Experimental AS developed in an established mouse model after wire-induced injury of the aortic valve. Haemodynamic changes and the severity of AS were assessed using echocardiography. Experimental AS animals were fed with either S. fusiforme extract or vehicle enriched chow. Histological sections through the aortic valves were stained to assess the cell types and extracellular matrix. Human aortic valve interstitial cells (VIC) were used to study the underlying molecular mechanisms of 24S-saringosterol treatment.
Results: In a murine model, treatment with S. fusiforme extract resulted in significant accumulation of 24S-saringosterol in liver tissue and an upregulation of downstream-targets of LXR was observed. Increased aortic valve peak velocity was attenuated in AS mice receiving S. fusiforme extract, accompanied by a reduction in valve area by hematoxylin & eosin staining. In vitro, 24S-saringosterol dose-dependently increased the transcription of ABCA1 and ABCG1 in VIC. Furthermore, 24S-saringosterol reduced the expression of RUNX-2 and ACTA-2 in procalcifing medium, suggesting less differentiation into osteoblastic and myofibroblastic phenotypes, respectively.
Conclusion: To our knowledge, the present study is the first to describe LXR signalling to be causally involved in both human and experimental aortic valve stenosis. Oral application of S. fusiforme extract attenuated aortic stenosis in vivo. LXR activation prevented adverse cell differentiation in vitro. Upregulation of LXR-associated pathways in human aortic valve stenosis may represent an escape mechanism and shows the therapeutic potential of S. fusiforme extract

Low Density Lipoprotein Cholesterol and Cardiovascular Disease Risk in Patients with Absence of Coronary Artery Calcification: The Western Denmark Heart Registry

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Aim: LDL-C plays a central role in the development of coronary heart disease (CHD). Absence of coronary artery calcification (CAC), as assessed by computed tomography (CT), is associated with a favorable outcome as it indicates a low burden of atherosclerosis. However, questions remain regarding the risk of non-calcified plaque and CHD in younger patients with the absence of CAC, as atherosclerosis tends to be non-calcified in earlier stages. We hypothesized that LDL-C is associated with the presence of non-calcified plaque and future CHD events in young patients with CAC zero.

Methods: We included 24,365 patients from Western Denmark Heart Registry who underwent CT and had a CAC score of zero. We assessed the association of LDL-C with presence of non-calcified plaque on CCTA and the risk for myocardial infarction and CHD across different age groups.

Results: Median follow-up time was 5.5 years. A total of 168 (0.7%) and 448 (1.8%) experienced myocardial infarction and CHD, respectively. Mean age was 54 years and the prevalence of non-calcified plaque was 11.2%. Higher LDL-C was associated with presence of non-calcified plaque with an odds ratio of 1.21 (95% CI 1.16-1.27) per 1 mmol/L higher LDL-C. Individuals aged <45 years had a higher odds ratio for non-calcified plaque per 1 mmol/L higher LDL-C compared to individuals aged 46-60 and >60 years; 1.36 (95% CI 1.22-1.52), 1.22 (95% CI 1.15-1.30) and 1.13 (95% CI 1.04-1.22), respectively. LDL-C was associated with higher risk for myocardial infarction and CHD despite CAC=0, with hazard ratios of 1.32 (95% CI 1.13-1.54) and 1.27 (95% CI 1.15-1.40) per 1 mmol/L higher LDL-C.

Conclusions: In younger patients with absence of CAC, elevated LDL-C is associated with presence of non-calcified plaque and increased risk for future cardiovascular events. These data are important for clinical practice, as they demonstrate the importance of managing LDL-C in younger individuals despite absence of coronary calcification.

Designer cytokine IC7Fc attenuates atherosclerosis development by targeting hyperlipidemia and bile acid metabolism in mice

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Background & aim: The chimeric designer cytokine IC7Fc conveys the metabolic signaling properties of the gp130 receptor cytokines interleukin 6 (IL-6) and ciliary neurotrophic factor (CNTF) without inducing a pro-inflammatory response. IC7Fc was previously shown to reduce obesity and slow the progression of type 2 diabetes mellitus. Here we aimed to delineate its effect on atherosclerosis development and established the underlying molecular mechanism.

Methods & results: In APOE*3-Leiden.CETP mice, an atherosclerosis-prone model with a humanized lipoprotein metabolism, IC7Fc treatment markedly lowered plasma triglyceride and total cholesterol levels over seven weeks of treatment. This was associated with increased AMP-activated protein kinase (AMPK) activity in the liver and the inhibition of markers of hepatic de novo lipogenesis. IC7Fc furthermore increased the synthesis of bile acids from cholesterol which resulted in decreased hepatic cholesterol secretion in very-low-density lipoprotein (VLDL) particles and an elevation of plasma bile acids as the hepatic re-uptake of circulating bile acids was simultaneously suppressed. As a consequence, IC7Fc treatment considerably reduced atherosclerotic lesion formation (-84%) and vascular inflammation while increasing the lesions stability index.

Conclusion: We here demonstrate that IC7Fc very effectively treats hyperlipidemia and counteracts atherosclerotic lesion formation through the modulation of hepatic cholesterol and bile acid metabolism. Combined with its previously shown anti-obesity and anti-diabetic effects this makes IC7Fc a promising new pharmacological treatment for cardiometabolic diseases targeting hyperlipidemia and inflammation.

Impaired renal function with elevated remnant cholesterol related to risk of atherosclerotic cardiovascular disease: The Copenhagen General Population Study

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Background: Chronic kidney disease (CKD) confers high risk of atherosclerotic cardiovascular disease (ASCVD), partly due to hyperlipidemia. Although statins reduce risk of ASCVD in CKD, residual risk persists. We hypothesized that in statin users and non-users with impaired renal function, elevated remnant cholesterol is associated with increased risk of ASCVD.

Methods: We included 108 258 individuals from the Copenhagen General Population Study, of which 10 440 had impaired renal function (eGFR<60mL/min/1.73m2). Remnant cholesterol was calculated from a standard lipid profile.

Results: In individuals with impaired renal function during up to 15 years follow-up, 597 were diagnosed with myocardial infarction, 618 with ischemic stroke, and 1182 with ASCVD. In these individuals, a 1 mmol/L (39 mg/dL) higher remnant cholesterol was associated with multivariable adjusted hazard ratios of 1.31 (95%CI: 1.12-1.52) for myocardial infarction, 1.22 (1.03-1.44) for ischemic stroke, and 1.27 (1.14-1.42) for ASCVD. Corresponding hazard ratios for ASCVD were 1.47 (1.14-1.90) in statin users and 1.22 (1.06-1.39) in non-users. Of the 1.36-fold excess risk of ASCVD in impaired versus normal renal function, elevated remnant cholesterol and elevated LDL cholesterol explained 25% (95%CI: 2.5-47%) and 0% in statin users, and 4.5% (0-9.7%) and 12% (5-18%) in non-users.

Conclusion: In statin users and non-users with impaired renal function, a 1 mmol/L (39 mg/dL) higher remnant cholesterol was associated with 1.5- and 1.2-fold risk of ASCVD. Of the excess risk of ASCVD in impaired renal function, elevated remnant cholesterol explained 25% and 5% in statin users and non-users.

Oral Presentations – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Hepatic disruption of Abca6 increases circulating non-HDL-cholesterol by decreasing hepatic uptake of VLDL remnants

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Background: ATP-binding cassette (ABC) transporters are transmembrane proteins that mediate the transport of a wide variety of substrates across biological membranes. Numerous studies have demonstrated the therapeutic potential of targeting ABC transporters in various diseases, such as cancer, Alzheimer's disease, and cardiovascular diseases. ABCA6, a member of the superfamily of ABC transporters, is expressed in hepatocytes and was assumed to be involved in lipid metabolism. In humans, a rare missense variant of ABCA6 was found to be strongly associated with hypercholesterolemia (van Leeuwen, Nat Commun 2015). To unravel the physiological function of ABCA6, we here aimed to evaluate the role of ABCA6 in lipoprotein metabolism.

Methods: We used liver-directed genome editing with CRISPR/Cas9 to target hepatic Abca6 in female APOE*3-Leiden.CETP mice, a well-established model for human-like lipoprotein metabolism. Mice received either adeno-associated virus 8 expressing the Cas9 protein and a sgRNA targeting Abca6 (Abca6 AAV-CRISPR) or an empty AAV vector expressing and a scrambled sgRNA (Control AAV-CRISPR). Following AAV-CRISPR delivery, the mice were fed a hyperlipidemia-inducing Western-type diet (16% fat, 0.15% cholesterol) for 6 weeks. Body weight, plasma lipid levels, and very low-density lipoprotein (VLDL) production and clearance were measured.

Results: Hepatic loss of Abca6 decreased body weight gain (-42%; P=0.013) and tended to decrease brown adipose tissue weight (-18%; P=0.084) and liver weight (-13%; P=0.092) over 6 weeks. Furthermore, it increased plasma non-high-density lipoprotein (non-HDL) cholesterol (+18%; P=0.022). This was explained by decreased hepatic uptake of VLDL-like particle remnants (-19%; P=0.020) rather than increased hepatic production of VLDL.

Conclusion: Our data suggest an important role of ABCA6 in regulating cholesterol homeostasis and we are currently investigating its mechanism of action further.

Lipoprotein(a) cardiovascular disease risk not captured by low density lipoprotein cholesterol and apolipoprotein B

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Background and aim: Elevated lipoprotein(a) affects 1 in 5 individuals and is considered a causal risk factor for atherosclerotic cardiovascular disease (ASCVD) and aortic valve stenosis (AVS). However, lipoprotein(a) measurements are not widely implemented in clinical practice. Low-density lipoprotein (LDL) cholesterol and apolipoprotein B (ApoB) measurements include contributions from lipoprotein(a), yet whether these measurements can replace measurements of lipoprotein(a) in assessing cardiovascular disease risk from this causal risk factor is unknown. Therefore, we tested the hypotheses that lipoprotein(a) risk of ASCVD and AVS is captured by LDL cholesterol or ApoB.

Methods: We included 70,189 individuals from the Copenhagen General Population Study, a contemporary prospective cohort study. Mediated risk by LDL cholesterol and ApoB in the association between lipoprotein(a) and risk of ASCVD and AVS was examined using four-way decomposition analyses stratified by statin treatment at baseline.

Results: During a median follow-up of 10.8 years, 7,127 developed ASCVD and 1,516 AVS. Per 50 mg/dL higher lipoprotein(a), multivariable adjusted hazard ratios were 1.22 (95% CI: 1.18-1.26) for ASCVD and 1.36 (1.28-1.46) for AVS. In non-statin users, LDL cholesterol mediated 19% (15%-24%) of the risk of ASCVD from lipoprotein(a) and 7.7% (1.5%-14%) of the risk of AVS from lipoprotein(a). Corresponding values for ApoB were 14.0% (11%-18%) and 6.6% (2.8%-10%). In statin users (n=9,570), LDL cholesterol did not mediate risk of ASCVD from lipoprotein(a) but mediated 8.0% (0.8%-15%) of the risk of AVS from lipoprotein(a). Correspondingly, ApoB mediated 9.8% (2.2%-17%) of the risk of ASCVD and 4.2% (0.5%-8.0%) of the risk of AVS from lipoprotein(a).

Conclusion: Lipoprotein(a) risk of ASCVD and AVS is not adequately captured by LDL cholesterol and ApoB. Therefore, lipoprotein(a) measurement is needed to capture the cardiovascular disease risk from this causal risk factor.

Polygenic risk of high LDL cholesterol and ischemic heart disease in the general population

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BACKGROUND: Elevated low-density lipoprotein cholesterol (LDL-C) is a causal risk factor for developing atherosclerosis. A person's LDL-C is partly determined by genetics. Polygenic risk scores (PRS) attempt to capture the total genetic risk by combining the effects of many variants into a single measure. The aims of this study were to test the association of an LDL-PRS with LDL-C and risk of IHD in the general population.

METHODS: We included a total of n=20,485 individuals from the Copenhagen General Population Study and Copenhagen City Heart Study. For each individual, an LDL-PRS was calculated based on 207 variants. We also genotyped four rare variants known to cause familial hypercholesterolemia (FH): LDLR Trp44Ter, Trp87Ter, and Trp577Ser, and APOB Arg3527Gln.

