28th Annual Scandinavian Atherosclerosis Conference

April 6th-9th at Krogerup Højskole, Humlebæk, Denmark

2022 Program

SCIENTIFIC COMMITEE

Milena Schönke (Netherlands)
Bo Angelin (Sweden)
Line Bisgaard (Denmark)
Pirkka-Pekka Laurila (Finland)
Ewa Ehrenborg (Sweden)
Jacob Juel Christensen (Norway)
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Organized by

SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH

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HOMEPAGE

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Wednesday, April 6th, 2022

16.00 – 18.00	Arrival, registration, and coffee (dining room until 17.45)
18.00 – 19.30	Dinner
19.30 – 19.35	Welcome **Mette Christoffersen** *(Denmark)*
THE NIKKILÄ MEMORIAL LECTURE	
19.35 – 19.40	Introduction of the 2022 Nikkilä Lecturer **Minna Kaikkonen-Määttä** *(Finland)*
19.40 – 20.25	*Atherosclerosis – from mice to men* **Bente Halvorsen** *(Norway)*
20.25 – 20.45	Discussion
20.45 –	Pub will be open

Thursday, April 7th, 2022

07.45 – 08.45	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Chaired by **Milena Schönke** (Netherlands) and **Bo Angelin** (Sweden)
08.45 – 09.10	*Invited speaker* Role of T-cell cholesterol efflux pathways in aging and atherosclerosis **Marit Westerterp** (Netherlands)
09.10 – 09.15	Discussion
09.15 – 09.30	Effect of semaglutide on dyslipidaemia and aortic plaque development in the GAN diet-induced obese LDLR-KO mouse model of atherosclerosis **Urmas Roostalu** *(Denmark)*
09.30 – 09.45	CCN2 Deficiency Causes VSMC De-differentiation Leading to Severe Aortapathy in Normo- and Hyperlipidemic Mice **Lasse Bach Steffensen** *(Denmark) - YIA*
09.45 – 10.00	Efferocytosis induces a transient burst of glycolysis in macrophages to promote lactate-driven binding and continuing removal