RESULTS: The LDL-PRS was strongly associated with LDL-C and explained 11.8% of the total variation in LDL-C in the cohort. Individuals in the lowest 1% of the PRS had a mean LDL-C of 2.33 mmol/L and those in the top 1% had a mean LDL-C of 4.39 mmol/L. For comparison, heterozygous carriers of FH-causing mutations in APOB or LDLR had a mean LDL-C of 5.38 and 5.99 mmol/L, respectively. An LDL-C above 4.9 mmol/L is widely used as a cutoff for hypercholesterolemia. Among those in the bottom 1% and top 1% of the PRS, 1.0% and 31.7%, respectively, had LDL-C >4.9 mmol/L. A higher LDL-PRS also conferred an increased risk of IHD. Compared to those in the middle 20-80% of the PRS, those in the bottom 1% and top 1% had odds ratios for IHD of 0.69 (95% CI, 0.47-1.04) and 1.22 (95% CI, 0.85-1.74), respectively.

CONCLUSIONS: An LDL-PRS was robustly associated with LDL-C, explaining nearly 12% of the variation in LDL-C. However, even in the extreme upper tail, the LDL-PRS conferred smaller effects on LDL-C and risk of IHD than those seen with rare FH-causing variants in LDLR or APOB. Taken together, these results demonstrate the potential clinical value (as well as limitations) of an LDL-PRS.

A rare gain-of-function variant of hepatic lipase attenuates hypercholesterolemia and atherosclerosis in mice independent of the LDL receptor

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Aim: We recently identified LIPC-E97G as a rare gain-of-function (GOF) variant in the LIPC gene, encoding for hepatic lipase (HL), as a new genetic cause of familial combined hypocholesterolemia in humans (Circulation 2022). Here, we investigated the lipid-lowering and anti-atherogenic properties of HL-GOF versus wild-type HL (HL-WT) in APOE*3-Leiden.CETP mice, and assessed dependence of these effects on the LDL receptor (LDLR) in LDLR-/- mice.

Methods: Female APOE*3.Leiden.CETP mice or male and female LDLR-/-mice received an intravenous injection of AAV8 expressing either eGFP (control), HL-WT or HL-GOF (3×1011 GC/mouse), and were subsequently fed a pro-atherogenic diet for 17 weeks or 14 weeks, respectively. Plasma cholesterol levels were measured monthly, and lipoprotein kinetics and aortic atherosclerotic lesion sizes were assessed at termination.

Results: HL-GOF largely decreased plasma total cholesterol levels in APOE*3-Leiden.CETP mice (-69% vs control; -63% vs HL-WT), at least partly explained by increased uptake of VLDL remnants and LDL by the liver. As a result, HL-GOF strongly decreased atherosclerotic lesion size (-98% vs control; -97% vs HL-WT) in the aortic root, without affecting body composition or liver lipids. Importantly, HL-GOF also strongly reduced plasma cholesterol in LDLR-/- mice, both in males (-82% vs control; -78% vs HL-WT) and females (-78% vs control; -80% vs HL-WT), and decreased aortic atherosclerotic lesion size in both males (-74% vs control; -62% vs HL-WT) and females (-73% vs control; -75% vs HL-WT).

Conclusions: LIPC-E97G, encoding a gain-of-function variant of HL, strongly reduces plasma cholesterol levels as well as atherosclerosis development in mice independently of the LDLR pathway, suggesting that improving LIPC function is a promising tool in patients with familial hypercholesterolemia.

High lipoprotein(a) as a cause of chronic kidney disease: a population-based Mendelian randomization study

High lipoprotein(a) as a cause of chronic kidney disease: a population-based Mendelian randomization study

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Aim: Previously it was thought that chronic kidney disease leads to high lipoprotein(a) levels; however, high plasma lipoprotein(a) could also lead to chronic kidney disease. We tested the hypothesis that high lipoprotein(a) is genetic, causally associated with increased risk of chronic kidney disease.

Methods: we studies 108439 individuals from the Copenhagen General Population Study with a median age of 58 years. Of these, 11152 had chronic kidney disease.

Results:
Kidney function estimated by eGFR was lower with higher plasma lipoprotein(a) levels. We found stepwise higher risk of chronic kidney disease with stepwise higher lipoprotein(a) levels with an odds ratio of 1.74 (95% confidence interval: 1.55-1.96) for individuals with lipoprotein(a) levels >95 mg/dl (>203 nmol/l;percentile 96th-100th) compared to individuals with lipoprotein(a) levels <10 mg/dl (<18 nmol/l;percentile 1st-50th) (P for trend across four groups=3*10-22). Similarly, lower LPA KIV-2 number of repeats were associated with higher risk of chronic kidney disease (P for trend across four groups=1*10-19). In instrumental variable analysis for a genetically 50 mg/dl (105 nmol/l) higher lipoprotein(a) level, the genetic causal risk ratio for chronic kidney disease was 1.30 (95% CI: 1.12-1.51) based on KIV-2 number of repeats and rs10455872 genotype combined; the corresponding observational odds ratio for plasma lipoprotein(a) was 1.25 (1.20-1.30). Finally, corresponding genetic and observational risk estimates were 1.53 (1.34-1.74) and 1.31 (1.25-1.38) for myocardial infarction and 1.30 (1.08-1.58) and 1.33 (1.24-1.43) for aortic valve stenosis, respectively.

Conclusion: In the general population, high lipoprotein(a) is associated observationally and genetic, causally with increased risk of chronic kidney disease.

Mitochondrial fatty acid synthesis is essential for coordinated energy transformation

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Next to the canonical cytosolic fatty acid synthesis, mitochondria can newly synthesize lipids in a process called mitochondrial fatty acid synthesis (mtFAS). Mitochondrial enoyl-CoA-reductase (MECR) is catalyzing the last step of mtFAS ,serving as a key mediator for lipoic acid production. In humans, genetic mutations in MECR disturb the mtFAS pathway and cause a neurometabolic mitochondriopathy known as MEPAN syndrome. However, how mtFAS affects systemic lipid and glucose metabolism has not been investigated. Whole exome sequencing and lipidomic analysis was performed in a 20-year-old male patient with muscle weakness and optic atrophy. To study the role of Mecr in systemic metabolism, mice lacking Mecr in liver (Mecrfl/fl-Albcre) and adipose tissues (Mecrfl/fl-Adipoqcre) were subjected to oral glucose-fat tolerance test with radioactive tracer uptake and indirect calorimetry, respectively. Metabolomic/lipidomic analysis, electron microscopy, western blotting, gene expression and ex vivo measurments of enzymatic activities as well as were performed. We identified a compound-heterozygous patient with loss-of-function and missense variants in MECR presenting lower levels of plasma lipid species while variant overexpression in vitro led to an opposite lipidomic fingerprint. In Mecrfl/fl-Albcre+ and Mecrfl/fl-Adipoqcre+ mice the mitochondria were irregularly shaped with critically affected cristae structures. Hepatic Mecr deletion led to profound insulin resistance, reduced lipoic acid synthesis as well as PDH activity. Adipose tissue Mecr ablation exacerbated tissue inflammation, attenuated brown fat thermogenic capacity and critically affected survival upon cold exposure. We show that MECR variants or Mecr-loss in vivo affect coordinated energy breakdown. 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.

Oral Presentations – Abstracts –

Other Topics

SESSION IV

Repurposing statin treatment for the prevention of gallstone disease

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AIM

Symptomatic gallstone disease (GSD) is one of the most common and costly gastrointestinal disorders, and the incidence is likely to increase due to the current global epidemic of obesity. The main cause of GSD is cholesterol hypersecretion in the bile, and observational studies have suggested that statin treatment may be associated with low risk of GSD. The aim of this study was to examine whether statins which lower plasma low-density lipoprotein cholesterol (LDL-C) and reduce the risk of ischemic cardiovascular disease, can be repurposed for the prevention of symptomatic GSD.

METHODS

We genotyped 102,205 individuals from the Copenhagen General Population Study (CGPS) for genetic variants in HMGCR with a strong association to plasma LDL-C. We first tested whether genetic variation in HMGCR, mimicking statin treatment, was causally associated with low risk of GSD. Second, we validated our findings in the UK Biobank and FinnGen studies (n>800,000).

RESULTS

Symptomatic GSD developed in 6,856 individuals (median follow-up 40.1 years) and 3,591 had a cholecystectomy performed (median follow-up 22.9 years). The strongest LDL-C lowering genetic variant in HMGCR (rs12654264) was associated with stepwise lower LDL-C of up to 3.9% (-0.13 mmol/L) in homozygotes for the LDL-C lowering allele versus wild-type (P-trend=8x10-63), and with corresponding stepwise lower risks of symptomatic GSD and cholecystectomy of up to 10% [hazard ratio (HR)=0.90(0.84-0.97) and 0.90(0.82-0.98), respectively]. In instrumental variable analysis, a 1 mmol/L genetic reduction in LDL-C was associated with a 50% lower risk of GSD [HR=0.50(0.30-0.84)]. In meta-analyses including CGPS, the UK Biobank, and FinnGen studies odd ratio for GSD per LDL-C lowering allele was 0.96 (0.95-0.97).

CONCLUSIONS

Genetic variants in HMGCR, mimicking statin treatment, causally reduced risk of GSD. These data therefore suggest that statins may be effective in the prevention of GSD.

Ube2h regulates insulin signaling in adipocytes via Akt phosphorylation.

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Proper insulin signaling and adipocyte health are cornerstones of cardiometabolic health. Adipocyte stress, inflammation, and insulin resistance are associated with diabetic dyslipidemia and cardiovascular disease. A molecular understanding of insulin action and stress resistance mechanisms is essential for targeting adipocyte health in obesity. Here, we focus on the ubiquitin-conjugating enzyme E2 H (Ube2h), which encodes a highly expressed protein in adipocytes implicated in the ubiquitin-proteasome system (UPS) and its role in insulin signaling.

To elucidate the role of Ube2h, we used multiple adipocyte cell lines and manipulated them by utilizing small interfering RNA to knockdown (KD) or plasmid cloning DNA to overexpress (OE) Ube2h. We assessed the effects on adipocyte function and health by combining catabolic and anabolic stimuli (starvation, insulin) or proteasome inhibition to stress the UPS. Phosphoproteomics was used to understand these responses on a global cellular scale and MS analysis of Ube2h IP to identify interaction partners.

Overexpression or loss of Ube2h did not affect global ubiquitin levels or the integrated stress response during proteasome inhibition. However, we found that loss of Ube2h led to lower insulin-stimulated Akt phosphorylation at Ser473, but not Thr308, in the PI3K/Akt/mTOR cascade, while OE did not cause any changes. These results are supported by the phosphoproteomic analysis, which revealed alterations primarily in the insulin-signaling pathway.

Our results identify Ube2h as a novel player in regulating intracellular insulin signaling in adipocytes. Rather than controlling UPS, Ube2h appears to have a specific target upstream of Akt, possibly controlling its activity through direct binding. Uncovering the precise mechanism of action by which Ube2h acts within these cells may aid in a better understanding of insulin-related downstream regulation and help develop effective treatments against insulin resistance during obesity.

Efficacy and safety of PCSK9 monoclonal antibodies in older adults: a real-world single center cohort study

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Background and aim: Proprotein Convertase Subtilisin/Kexin type 9 monoclonal antibodies (PCSK9 mAbs) are established as standard of care in lipid lowering therapy (LLT) in adults. Until recently, limited real-world data were available on the long-term effects of PCSK9 mAbs treatment in older patients. Our aim was to investigate efficacy and safety of PCSK9 mAbs in patients ⩾70 years compared to younger patients with very high cardiovascular risk and elevated LDL-cholesterol (LDL-C) levels.

Methods: Patients ⩾ 18 years who initiated PCSK9 mAbs treatment at our hospital clinic were included in a prospective registry. After PCSK9 mAbs initiation, LDL-C levels were measured every three months during the first year of follow-up. Patients were grouped based on age at baseline: below and ⩾70 years. Mean LDL-C decrease was calculated on an individual and group level. Side effects and PCSK9 mAbs discontinuation were registered at each follow-up visit.

Results: Of the 436 patients, 66 patients (64% women, 62% with FH, 80% with ASCVD) were ⩾70 years (mean ± SD age of 72 ± 2 years). Average LDL-decrease was comparable between age groups at different follow-ups: 6 months (70+ yrs.: -56% vs <70 yrs.: -55%), 12 months (70+ yrs.: -54% vs <70 yrs.: -53%), and 24 months (70+ yrs.: -55% vs <70 yrs.: -52%) from baseline. Patients ⩾ 70 years reported side effects (35% vs 28%) more frequently and discontinued PCSK9 mAbs more often than patients <70 years (18% vs 11%).

Conclusions: In a real-world patient population, we observed a comparable efficacy of PCSK9 mAbs in patients ⩾ 70 years compared to patients < 70 years. However, older patients reported more side-effects and showed a higher discontinuation rate than patients <70 years in the first 6 months follow-up. PCSK9 mAbs are a valuable addition to other LLT in elderly patients with very high cardiovascular risk and elevated LDL-C.

Adipokines mediating stress-dependent adipose-liver endocrine crosstalk

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Adipose tissue is an energy storage depot and endocrine organ with metabolic and immunomodulatory functions. In obesity, adipocyte dysfunction is linked to proteotoxic stress, aberrant endocrine activity, and increased risk for cardiovascular disease. However, the impact of proteotoxic stress-dependent on plasma adipokines is insufficiently well understood. Here, we analyze plasma adipokines, adipose tissue, and liver in mice and cultured adipocytes after inducing proteotoxic stress via the ubiquitin-proteasome system.
We established a siRNA-based knockdown of the proteasome subunit Psmb4 or the transcription factor Nfe2l1 to induce proteotoxic stress in cultured adipocytes. Cells and supernatant were analysed by qPCR, Western blot, and ELISA profiling. In mice, adipocyte Ucp1-Cre-flox knockout of Nfe2l1 was studied regarding systemic metabolism, adipokine levels, and liver phenotypes.
Proteotoxic stress had a profound impact on the adipokine profiles in vitro and in vivo. Analysis of plasma and tissue show a distinct impact on the secretome by proteotoxic stress. The most profoundly upregulated cytokines in plasma were IGFBP-1 and resistin, which was also found in the cell culture. Furthermore, we found several known adipokines such as Fgf21, Timp1, and Lipocalin being induced by proteotoxic stress. In the liver, Moxd1 and Ces2c, which have been linked to steatosis, induced in the livers of knockout mice, indicating adverse crosstalk between adipose tissue and the liver.
In conclusion, we found that adipose tissue communicates stress, such as proteasomal imbalance and proteotoxicity through secreted endocrine factors, and that these may influence systemic metabolism. Elucidating the regulation of resistin and Timp1 and their impact on Moxd1 and Ces2c will help to understand the impact of adipocyte tissue dysfunction on hepatic lipid metabolism. This may lead to new ways of correcting adverse endocrine effects of adipose tissue dysfunction in obesity.

Integrative common and rare variant analyses provide insights into the genetic architecture of liver cirrhosis

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We report a multi-ethnic genome-wide association study on liver cirrhosis and its associated endophenotypes, alanine aminotransferase (ALT) and gamma-glutamyl transferase. Using data from 12 cohorts, including 18,265 cases with cirrhosis, 1,782,047 controls, up to 1 million individuals with liver function tests, and a validation cohort of 21,689 cases and 617,729 controls, we identify and validate 20 risk associations for liver cirrhosis. Many variants are located near genes encoding proteins involved in hepatic lipid metabolism, underscoring the importance of fatty liver disease in cirrhosis pathophysiology. One of the 36 variants, p.Ile148Met (rs738409) in PNPLA3, significantly interacts with alcohol intake, obesity, and type 2 diabetes on the risk of cirrhosis and hepatocellular carcinoma (HCC). We develop a cirrhosis polygenic risk score (PRS) that strongly associates with progression from cirrhosis to HCC in the UK Biobank. We investigate the association of rare coding variants with ALT and cirrhosis by focusing on prioritized genes from common variant analyses. We find that rare coding variants in GPAM associate with lower ALT levels, supporting GPAM as a potential target for therapeutic inhibition. In conclusion, this study provides new insights into the genetic underpinnings of cirrhosis.

Deletion of the murine ortholog of human 9p21.3 CAD risk locus leads to insulin resistance and obesity in mice

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Background and aim

9p21.3 genomic locus is a hot spot for disease-associated single nucleotide polymorphisms (SNPs), with strongest associations with coronary artery disease (CAD) and type 2 diabetes (T2D). The disease associated SNPs at the 9p21.3 are located in a sequence of a long non-coding RNA ANRIL, which potentially affects atherosclerosis by regulation of vascular cell proliferation and inflammatory response. However, less is known about its contribution to metabolic regulation. In the present study, we aimed to characterize the effects of 9p21.3 ortholog in metabolic phenotype in hyperlipidemic mice.

Methods

Hyperlipidemic mice deficient in murine ortholog of 9p21.3 risk locus (Chr4Δ70/Δ70Ldlr-/-ApoB100/100) were aged 6 or 12 months on a standard laboratory diet. Body weight, insulin response, and gene expression and histopathology of liver, skeletal muscle and white adipose tissue (WAT) of the mice were analyzed. The role of ANRIL in energy metabolism in human hepatocytes (HEPG2) was studied by silencing ANRIL with siRNA and by using Seahorse XF extracellular flux analyzer (Agilent).

Results

Aged Chr4Δ70/Δ70Ldlr-/-ApoB100/100 mice showed increased body weight, adipocyte size and resistance to insulin. The expression of insulin receptor was reduced only in WAT, not in liver or in skeletal muscle. In addition, the expression of Pparγ, Sirt1, Sirt2 and Ucp2 was downregulated in WAT. Also, the expression of Insr was significantly downregulated by Chr4Δ70/Δ70 in WAT of young mice.

Conclusions

Chr4Δ70/Δ70 leads to obesity and insulin resistance in Ldlr-/-ApoB100/100 mice. These results suggest ANRIL's contribution on fatty acid and glucose metabolism in white adipose tissue.

Circulatory hsa-miR-339-5p is associated with later development of metabolic dysfunction-associated steatotic liver disease (MASLD) in the prospective Young Finns Study

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Introduction: The often suboptimal treatment outcomes of MASLD emphasize the need for novel tools for earlier diagnosis. Several micro-RNAs (miRNAs) have been associated with MASLD in cross-sectional studies, but the utility of these or other miRNAs as early predictive biomarkers for the condition remains unclear. We investigated this issue in a large longitudinal cohort with a follow-up of up to 10 years.
Materials and Methods: Altogether 651 adult participants of the Young Finns Study without signs of MASLD in hepatic imaging in 2011 underwent a comprehensive re-evaluation in 2018-2020. Profiles of 243 circulatory miRNA obtained with TaqMan OpenArray in 2011 were compared between those who maintained a healthy liver and those who developed MASLD using Mann-Whitney U test. Multivariate logistic regression model considering previously established metabolic and other risk factors for MASLD was used to further evaluate possible associations.
Results: Median age of the participants in 2011 was 43 years and 62% were females. Altogether 122 (18.7%) developed MASLD during the follow-up period, with a higher proportion of men being affected (33% vs. 18%, p=0.003). Those who developed MASLD had significantly higher BMI (24.5 vs. 27.8, p<0.001), whereas the groups did not differ in age or alcohol usage. The individuals who developed MASLD had significantly lower levels of miR-339-5p than those without later MASLD (fold change -1.11, pFDR<0.07). In the multivariate logistic regression model, miR-339-5p was an independent protective factor for MASLD (odds ratio 0.64, 95% confidence interval 0.50-0.81) and contributed to an improvement of its prognostic performance. None of the miRNAs previously associated with MASLD demonstrated similar prognostic potential.
Conclusions: As a possible novel early biomarker, lower levels of miR-339-5p predicted the later development of MASLD even after considering other known risk factors in a multivariate model.

Remnant cholesterol, not LDL cholesterol, explains peripheral artery disease risk conferred by apolipoprotein B: a cohort study

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Background: Elevated apolipoprotein B (apoB)-containing lipoproteins (=remnants + low-density lipoproteins [LDL]) are a major risk factor for atherosclerotic cardiovascular disease, including peripheral artery disease (PAD) and myocardial infarction. We tested the hypothesis that remnants and LDL both explain part of the increased risk of PAD conferred by elevated apoB-containing lipoproteins. For comparison, we also studied risk of chronic limb-threatening ischemia and myocardial infarction.

Methods: ApoB, remnant cholesterol, and LDL cholesterol were measured in 93,461 individuals without statin use at baseline from the Copenhagen General Population Study (2003-2015). During up to 15 years of follow-up, 1,207 had PAD, 552 had chronic limb threatening ischemia, and 2,022 had myocardial infarction in the national Danish Patient Registry. Remnant and LDL cholesterol were calculated from a standard-lipid profile. Remnant and LDL particle counts were additionally measured with nuclear magnetic resonance spectroscopy in 25,347 of the individuals. Results were replicated in 302,167 individuals without statin use from the UK Biobank (2004-2010).

Results: In the Copenhagen General Population Study, multivariable adjusted hazard ratios for risk of PAD per 1 mmol/L (39 mg/dL) increment in remnant and LDL cholesterol were 1.9 (95% confidence interval: 1.5-2.4) and 1.1 (1.0-1.2), respectively; corresponding results in the UK Biobank were 1.7 (1.4-2.1) and 0.9 (0.9-1.0), respectively. In the association from elevated apoB to increased risk of PAD, remnant and LDL cholesterol explained 73% (32-100%) and 8% (0-46%), respectively; corresponding results were 63% (30-100%) and 0% (0-33%) for risk of chronic limb-threatening ischemia, and 41% (27-55%) and 54% (38-70%) for risk of myocardial infarction; results for remnant and LDL particle counts corroborated these findings.

Conclusions: PAD risk conferred by elevated apoB-containing lipoproteins was explained mainly by elevated remnants, while myocardial infarction risk was explained by both elevated remnants and LDL.

Posters – Abstracts –

Inflammation and Vascular Biology

SESSION I

Blockage of endoglin prevents endothelial dysfunction development in type 2 diabetic coronary artery endothelial cells

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Endoglin (Eng) is a cell membrane glycoprotein expressed in the vascular endothelium, which is related to endothelial dysfunction and inflammation. TRC105 (carotuximab) is a monoclonal antibody that binds to membrane endoglin, and it was published that direct blockage of Eng can potentially prevent hypercholesterolemia- or hyperglycemia-induced development of endothelial dysfunction. Therefore, we hypothesized that TRC105 prevents endothelial dysfunction development in coronary artery endothelial cells from type 2 diabetes mellitus patients.

We aimed to evaluate the impact of TRC105 treatment on Eng expression and function (adhesion and transnigration of THP-1 monocytes) with respect to endothelial dysfunction and inflammation by comparing human coronary artery endothelial cells from healthy donors (HCAEC) with human coronary artery endothelial cells from type 2 diabetes mellitus patients (D-HCAEC).

HCAEC and D-HCAEC, passage 6, were cultured in EGM-2 media with appropriate supplements and 10% FBS until reaching 90% confluence. Cells were treated with TRC105 (300μg/ml) for 12 hours. For protein analysis, samples were analyzed by flow cytometry, using anti-Eng, anti-VCAM-1, and anti-ICAM-1 antibodies. For functional analysis, THP-1 monocytes were added to the medium for 1 hour. Afterward, cells were washed with PBS, and monocytes adhering or transmigrated to endothelial cells were quantified by flow cytometry.

The protein analysis showed that Eng, inflammatory markers such as VCAM-1 and ICAM-1, soluble endoglin in cuture media, as well as adhesion and tramsmigration of THP-1 monocytes to endothelial monolayer, are significantly increased in D-HCAEC compared to HCAEC. TRC105 treatment significantly reduced Eng expression, which resulted in decreased adhesion of monocytes to endothelium.

These results suggest the crucial role of Eng in endothelial dysfunction development in endothelial cells from type 2 diabetes mellitus patients.

Degradomics analysis of human atherosclerotic plaques

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Atherosclerosis is the major underlying cause of cardiovascular disease, and a leading cause of morbidity and mortality. Destabilization and rupture of atherosclerotic plaques, which are often asymptomatic, can arise suddenly and result in acute arterial occlusion, peripheral embolization, myocardial infarction, stroke and lower limb ischaemia. Data indicate that plaque destabilization is associated with extracellular matrix (ECM) modification and remodelling, arising from damage and protease activity. We hypothesized that plaques would contain fragmented proteins with new N-termini. Plaques from 21 patients who underwent carotid surgery following symptomatic carotid artery stenosis were examined. These were solubilized, digested, enriched for N-terminal fragments and analyzed by liquid chromatography-mass spectrometry. This allowed detection of 35349 peptides, with 19543 being N-terminal species; 6561 were subsequently identified and quantified. Multidimensional scaling analysis and hierarchical clustering indicate the presence of three distinct clusters, which correlate with macroscopic plaque morphology (soft/unstable, mixed, and hard/stable), ultrasound classification and presence of hemorrhage/ulceration. Major differences were identified in the complement of peptide fragments, consistent with alternative protein turnover and degradation pathways that are dependent on plaque type. Identified peptides include signal and pro-peptides from ECM synthesis and turnover, and large number of peptides with neo-N-termini from fragmentation. Sequencing indicates the targeted proteins (including apolipoprotein B and multiple ECM species) and the proteases in fragment generation (including meprins, cathepsins, matrix metalloproteinases, elastase, kallikreins). This study provides a large dataset of peptide fragments and proteases involved in plaque (in)stability, mechanistic insights into remodelling processes, and possible biomarkers for risk profiling of atherosclerosis.

YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

The Immunometabolic Role of Pyruvate Dehydrogenase Kinase-1 in Smooth Muscle Cells in Atherosclerosis

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Background and aims. Acting as a gatekeeper between glycolysis and oxidative phosphorylation, the PDK/PDH axis is shown to regulate immune cell polarization and inflammation. Recently, we showed that PDK1 expression inversely correlated with carotid plaque vulnerability and predicted major cardiovascular outcomes. Whether the PDK1 isoenzyme can influence atherosclerosis by skewing smooth muscle cell (SMC)-mediated responses is unknown.
Methods. This project examined PDK1 expression regulation in vitro in human aortic smooth muscle cell (haSMC) cultures. Pdk1flox and Myh11CreERT2 mice were crossed to create a specific inducible SMC-Pdk1KO strain; littermate WT (Pdk1+/+) mice were used as controls. These mice were made hyperlipidemic and prone to atherosclerosis by overexpressing the gain-of-function mutated Pcsk9 using AAV and Western diet feeding.
Results. PDGFB-mediated dedifferentiation of haSMCs led to a 21% upregulation in PDK1 expression and increased lactate secretion (19.4±0.5 and 12.9±0.5, PDGFBvs.control). Interestingly, oxLDL stimulation also increased PDK1 mRNA expression (1.4±0.2 and 0.7±0.1, oxLDLvs.control). At 12 weeks post-Pcsk9-AAV injection and the start of western diet feeding, SMC-Pdk1KO and WT presented high plasma cholesterol (9.7±2.0 and 14.2±3.0) and triglycerides (3.3±0.6 and 3.1±0.6), but no difference between the groups was observed. No difference in plasma lactate levels was observed between groups (3.4±0.1 and 3.1±0.2,SMC-Pdk1KO vs. WT). Hence, in this time point, mice showed no difference in the aortic arch (14.3±3.2 and 14.3±2.0, SMC-Pdk1KOvs.WT) and root (7.6±1.2 and 5.2±1.7,SMC-Pdk1KO vs.WT) atherosclerotic burden.
Conclusion. We demonstrate that dedifferentiation and oxLDL increase PDK1 expression in SMCs in vitro. However, genetic targeting of Pdk1 in SMCs did not reduce atherosclerosis in mice with 12-week hyperlipidemia. Further studies and plaque characterization will provide definitive proof of SMC-PDK1's role in atherogenesis.

Adipose Tissue's Eosinophils and Macrophages - The Untold Story Exposed

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Introduction: The significance of eosinophils (Eos) in regulating the metabolic homeostasis of adipose tissue (AT) has been demonstrated in an animal model; however, data obtained from humans are limited. Obese adipose tissue is often associated with the development of subclinical inflammation, and in this context, M2 polarization represents a crucial factor contributing to the preservation of physiological status. This project builds upon and extends our laboratory's research on innate immunity mechanisms in atherogenesis. Methods: Preoperatively, blood (EDTA) was collected. Visceral AT was obtained intraoperatively during nephrectomy. Using flow cytometry, leukocytes (CD45+) were identified from the obtained non-adipocyte stromovascular fraction and whole blood, followed by the identification of eosinophil populations (CD14- CD15+ CD16-) and macrophages (CD14+). The anti-inflammatory (M2; CD16- CD36low) and pro-inflammatory, metabolically activated (M1; CD16+ CD36high) subpopulations were determined within the macrophage population. Results: The study included 53 living kidney donors. The representation of eosinophils was compared in various types of AT and blood. The proportion of Eos in visceral AT significantly increased with the increasing number of M2. The opposite trend was observed in relation to M1. The proportion of Eos in perivascular AT also significantly increased with the increasing number of M2, but no relationship with M1 was observed. Furthermore, the proportion of Eos in blood significantly increased with the proportion of M2 in AT. Conclusion: The study provides entirely new data describing the representation of eosinophils in human AT and their involvement in immune processes in situ. The demonstrated positive correlation between the number of eosinophils and M2 in the AT of healthy individuals, along with other results, indirectly confirms the paradigm of the likely importance of eosinophils in maintaining the homeostasis of AT metabolism.

Human atherosclerotic plaques contain oxidant-modified proteins

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Compared to stable atherosclerotic plaques, unstable rupture-prone plaques contain higher levels of activated inflammatory cells that release the enzyme myeloperoxidase (MPO). MPO generates potent oxidants, including hypochlorous acid (HOCl), which may chlorinate/nitrate amino acid residues and thereby damage extracellular matrix (ECM) proteins, potentially contributing to plaque destabilization and rupture. As MPO is a source of oxidants, we hypothesized that MPO would also be a target for these species, and thus become modified. The type, extent, and consequences of such modification remains unclear. We have therefore examined whether human carotid artery atherosclerotic plaques contain both oxidant-modified MPO and ECM proteins.

Proteins were extracted from unstable and stable human carotid artery plaques, digested to peptides, and subjected mass spectrometry to identify parent peptides and oxidation products. In vitro studies were carried out with purified human MPO and proteins extracted from unstimulated and stimulated differentiated PLB-985 cells, a model of human neutrophils.

Purified human MPO exposed to its own oxidants, as well as MPO released from PLB-985 cells, was found to be chlorinated and nitrated at multiple sites. More than 9000 proteins were identified from human carotid plaques, many of which were shown to contain oxidative modifications, including those generated by MPO. Thus our data indicates that 3 tyrosine and 2 tryptophan residues within the MPO sequence can be chlorinated and/or nitrated in vivo. Our data further indicate that modification occurs on ECM proteins, with differences in the pattern of these modifications detected between stable versus unstable plaques.

Our data indicate that human carotid plaques contain oxidant-modified proteins. These oxidative modifications may alter protein structure and function, weaken plaque structure and enhance susceptibility to rupture.

Role of selenocyanate to modulate cellular damage resulting from chronic inflammation in atherosclerosis.

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An important factor in atherosclerotic lesion development is chronic inflammation, which results in the infiltration of immune cells into the vessel wall. During inflammation activated immune cells release myeloperoxidase (MPO), which produces the strong oxidant hypochlorous acid (HOCl), as a means to kill pathogens. However, excessive HOCl production, as seen in chronic inflammation, leads to severe and irreversible host tissue damage, which contributes to disease, particularly atherosclerosis. As a result, decreasing the extent of HOCl mediated damage could be a useful therapeutic strategy. This study explores the use of selenocyanate (SeCN-) to decrease and prevent the cellular damage induced by HOCl seen in chronic inflammation. This approach could be advantageous, as SeCN- is a competitive substrate for MPO and can also actively scavenge HOCl. These reactions favour formation of hyposelenocyanous acid (HOSeCN), which is thought to be a milder oxidant than HOCl that retains antibacterial properties. The reactivity of SeCN- and HOSeCN was assessed in macrophage cell models, as this is a key cell type in driving lesion formation. Exposure of J774A.1 and THP-1 macrophages to HOCl resulted in a dose-dependent loss of viability, assessed by metabolic activity assays and release of lactate dehydrogenase. With HOSeCN, a greater loss of viability was seen, together with an altered redox balance as reflected by a decrease in intracellular thiol concentrations. Current studies are examining the reversibility of this cytotoxic effect of HOSeCN, and whether this oxidant can activate inflammatory signaling in macrophages. The results contrast with previous studies with human coronary artery smooth muscle cells, where HOSeCN was less damaging and prevented HOCl-induced damage. Taken together, these results suggest further experiments are necessary to establish the utility of SeCN- supplementation in modulating cell damage in atherosclerosis.

Posters – Abstracts –

Cardiovascular Disease

SESSION II

Ex vivo culture of atherosclerotic plaques provides a new model for the study of plaque stability

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While some atherosclerotic plaques remain stable and symptom-free for years, others become unstable and suddenly rupture to form blood clots with serious complications (e.g. heart attack and stroke). The underlying mechanisms of plaque instability are poorly understood and treatments that prevent plaque rupture are lacking. Mouse models have been used extensively in the study of plaque formation and progression, yet several limitations of these models exist. Importantly, mice are inherently resistant to atherosclerosis and must be genetically modified to develop the disease, and even then, murine atherosclerotic plaques usually do not rupture. We propose that the culture of human atherosclerotic plaques provides a new model that more accurately reflects the mechanisms behind plaque instability. Human carotid artery atherosclerotic plaques, excised during endarectomy, will be collected and placed in growth medium for culture. We will analyze plaque structure and composition by a combination of histological methods and proteome analysis. Currently, we have cultured plaques (n = 4) for up to 13 days, during which they remained structurally intact. Preliminary proteomics data (n = 1) comparing cultured plaque pieces (4-7 days) to non-cultured (frozen upon collection) has revealed few changes to the plaque proteome (229 differentially expressed proteins out of 4301 detected). The expression of protein markers of vascular and inflammatory cells were unchanged in culture (e.g. ACTA2 and TAGLN for vascular smooth muscle cells), indicating that plaque cells survive in culture. Once established, we believe this model will be useful to study the effect of cardiovascular risk factors, while allowing us to test new potential drugs, with the aim of increasing plaque stability and reducing serious complications in patients with cardiovascular disease.

Lipoprotein(a) during early life course in girls and boys with genetically verified familial hypercholesterolemia.

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Background and aim: Many individuals with a familial hypercholesterolemia (FH) mutation may also exhibit elevated lipoprotein(a) (Lp[a]) levels, which is an independent risk factor for atherosclerotic cardiovascular disease. Studies have reported higher levels of Lp(a) in adult and middle-aged women compared to men. However, there is limited knowledge on the concentration and variation of Lp(a) during childhood and adolescence among individuals with FH. We therefore investigated Lp(a) level at baseline and follow-up in girls and boys with FH and explored age-related changes in Lp(a) level during follow-up of 10 years at a specialized lipid clinic.

Methods: We retrospectively reviewed medical records of 438 patients with heterozygous FH that started follow-up below the age of 19 years at the Lipid Clinic, Oslo University Hospital in Norway. To analyze the effect of sex and changes over time, we fitted a linear mixed effect model with linear splines on log-transformed Lp(a).

Results: Three-hundred and eighty-six patients had at least one measurement of Lp(a) and of these, 185 patients had at least two measurements. Mean (SD) age at baseline was 13.8 (7.3) years and the age was similar between sexes. Girls had a higher Lp(a) level than boys at baseline: median (25-75th percentile) 45.7 (20.6-103.0) vs. 30.4 (13.8-74.4) nmol/L, respectively ($p<0.01$). Based on repeated measures, females had 30.9% higher Lp(a) levels on average per year compared to males. On average, Lp(a) levels increased 19.1% annually from 0 to 5 years, and thereafter decreased 1.3% annually from 5 years onwards.

Conclusions: Lipoprotein(a) level at baseline and over time was higher in girls compared to boys with FH. Further research is needed to elucidate whether this could result in increased lifetime risk of cardiovascular disease, and if patients with FH could benefit from Lp(a)-lowering therapies, that are currently being investigated.

YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION II

Saturated fatty acids and total and cardiovascular disease mortality in Norway: A prospective cohort study with up to 45 years of follow-up

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Background: The contribution of dietary saturated fat (SFA) to cardiovascular disease (CVD) and mortality is still debated after decades of research. Most studies to date lack repeated dietary assessments and had low power to assess mortality. Evidence on individual SFA and CVD mortality is limited.
We aimed to assess associations between intake of total SFA and individual SFA and risk of total and CVD mortality in a large population-based cohort of young to middle-aged adults in Norway.
Methods: All adults 35–50 years of age, and random samples of younger adults, in three Norwegian counties were invited to repeated health screenings between 1974 and 1988 (>80 % attendance). Diet was assessed at each screening with semi-quantitative food frequency questionnaires. We calculated cumulative average intakes of fats and other macronutrients. Multivariable Cox regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for total mortality, mortality from CVD, ischaemic heart disease (IHD), and acute myocardial infarction (AMI).
Results: Among the 78,725 subjects included in the analyses, 28,555 deaths occurred during a median follow-up of 33.5 years (2,489,121.1 person-years). 9,318 died of CVD. Median (inter-quartile range) intake of SFA was 14.6% (12.8% to 16.6%) of total energy (E%). Intake of SFA at the expense of carbohydrates was positively associated with all mortality endpoints, including total mortality (HR per 5 E% increment, 1.18; 95% CI 1.13 to 1.23) and CVD mortality (1.16; 1.07 to 1.25). Theoretical isocaloric substitution of SFA with carbohydrates or monounsaturated fatty acids was associated with lower risk. Of individual SFA, myristic (C14:0) and palmitic acid (C16:0) were also positively associated with mortality.
Conclusion: Long-term intake of dietary SFA intake was strongly associated with total and CVD mortality in this Norwegian cohort study. This supports the dietary policies to limit the intake of SFA.

Excess apoB in risk of cardiovascular disease and mortality in women and men

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Background and aims: Low-density lipoprotein cholesterol (LDL-C) and apolipoprotein-B (apoB) are highly correlated measures of atherogenic lipoproteins. We hypothesized that excess apoB is associated with increased risk of myocardial infarction (MI), atherosclerotic cardiovascular disease (ASCVD), and all-cause mortality.

Methods: We included 53,484 women and 41,624 men not taking statins from the Copenhagen General Population Study. Associations of excess apoB with risk of MI, ASCVD, and all-cause mortality were estimated by Cox proportional hazards regressions with 95% confidence intervals. Excess apoB was defined as measured levels of apoB minus expected levels of apoB from LDL alone; expected levels were defined by linear regressions of LDL-C levels versus apoB levels in individuals with triglycerides $\leqslant$1mmol/L (89mg/dL).

Results: During a median follow-up of 9.6 years, 2048 MIs, 4282 ASCVD events, and 8873 deaths occurred. There was a dose-dependent association between excess apoB with risk of MI and ASCVD in both women and men, and with risk of all-cause mortality in women. For ASCVD in women compared to those with excess apoB<11 mg/dL, the multivariable adjusted hazard ratio was 1.08 (95% confidence interval 0.97-1.21) for excess apoB 11-25mg/dL, 1.30 (1.14-1.48) for 26-45 mg/dL, 1.34 (1.14-1.58) for 46-100 mg/dL, and 1.75 (1.08-2.83) for excess apoB>100 mg/dL. Corresponding hazard ratios in men were 1.14 (1.02-1.26), 1.41 (1.26-1.57), 1.41 (1.25-1.60), and 1.52 (1.13-2.05), respectively. Results were robust across the entire LDL-C spectrum.

Conclusion: Excess apoB, that is, the value of apoB above that contributed by LDL levels alone, is associated dose-dependently with increased risk of MI and ASCVD in women and men; demonstrating that apoB provides important predictive value beyond LDL-C across the entire LDL-C spectrum.

LDL-cholesterol burden in elderly patients with familial hypercholesterolemia: insights from real-world data

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Background and aims: Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated low-density lipoprotein cholesterol (LDL-C) from birth and increased risk of coronary heart disease (CHD). The cumulative exposure to LDL-C (the LDL-C burden) is suggested to offer a more comprehensive understanding of cardiovascular risk in FH patients than singular LDL-C measurements. Using real-world data, this study aimed to estimate the LDL-C burden in FH patients at CHD onset in females and males, and to compare LDL-C burden in FH patients with and without CHD at different ages.

Methods: Data was retrospectively collected from the medical records of elderly (>60 years) FH patients at the Lipid Clinic in Oslo. The LDL-C burden (mM-years) was estimated based on repeated LDL-C measurements and detailed information on lipid-lowering medication.

Preliminary results: We included 110 genetically verified FH patients in this study, with 56 (51%) females, median age 68 (62-79; range) years, and median 9 (2-14; range) available LDL-C measurements. Among subjects with CHD (n=53 [48%]), median age and LDL-C burden at CHD onset were 50 years and 323 mM-years. Females had lower age (45 vs. 51 years, $p<0.05$) and LDL-C burden (318 vs. 349 mM-years, $p<0.05$) at CHD-onset than males. Moreover, the CHD group had higher LDL-C burden at different ages compared with the non-CHD group ($p=0.038$).

Preliminary conclusions: Among FH patients, females suffered from CHD at a lower age and with a lower estimated LDL-C burden compared to males. Furthermore, elderly FH patients with CHD had higher estimated LDL-C burden than their non-CHD counterparts. These findings underscores the importance of maintaining optimal LDL-C levels at an earlier age to minimize cumulative LDL-C exposure and reduce the lifetime risk of CHD. We emphasize the need for careful follow-up and early intervention in children with FH, in particular in girls and young women.

A Comprehensive Analysis of Women's Participation in Cardiovascular Trials

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Aim

Historically, women have faced underrepresentation in cardiovascular disease clinical trials, potentially hindering the accurate measurement of the safety and efficacy of therapies for women. Furthermore, enrolling a more representative population may lead to the broader and more rapid adoption of the study results in society. Our study aimed to investigate women's participation in trials based on cardiovascular disease prevalence in the population.

Methods

We assessed the percentage of female participants in all completed interventional trials in which the largest research network in the Netherlands (Working Group Cardiology Centres Netherlands) collaborated, between 1996 and 2020. Trials were categorized according to the domains: atherosclerotic cardiovascular disease (ASCVD), arrhythmia, diabetes mellitus, heart failure or primary prevention. Gender imbalances were assessed using the Participation to Prevalence Ratio (PPR), which is calculated by dividing the percentage of women enrolled in a trial by the prevalence of women affected by the disease, as mentioned in the inclusion criteria, in the general population. A PPR between 0.8 and 1.2 indicates unbiased enrollment with respect to the number of women included in the trial.

Results

In total 114 cardiovascular trials were eligible for analyses with a total of 779,738 participants, of whom 29.2% were women. Women were underrepresented (PPR <0.8) in 59.6% of the included trials, especially in ASCVD see figure 1.

Conclusions

The underrepresentation of women in cardiovascular trials relative to the diseased population of interest is concerning. Our study emphasizes the urgency of investigating factors contributing to female trial non-participation. Implementing strategic initiatives, guided by these insights, is imperative for promoting the higher inclusion of women in cardiovascular trials.

DNA methylation differences between Eastern- and Western-originating Finns

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The coronary heart disease (CHD) mortality rate is unequally distributed in Finland, with a higher risk in the East than in the West. Eastern and Western Finns are also genetically distinct, which may partly explain the difference in CHD risk. This study aims to investigate whether there is a difference in DNA methylation profiles between Eastern and Western Finns and whether this might be involved in mediating the discrepancy in CHD.

We utilised a subset (n=969) of the Young Finns Study (YFS) cohort, containing participants who have 3-4 grandparents originating either from Eastern or Western regions of Finland. Genome-wide DNA methylation levels were compared between the two groups to identify differentially methylated positions (DMPs) and regions (DMRs). We then used regression analysis to investigate the association between methylation at the identified loci and genetics, gene expression and cardiometabolic disease (CMD) risk-associated phenotypes.

We identified 21 DMPs (FDR < 0.05 and $\Delta\beta > 2.5\%$) and 7 DMRs between Eastern- and Western- originating Finns. Methylation at all DMPs and DMRs associates with underlying genetic variants ($p < 5e\text{-}8$). However, a nominally significant difference between the methylation levels of the two groups is retained at 13 DMPs after adjusting the model with the respective top SNP, indicating that factors other than genetics may also be involved in DNA methylation establishment at these loci. Association with proximal gene expression was identified for 10 DMPs and 4 DMRs ($p < 0.05$), independently of genetics. We also found an association, independent of genetics, between 5 DMPs and 4DMRs ($p < 0.05$) with CMD risk phenotypes including triglyceride, glucose and cholesterol levels as well as blood pressure and a metabolic syndrome diagnosis. This suggests that the DNA methylation differences could have biologically relevant functions and may partly account for the difference in CHD risk between Eastern and Western Finns.

High intake of omega-3 fatty acids reduces the odds to subclinical atherosclerosis in German descendants living in Brazil: SHIP-Brazil Study

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Aim: To investigate whether the consumption of omega-3 fatty acids is associated with the presence of subclinical atherosclerosis in German descendants living in Brazil. Methods: This cross-sectional study investigated demographic, lifestyle and clinical data at baseline time of the "Life and Health Cohort in Pomerode (SHIP-BRASIL, 2014 to 2018). The presence of plaques in the common, internal, and external carotid arteries bilaterally (14 parameters) was identified through ultrasound. Subclinical atherosclerosis was grouped according to: 0 to 2 lesions (no atherosclerosis/mild), 3 to 5 lesions (moderate atherosclerosis), and ⩾ 6 lesions (severe atherosclerosis). Results: The sample comprised 597 individuals, 56.8% female and a mean age of 52.03 (SD=15.08) years. 70.1% of descendants preserved the Germanic culture and were predominantly white skin (93.8%). Regarding lifestyle, 9% of Germanic descendants were smokers, 10.2% consumed alcohol, and 33.5% were sedentary. The average intake of omega-3 fatty acids was 1.82 (SD=1.04) grams/day, with the highest intake (⩾P50=1.56 grams/day) being directly associated with males (58.5%) and the 18-59 age group (54.7%) and lower prevalence of altered waist circumference (WC), hypertension and diabetes mellitus (P<005; for all). Higher omega-3 intake (⩾P50) was associated with lower changes of subclinical atherosclerosis, 55.5% no atherosclerosis/mild, 39.5% moderate atherosclerosis and 41.2% severe atherosclerosis (P=0.027). In the multiple analyses, consumption of omega-3 ⩾ 1.56 grams/day (⩾ P50) reduced the odds to subclinical atherosclerosis (moderate to severe atherosclerosis) by 52%, regardless of gender (OR=0.48; 95%CI=0.29-0.78; P=0.003). However, when adjusted for age, this association lost its significance (OR=0.66; 95%CI=0.37-1.17; P=0.153). Conclusion: High intake of omega-3 in German descendants living in Brazil was associated with less subclinical atherosclerosis.

Posters – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Empowering In-depth definition of Simvastatin and Ezetimibe Effects in Humans by Intelligible Heterogeneous Networks

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Increasing technological capability and computational advances give access to a large quantity of different types of data (e.g., genomic, epigenomic, transcriptomic, proteomic, and metabolomic), and create the opportunity to understand both the mechanistic complexity of diseases and the multiplicity and heterogeneity of the effects induced by different treatments. Yet, access to these large amounts of data of different types also creates challenges, one of which is the absence of approaches that reduce the dimensionality and render the information extracted from these big data sets in a clinically interpretable format that informs patient care. To facilitate interpretation and clinical implementation, we created heterogeneous networks able to characterize the complex interactions between the molecular effects in the liver (endophenotype), plasma, and bile (peripheral phenotype) exerted by two of the most prescribed lipid-lowering drugs for prevention and management of ASCVD in humans, i.e., simvastatin and ezetimibe. We refer to such efforts as the creation of intelligible heterogeneous networks. The Stockholm Study, in which patients eligible for cholecystectomy were randomized to simvastatin, ezetimibe, combined treatment (simvastatin and ezetimibe), or placebo for 4 weeks prior to surgery, generated different types of data: liver transcriptomics and methylomics, and biochemical parameters such as biliary lipids, lipoprotein lipid composition, and atherogenic characteristics. Identification of unique modules of heterogenous information increases the interpretability and leads to the identification of putative unique effects of the different lipid-lowering drugs. Proof-of-concept validation in a pre-clinical human model confirms e.g., TMBIM6 to be a target gene of both statin and ezetimibe in combination, supporting the validity of our network medicine-based approach at defining biological interactions and discovering new drug targets.

What happens to Lp(a) when stored in a freezer for many years? Longitudinal analyses of the HUNT Study

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Background and aim: Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein known to be a causal risk factor for cardiovascular disease. In the 1990s, its role was debated after conflicting results emerged from various studies. The discrepancies were later attributed to degradation of Lp(a) in long-term frozen samples (and other confounding factors), which was dependent on temperature, time, and isoform.

The Trøndelag Health Study (HUNT) is a comprehensive database of questionnaire data, clinical measurements and biomaterial collected in 4 rounds (HUNT1-4), with an additional round during COVID-19. The aim of this study was to estimate Lp(a) degradation, as indicated by individual -level changes in Lp(a) concentrations between stored blood samples from HUNT4 (2017-2019) and HUNT3 (2006-2008) and samples collected <3 months before analysis, and to develop an algorithm to correct for degradation.

Methods: We randomly selected 2000 individuals who had participated in HUNT3, HUNT4 and the ongoing HUNT COVID and analyzed samples using the Roche Tina-Quant Lipoprotein(a) 2. generation assay.

Results: We found evidence of a time-dependent degradation of Lp(a) when stored at -80°C. The mean Lp(a) level declined from 49.5 nmol/L in recently collected samples to 46.9 nmol/L in samples from HUNT4 and 36.9 nmol/L in samples from HUNT3, corresponding to a decline of 1.73% per year. Unlike previous studies, we observed that the relative degradation was independent on the initial Lp(a) level. When correcting for years of storage, we found that 87% of individuals (and 98% in the top decile) were categorized in the same or neighboring Lp(a) decile as before.

Conclusion: Lp(a) degrades over time when stored at -80°C. The degradation is not isoform-dependent when measured using a particle-based assay and can be easily adjusted for. These findings emphasize the need to consider degradation when measuring Lp(a) in biobank samples, especially if samples are collected over a long period.

Leukocyte lipid uptake and storage profiles along the lipid-lowering treatment journey: an observational study

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Aims: Outcomes of lipid-lowering therapy (LLT) vary between individuals. Previously we showed that leukocyte lipid uptake and storage (hereafter referred to as lipid trafficking) vary between individuals and are associated with on-treatment LDL-C levels. Here we investigated whether cellular lipid trafficking is influenced at different stages of LLT and whether it can be used to define an optimal LLT strategy.
Methods: Naive persons with an LDL-C >5 mmol/l (n=43), or patients undergoing LLT (n=25) were included in this study. Blood lipid values were assessed during the visit and LLT was prescribed or adjusted according to the best practices. The patients returned to one or two follow-up visits during which the therapy was adjusted if cholesterol targets had not been reached. Samples for PBMC isolation, NMR-metabolomics, statin adherence and sterol metabolite analysis were collected at each visit. 26 readouts for cellular lipid trafficking were derived from PBMC cell samples and correlated with the other biomarkers.
Results: In subjects on statin monotherapy (n=26), monocyte lipid trafficking was negatively associated with concentrations of XS-VLDL (rs =−0.36, p=0.06), IDL (rs=−0.35, p=0.08), L-LDL (rs=−0.42, p=0.03), M-LDL (rs=−0.38, p=0.05), S-LDL (rs =−0.44, p=0.02). LLT-naive patients that were prescribed combination therapy at their first visit displayed lower lipid trafficking scores than those prescribed statin monotherapy (0.14±0.06 vs 0.25±0.07, p<0.01). Moreover, of those statin users who were escalated to statin and ezetimibe combination therapy (n=13), the ones with higher lipid trafficking scores achieved larger LDL-C reductions (rs =0.58, p=0.04) and lower absolute LDL-C values (rs =−0.47, p=0.1).
Conclusion: Cellular lipid trafficking scores provide novel insight into interindividual variation of LLT outcomes, providing new opportunities for treatment optimization.

Apolipoprotein M and incident cardiovascular events in patients with chronic kidney disease

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Background: Cardiovascular disease (CVD) poses a significant risk in patients with chronic kidney disease (CKD), with traditional risk factors often failing to predict outcomes. Identifying alternative markers for high-risk patients is crucial, especially as CVD is a leading cause of morbidity and mortality in CKD. High-density lipoproteins (HDL) represent potent anti-atherogenic factors in humans. Recent research has linked some of the vasoprotective properties of HDL to a specific subset – HDL particles containing apolipoprotein M (apoM). Plasma apoM is primarily linked with HDL, serving as a carrier for the bioactive lipid sphingosine-1-phosphate, and exhibiting robust vascular-protective effects

Methods: In the CARE FOR HOME study, plasma apoM levels were measured by enzyme-linked immunosorbent assay. The study included non-dialysis CKD patients with CKD stages G2-G4. 125 patients met the predefined cardiovascular endpoint, a composite of all cardiovascular events including cardiovascular death after a median follow-up of 4.5 ±2.4 years. The relationship between plasma apoM and adverse outcomes in patients with CKD was investigated.

Results: In the present study, plasma apoM levels were reduced in patients with advanced CKD. Interestingly, patients with prevalent CVD and diabetes mellitus had lower apoM levels compared with non-diseased patients. Cox regression analyses revealed a significant inverse association of plasma apoM with the cardiovascular endpoint. Even after adjustment for traditional cardiovascular risk factors, apoM remained a significant predictor for the cardiovascular endpoint.

Conclusions: Here we could show that in a cohort of CKD patients, plasma apoM was independently associated with cardiovascular events. Therefore, our study supports the role of apoM as a potential predictor of CVD in CKD patients.

Lipoprotein metabolism and inflammation in healthy young subjects – exploring the postprandial and postabsorptive phases following intake of a standardized meal

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Aims: To describe how a standard meal affected the postprandial and postabsorptive lipoprotein metabolism and inflammatory response, and differences in response by sex, age, and BMI.
Methods: We conducted a trial with 34 normal weight subjects aged 20-30 y. Subjects fasted 12 hrs, had blood sampled, consumed a standardized breakfast meal, and had blood sampled 13 more times over 24 hrs. NMR spectroscopy was used to quantify lipoprotein subclasses and various biomarkers, and ELISA to analyse VCAM-1, ICAM-1, E-selectin, and IL-6. We characterized the responses using visualizations and non-linear mixed effects models.
Results: Six VLDL subclasses increased 11-429% within two to four hrs postprandially, fell to normal levels again, followed by another increase after eight to ten hrs. IDL and three LDL subclasses increased around 10-12% over 14 hrs. Four HDL subclasses showed an inverse association with VLDL subclasses, reaching their lowest levels about one hr after the meal and peaking after eight to ten hrs with a 5-15% increase from baseline. Cholesteryl esters and other lipid fractions within subclasses followed the overall pattern of the lipoprotein particles. Standard lipid markers also displayed consistent time-related responses; for example, LDL-C increased 11% from baseline over 24 hrs. Inflammatory markers VCAM-1, ICAM-1, IL-6, and GlycA had significant but small changes over time, while E-selectin remained unchanged. For most biomarkers, males generally exhibited higher concentrations and more prominent responses compared to females, except for HDL particles, where females tended to have higher concentrations and more prominent responses. Age generally had little effect on concentrations or responses, while subjects with highest BMI exhibited higher absolute concentrations but similar responses.
Conclusion: Food intake induced postprandial and postabsorptive responses in lipoprotein metabolism and inflammatory biomarkers, reflecting normal physiology.

Altered Functionality of Lipoprotein(a) Impacts on Angiogenesis in Diabetic Retinopathy erative Diseases

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Purpose: Diabetic retinopathy (DR) is a complication of type 2 diabetes mellitus (T2DM). Lipoprotein(a) (Lp(a)) contributes to the progression of DR, but how is unclear. In homeostasis of the retinal microvasculature, myeloid-derived pro-angiogenic cells (PACs) also play a pivotal role, and fail to function properly in diabetic conditions. Here, we explored the putative contribution of Lp(a) from patients with T2DM with/without DR and healthy controls on inflammation and angiogenesis of retinal endothelial cells (RECs), and on PAC differentiation. Subsequently, we compared the lipid composition of Lp(a) from patients to that from healthy controls.

Methods: Lp(a)/LDL obtained from patients and healthy controls were added to TNF-alpha-activated RECs. Expression of VCAM-1/ICAM-1 was measured using flowcytometry. Angiogenesis was determined in REC-pericyte co-cultures stimulated by pro-angiogenic growth factors. PAC differentiation from peripheral blood mononuclear cells was determined by measuring expression of PAC markers. The lipoprotein lipid composition was quantified using detailed lipidomics analysis.

Results: Lp(a) from patients with DR (DR-Lp(a)) failed to block TNF-alpha-induced expression of VCAM-1/ICAM-1 in REC whereas Lp(a) from healthy controls (healthy control [HC]-Lp(a)) did. DR-Lp(a) increased REC angiogenesis more than HC-Lp(a) did. Lp(a) from patients without DR showed intermediate profiles. HC-Lp(a) reduced the expression of CD16 and CD105 in PAC, but T2DM-Lp(a) did not. Phosphatidylethanolamine content was lower in T2DM-Lp(a) than in HC-Lp(a).

Conclusions: DR-Lp(a) does not show the anti-inflammatory capacity seen with HC-Lp(a), but increases REC angiogenesis, and affects PAC differentiation less than HC-Lp(a). These functional differences in Lp(a) in T2DM-related retinopathy are associated with alterations in the lipid composition as compared to healthy conditions.

A Novel Flag Epitope Tag Knock-In ApoB Mouse Model to Investigate Intracellular ApoB/VLDL Biology

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Background and aim: ApoB/VLDL metabolism disturbances are associated with metabolic-associated steatotic liver disease and atherosclerosis. We have generated a new knock-in mouse model for in-depth studies to enhance our understanding of intracellular VLDL metabolism.

Methods: Employing CRISPR/Cas9, we integrated a 24-nucleotide sequence coding for a Flag epitope tag (DYKDDDDK) before the stop codon of the murine ApoB gene, creating ApoB-Flag tag knock-in (KI) mice. Male and female homozygotes for ApoB-Flag and wild-type were fed a chow diet. Immunoblotting was used to assess ApoB-Flag production by the KI mice. Characterization included quantifying plasma lipids and lipoproteins, body weight, and food intake. Liver histology was studied using haematoxylin and eosin (H&E) staining, and hepatic lipids were stained using Oil Red O. The subcellular localization of ApoB-Flag in primary hepatocytes was studied with immunofluorescence (IF) using a commercially available anti-Flag monoclonal antibody.

Results: Compared to controls, ApoB-Flag mice displayed no differences in food intake, body weight gain, liver and plasma lipids, or liver histology, and the ApoB-Flag protein was detected in both the livers and plasma of the KI mice. We showed that their primary hepatocytes produce and secrete ApoB-Flag into the culture medium. IF studies revealed a perinuclear localization of ApoB-Flag (Fig. 1).

Conclusion: C-terminal tagging of ApoB with a Flag tag does not impact lipid metabolism in mice. We confirmed ApoB-Flag expression in primary hepatocytes, liver, and plasma using anti-Flag antibodies, opening the door for ultrastructural analysis of hepatic VLDL biogenesis by state-of-the-art correlated light and electron microscopy in liver sections and primary hepatocytes (underway). This versatile model is expected to advance our understanding of intracellular VLDL biology.

YIA Poster Walk – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

SMLR1 is a novel player in chylomicron metabolism

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Background and aims: The intracellular assembly, trafficking, and secretion of lipoproteins is incompletely understood. One of the most recent novel players in lipoprotein metabolism is SMLR1: a protein primarily expressed in the liver and the small intestine. Previously, we have shown that hepatic SMLR1 ablation in mice attenuates VLDL secretion, increases hepatic steatosis, and protects against atherosclerosis (van Zwol et al., Hepatology, 2023). The current study aims at exploring the contribution of intestinal SMLR1 to lipoprotein metabolism.

Methods: A Smlr1 whole-body knockout mouse model (Smlr1-KO) was generated by ablating exon 2 of the gene using CRISPR/Cas9 technology. Ablation was confirmed using qPCR in the liver and the small intestine. Mice were fed a chow diet and plasma lipids were measured using commercially available kits. Liver and intestinal jejunum histology were studied using haematoxylin and eosin (H&E) staining. SMLR1 was ablated in Caco-2 cells using CRISPR/Cas9. Intracellular lipids were extracted using Bligh & Dyer. For immunofluorescence, cells were stained with DAPI and Lipidtox for the nucleus and lipids, respectively.

Results: In a first study, whole-body ablation of SMLR1 resulted in a reduction in plasma cholesterol as previously seen in liver-specific KO mice. Histochemical analysis revealed lipid accumulation in the liver and intestinal jejunum of Smlr1-KO mice as shown in Figure 1. Additionally, lipid accumulation occurred in Caco-2 SMLR1-KO cells, as seen in Figure 2.

Conclusions: This study suggests that next to its role in the liver, SMLR1 also plays a role in chylomicron metabolism. Ongoing studies focus on the effects of the whole-body loss of SMLR1 on body weight gain, fecal caloric loss, and the molecular mechanism underlying the liver and intestinal phenotype.

VACUOLAR H+-ATPASE AS A NOVEL TARGET TO LOWER LOW DENSITY LIPOPROTEIN CHOLESTEROL

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Objective: Low density lipoprotein cholesterol (LDL-c) is cleared from the circulation through binding to the hepatic LDL receptor (LDLR). The (pro)renin receptor [(P)RR, also known as vacuolar H+-ATPase (V-ATPase) accessory protein 2 or ATP6AP2)] is a regulator of this process. Indeed, (P)RR silencing markedly reduced LDLR protein abundance and thereby LDL uptake. Since V-ATPase is a multi-subunit complex (consisting of 13 subunits), in this study we evaluated the role of subunits other than the (P)RR.

Methods: siRNAs were designed against all V-ATPase subunits, transfected into HepG2 cells, and evaluated for their capacity to affect cell surface LDLR. Given that silencing subunit ATP6V1B2 upregulated LDLR most, we next designed an adeno-associated virus expressing shAtp6v1b2 and injected this into C57BL/6 mice and then investigated the effects of Atp6v1b2 knocking-down on LDL metabolism in vivo.

Results: Silencing subunit ATP6V1B2 in HepG2 cells increased surface LDLR and total LDLR protein abundance by 1.5- and 2.3-fold, respectively. This was accompanied by both LDLR mRNA upregulation, suggestive for de novo protein synthesis, and enhanced LDL uptake following exposure to excess LDL. Silencing ATP6V1B2 significantly increased the mRNA levels of sterol regulatory element-binding protein 2 (SREBP2)-targeted genes (SQLE, HMGCR, and NPC1) as well as the nuclear SREBP2 protein level. In vivo, knocking-down Atp6v1b2 decreased plasma total cholesterol by 23%, without affecting total triglycerides. This was accompanied by LDLR upregulation in the liver, both at the mRNA and protein levels.

Conclusions: The V-ATPase subunit ATP6V1B2 is an important regulator of LDLR expression. This involves the transcription factor SREBP2, and allows substantial LDL-c lowering. V-ATPase might be a novel pharmacological target to lower cholesterol.

Sex differences of Lp(a) in individuals aged 18-50 years – a nationwide study of 185,000 individuals

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Background: Lipoprotein(a) [Lp(a)] is an independent and causal risk factor for atherosclerotic cardiovascular disease (ASCVD). Although women above 50 y have higher Lp(a) levels than men, there are few studies with a significant sample size that have studied sex differences in younger individuals.

Aim of study: To investigate the association between Lp(a), age and sex in young people.

Methods: We used nationwide clinical laboratory data from Fürst Medical Laboratory with 272,463 Lp(a) measurements from 185,493 unique individuals <50 years of age, measured between 2000 and 2019. The data material was restricted to individuals <50 years due to a low use of medication in this age group. The association between sex and age on Lp(a) levels were determined using linear models with log transformed Lp(a) as dependent variable and age and sex and their interaction as independent variables. Estimated marginal means were obtained to study the average Lp(a) level at each age group level, adjusted for multiplicity.

Results: Women had significantly higher Lp(a) levels than men in age group 18-31.5 and 31.5-37.7 ($P < 0.001$). Mean Lp(a) was 27.4 mg/dL in women and 25.8 mg/dL in men aged 18-31.5 y, and 28.1 mg/dL in women and 26.8 mg/dL in men aged 31.5-37.7 y. Estimated marginal means of Lp(a) levels increased by 7.5% in women and 11.1% in men between age group 18-31.5 to 46.5-50. eGFR did not significantly affect the Lp(a) levels in this age range.

Conclusion: This is the first study that shows that women have higher levels of Lp(a) than men in individuals aged 18-38 years. There was also a modest increase of Lp(a) levels by age from 18 to 50 y. The clinical impact of these findings is not known, but are nevertheless biologically interesting.

The choice of lipoprotein(a) immunoassay can affect clinical decisions

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Background and aims: Different assays have been used to analyze lipoprotein(a) over time, and the need for standardization of Lp(a) assays is well-known. Nonetheless, the impact of the use of different assays for clinical decision making and risk stratification of individuals is understudied. This study aimed to investigate how the change from one commercially available assay to another at a large central laboratory affected the proportion of individuals with Lp(a) laboratory result over specific thresholds.

Methods: We used nationwide clinical laboratory data from Fürst Medical Laboratory with 272,463 Lp(a) measurements from 185,493 individuals measured between 2000-2019. The data material was restricted to individuals <50 years due to a low use of medication in this age group. Lp(a) was analyzed in fresh serum samples using immunoturbidimetric methods from 1) Roche Tina-quant assay in 2000–09 (N individuals = 75,221) and 2) Siemens Lipoprotein(a) [LPA] assay (N individuals = 123,824) in 2009-19 in mg/dL units. Due to changes in the cutoff that was used when reporting Lp(a) concentration, the main analyses are based on measurements from 2000-09 and 2018-19.

Results: Roche assay detected 1) 20% more individuals with Lp(a) >50 mg/dL than Siemens assay (23%, N = 17,183 vs. 19%, N = 3,627), 2) 40% more individuals with Lp(a) >100 mg/dL than the Siemens assay (7.1%, N = 5,343 vs. 5.1%, N = 990) and 3) 200% more individuals with Lp(a) > 180 mg/dL than the Siemens assay (0.86%, N = 645 vs. 0.43%, N = 83). Results were similar when repeating the analysis with data only for individuals that had measurements analyzed by both assays.

Conclusion: The transition from one commercially available Lp(a) assay to another can significantly impact the number of individuals who receive laboratory results exceeding specific thresholds. This has notable clinical implications as these thresholds are frequently used in risk assessment and initiation of preventive treatment.

Effects of Intermittent Fasting on HDL Function in Individuals with Type 2 Diabetes Mellitus

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Background:
Cardiovascular disease is a significant risk factor for mortality in people with type 2 diabetes mellitus (T2DM). High-density lipoprotein (HDL) is thought to play a central role in maintaining cardiovascular health through its multiple atheroprotective effects. Dietary interventions and specific dietary components have been shown to improve HDL function, but the effect of fasting on HDL efficacy remains unclear. This study aimed to evaluate the effects of intermittent fasting as a strategy to safely improve glycemic control and reduce body weight on functional parameters of HDL in T2DM patients.

Methods:
In a 12-week intervention study, we investigated the effects of intermittent fasting (IF) on HDL function, considering its anti-inflammatory, antioxidant, compositional, and metabolic aspects. This study compared a fasting group to a control group. Both groups were advised to maintain a well-balanced diet meticulously designed by a qualified dietician. The fasting group followed an alternate-day fasting routine, reducing their calorie intake by 75% on fasting days. Serum samples were collected before and after the intervention to evaluate HDL function and metabolism.

Results:
Substantial enhancements in HDL cholesterol efflux capacity and elevated levels of apolipoprotein M were observed in the IF group following a 12-week intervention involving a well-balanced diet. Intriguingly, the control group also demonstrated improvements in cholesterol efflux capacity, accompanied by remarkable increases in paraoxonase-1 activity and lecithin-cholesterol acyltransferase activity.

Conclusions:
We conclude that a well-balanced diet and well-controlled insulin therapy affect HDL function and appear to be advantageous for cardiovascular health in patients with T2DM. Additional IF does not further improve HDL functionality but rather weakens the effects of a well-balanced diet.

Posters – Abstracts –

Other Topics

SESSION IV

Quantification of Hospitalization or Outpatient Consultations Caused by Pre-Selected Diagnostic Codes in Individuals with Familial Hypercholesterolemia (FH).

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Background: Could FH be associated with any non-atherosclerotic diseases? Our objective was to investigate the occurrence of various pre-specified diagnoses in a large cohort of individuals with a verified FH mutation.

Methods: We followed 5609 individuals with FH, and 112,183 age- and sex-matched controls during 2008 to 2019. In adherence to data protection regulations in Norway, data extraction was constrained to a predefined set of ICD-10 diagnoses. We decided to study a restricted number of ICD-10 diagnostic codes prior to the data access. In addition to atherosclerotic diseases, we requested data on a subset of diagnoses with an undetermined relationship to FH, such as musculoskeletal system disorders, hypothesizing a potential overrepresentation possibly linked to aggressive statin use over many years.

Results: Data in Table 1 presents the number of hospitalizations or outpatient clinic contacts, not assessing the individual-level risk between the FH and control groups. As expected, the FH group exhibited a pronounced overrepresentation of various cardiovascular diseases as shown in Tables 2-3 using aortic aneurism as an example. 74 incident aortic aneurysm were observed in the FH population, versus 824 in controls (Tab 2). The incidence rate per 1000 person years (95% CI) was 1.50 (1.19-1.88) in FH and 0.84 (0.79-0.90) in controls, yielding a HR (95%CI) of 1.71 (1.34-2.17). Individuals with FH also had an almost 2-fold higher risk of surgical treatment of AA (HR 1.99 (1.29-3.06).

Conclusion: Several forms of CVD were prevalent in the FH group, as expected. In most other disease categories, the frequency of events appeared comparable between the FH group and the control group, however data suggests a potential need for additional investigations into the risk of muscle diseases (M60-M63), congenital malformations of the circulatory system (Q20-Q28), and diabetes (E10-E14).

Cardiovascular risk factors and risk of vascular-related dementia in high-risk and low-risk prospective cohorts

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Aim: Dementia is the 2nd most common cause of death in high-income countries. Up to 40% of all dementia cases may be preventable, primarily by treating or acting on well-established cardiovascular risk factors. Here we explore which midlife cardiovascular risk factors are most important for risk of late-life vascular-related dementia, in a white general population setting of high-risk individuals. Subsequently, we validated these risk factors in a contemporary low-risk population.

Methods: In two prospective cohort studies; a cohort of high-risk individuals including 15,746 individuals (54% women) from the Copenhagen City Heart Study's first two examinations in the 1970s and 1980s with up to 43 years of follow-up, and a contemporary cohort study of low-risk individuals including 409,506 individuals (54% women) from the UK Biobank with up to 16 years of follow-up, we investigated the association between modifiable cardiovascular risk factors and risk of late-life vascular-related dementia using multifactorially adjusted Cox proportional hazards regression models.

Results: In a high-risk population midlife diabetes, smoking, low physical activity, low educational level, hypertension, high body mass index (BMI), and triglycerides were associated with increased risk of late-life vascular-related dementia with hazard ratios of 1.12-2.42. In a low-risk contemporary population, midlife diabetes, smoking, low physical activity, low educational level, and high BMI were associated with increased risk of late-life vascular-related dementia with hazard ratios of 1.11-3.99. Results were similar when assessing women and men separately.

Conclusion: Midlife diabetes, smoking, low physical activity, low educational level, and high BMI all associate with late-life vascular-related dementia in both a high-risk population and a contemporary low-risk population. These findings emphasize the importance of early prevention of cardiovascular risk factors for improved brain health.

Identifying biomarkers of fat-specific dietary patterns using a multi-omics approach

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Aim: To identify potential biomarkers of fat-specific dietary patterns using a "multi-omics" approach.
Methods: We used data from an 8-week double-blind RCT where 6 E% saturated fatty acids was exchanged by polyunsaturated fatty acids; data from the screening visit will be used in a discovery phase (n=240) while the intervention visits at baseline and end-of-study will be used in a test phase (n=100). Data used are dietary intake based on FFQ/24 hr recalls (53 food groups), PBMC gene expression (13 968 mRNA transcripts), targeted metabolomics (225 variables for NMR, > 100 for non-NMR), and untargeted UPLC-HRMS metabolomics (740 negative and 2120 positive mode metabolites). So far, we have derived and characterized three fat-specific dietary patterns (DPs) using PLS regression based on data from the screening visit. In further analyses, we will use LASSO regression to associate the DPs with original gene expression variables and dimensionality-reduced features, the latter including CIBERSORT-predicted cell types and WGCNA-based gene clusters. We will also use LASSO regression to associate the DPs with original metabolomics variables and dimensionality-reduced features based on PCA. All top hits will be considered candidate biomarkers, including gene transcripts, gene set-derived biological processes, metabolites, and metabolite set-derived metabolic pathways, and thus subjected to further testing using the data from the intervention visits. Positive hits will be regarded as potential biomarkers of fat-specific DPs, which should be further investigated in external cohorts for possible academic or clinical utility.
Results and conclusions: Analyses are ongoing, and results will be presented at the meeting.

YIA Poster Walk – Abstracts –

Other Topics

SESSION IV

Regulation of visceral adipose tissue inflammation and metabolism by signals in the vagus nerve

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BACKGROUND: Excessive accumulation of visceral fat and adipose tissue inflammation are hallmarks of the metabolic syndrome. Recent advances indicate that adrenergic signals originating in the sympathetic nervous system regulate adipose tissue metabolism, but whether cholinergic signals in the parasympathetic nervous system regulate adipose tissue metabolism and inflammation is unknown. Accordingly, we hypothesized that signals in the vagus nerve regulate adipose tissue inflammation and metabolism.

METHODS: C57BL/6J male mice and ChAT-eGFP reporter mice, respectively, were subjected to either sham surgery or left cervical vagus nerve stimulation (VNS) or vagotomy. Mice were euthanized 12 h thereafter. Epididymal adipose tissue and plasma were collected and analyzed by flow cytometry and ELISA.

RESULTS: Neutrophil numbers in epididymal adipose tissue were significantly higher in vagotomized mice compared with sham. Moreover, the level of MCP1 in plasma measured by ELISA was significantly higher in vagotomized mice compared to sham. The weight of fat pads was lower 7 days after vagotomy compared with sham. Also, phosphorylation of Hormone-Sensitive Lipase (HSL) in adipocytes was increased in vagotomized mice compared with sham.

In ChAT-eGFP reporter mice, the percentage of GFP+ immune cells in epididymal adipose tissue was significantly higher in vagotomized mice as compared with sham-treated mice. In ChAT-eGFP reporter mice injected i.p. with the TLR2 agonist zymosan, we observed that frequencies of GFP+ immune cells in epididymal adipose tissue were significantly lower in vagotomized mice, and frequencies of GFP+ immune cells in epididymal adipose tissue significantly higher in vagus nerve stimulated mice compared with sham.

CONCLUSIONS: These observations indicate that signals in the vagus nerve regulate immune cell infiltration, including cholinergic immune cell frequencies, and metabolism-associated outcomes in epididymal adipose tissue.

Lifestyle characteristics and plasma biomarkers for risk of NAFLD differ by sex in the general population

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Aim of study: The rise in global prevalence of non-alcoholic fatty liver disease (NAFLD) has highlighted the importance for development of sex-specific risk profiles for NAFLD. This study aims at developing sex-specific risk profiles for NAFLD according to lifestyle characteristics and plasma biomarkers.
Methods: 3,282 women and 2,167 men from the Copenhagen General Population Study who had a computed tomography (CT) scan of the thorax and abdomen were included; all these individuals had measured information on 17 lifestyle characteristics and plasma biomarkers. Severe and severe+moderate NAFLD were defined as liver attenuation of <48 HU and <56 HU (lower number indicating more fat).
Results: We found that for most characteristics, women had less liver fat than men at the same values for the given characteristics. However, odds ratios for NAFLD were more pronounced in women than in men for similar abnormal categories of most lifestyle characteristics and plasma biomarkers (p-values for interaction 4×10-7 to 3×10-25). Risk profiles for severe NAFLD (<48 HU) included waist circumference, smoking, and HDL cholesterol for both sexes. For women, risk profiles further included body mass index, systolic blood pressure, and remnant cholesterol, while for men, risk profiles further included alanine transaminase. Risk profiles for severe+moderate NAFLD (<56 HU) included waist circumference, body mass index, smoking and remnant cholesterol for both sexes. For women, risk profiles also included physical activity, while for men, risk profiles further included alanine transaminase and C-reactive protein.
Conclusion: Risk profiles for NAFLD according to 17 lifestyle characteristics and plasma biomarkers differ by sex. This knowledge is important when screening individuals for signs of NAFLD.

Large-scale gene-age interaction analyses of cardiometabolic risk factors in 270,276 Europeans

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Background and aims: Although the genetic landscape of cardiometabolic risk factors have been explored extensively, investigations on whether the genetic effects remain constant over the life course are scarce. This study aims to investigate the interactions between single nucleotide polymorphisms (SNPs) and age on cardiometabolic risk factors.

Methods: We conducted genome-wide interaction studies (GWIS) in 270,276 unrelated European-ancestry participants from UK Biobank (54.2% women, mean [standard deviation] age 56.7 [8.0] years). Analyses with linear regression models including a multiplicative SNP-age interaction term were performed on apolipoprotein B (ApoB) (g/L), low-density lipoprotein-cholesterol (LDL-C) (mmol/L), log-transformed triglycerides (TG) (mmol/L), body mass index (BMI) (kg/m2), and systolic blood pressure (mmHg).

Results: Only few genetic variants were identified to have genome-wide significant SNP-age interactions (P < 1e-8 for the interaction term) for some of the traits. In detail, rs429358 (mapped to APOE) was identified for both ApoB (P = 9.0e-14) and TG (P = 5.4e-16). Three additional lead SNPs were identified for ApoB: rs11591147 [PCSK9] (P = 3.9e-09), rs34601365 [TDRD15] (P = 8.4e-09), and rs17248720 [LDLR] (P = 2.0e-09). No significant SNP-age interaction effects were found for LDL-C, systolic blood pressure and BMI. The effect sizes of identified lead SNPs decreased with age, except for rs11591147.

Conclusions: Awaiting replication of the main study results, the present project indicated that the genetic effects on cardiometabolic risk factors vary little with age. However, the exceptions for ApoB and TG illustrate the importance of understanding the aetiology of cardiometabolic disease throughout different stages of the life course.

Expression and characterization of modified recombinant human acid sphingomyelinase variants for LDL aggregation assay

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In 2018, Ruuth et al. developed a method to measure low-density lipoprotein (LDL) aggregation susceptibility in vitro, which demonstrated the correlation with cases of atherosclerotic cardiovascular diseases (ASCVD). The method includes the use of human recombinant sphingomyelinase (hrSMase), an enzyme that hydrolyzes sphingomyelin. This reaction is thought to influence the conformation of apolipoprotein B within the LDL particles leading to their aggregation, which can be measured and followed in vitro.

The general aim of the project is to optimize the components and reproducibility of the described method. The previously used full-length hrSMase was rather unstable, thus, one of the objectives was to optimize its amino acid sequence, produce it and test its activity and functionality within the assay. The sequence modifications were expected to increase the enzymatic activity of hrSMase based on the previous approaches, where it was achieved through the modification and deletion of C-terminal cysteine. Our versions of hrSMase represent truncated sequences of the enzyme with N- or C-terminal polyhistidine tags.

The produced hrSMases have demonstrated significantly higher levels of specific activity and stability than the previously used hrSMase. New hrSMases were used to analyze LDL aggregation susceptibility in different patient samples and have shown certain differences in the enzymatic behaviour, resulting in dissimilar LDL aggregation levels, although with high inter-assay reproducibility.

We will further investigate the differences in the functions and lipolysis of the two hrSMase versions considering their structural features as well as the influence of the reaction conditions and the substrate composition. As a result, a more active and stable version of hrSMase can be used for further studying of LDL aggregation susceptibility and potentially in high-throughput clinical settings for early assessment of an elevated ASCVD risk.

The lifestyle and cardiovascular risk factors in Norwegian patients with a severe mental illness

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BACKGROUND: Patients with severe mental illness have increased risk for cardiovascular disease, and modifiable risk factors are common. As a result, these patients have a shortage of 15-20 years in life expectancy compared to the general population. However, the lifestyle of Norwegian patients with severe mental illness has not been studied previously.

METHODS: Patients with schizophrenia or bipolar disease 1 were recruited from a municipality Psychiatric ward in Asker, Norway. Anthropometry and blood parameters were measured or obtained from medical journals. A digital questionnaire (DIGIKOST) measured dietary habits, exercise, and tobacco- and alcohol habits. Qualitative statements related to diet and lifestyle were recorded to nuance the findings.

RESULTS: 42 patients, 23 men and 19 women, were included in the study. Mean age was 41 and 42 years, respectively. Average body mass index corresponded to obesity, and more than half of the patients had abdominal obesity measured as waist circumference. Prevalence of metabolic syndrome was 50%. Dietary quality was low to moderate. Patients reported little exercise and frequent smoking. Barriers for a healthy lifestyle related to psychiatric treatment was described, and the patients wanted dietary tutoring.

INTERPRETATION: The lifestyle of severe mentally ill patients in Asker increased cardiovascular risk. They are thought to benefit from lifestyle- and dietary tutoring. Results from this assessment revealed potential to reduce cardiovascular risk in Norwegian patients with severe mental illness.

Tspan18 regulates preadipocyte expansion during adipogenesis

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Healthy adipocytes and adipose tissue expansion are important pillars of cardiometabolic health. Conversely, adipocyte hypertrophy in obesity or limited development in lipodystrophy are linked to inflammation, insulin resistance, and ectopic lipid deposition, which promote dyslipidemia and favor the development of atherosclerosis. Adipogenesis, the differentiation of preadipocytes into mature adipocytes is vital for maintaining healthy adipose tissue expansion. However, the intricacies of adipogenesis involve a complex interplay of multiple molecular entities that are not yet fully understood. Here, we explore the potential regulatory role of Tspan18, a transmembrane protein of the tetraspanin family, in adipocyte differentiation.
Employing both immortalized brown adipocytes (imBAT) and 3T3-L1 cells, we conducted siRNA-mediated knockdown (KD) and CRISPRa-SAM overexpression (OE) experiments targeting Tspan18. Tspan18 gene expression is highly induced early in adipocyte differentiation before classical adipocyte genes such as Fabp4 or Lpl. No discernible effects were observed when Tspan18 was silenced in mature adipocytes, hinting at an involvement of Tspan18 in mitotic clonal expansion during the early phases of adipogenesis. Interestingly, glucocorticoids were required but insufficient for the induction of Tspan18. Silencing of Tspan18 in preadipocytes resulted in decreased cell proliferation during early adipogenesis, resulting in less adipocytes with unchanged function. In addition, utilizing Fluo-8, a fluorescent Ca2+ indicator, we noted an elevation in cytoplasmic Ca2+ in cells with Tspan18 KD following thapsigargin treatment.
Our results introduce Tspan18 as a novel regulator of early adipogenesis and preadipocyte expansion with a role in Ca2+ signaling. By influencing adipocyte amount and fostering proper adipose tissue development, Tspan18 emerges as a potential candidate to therapeutically facilitate beneficial
hyperplasia in obesity-related diseases.

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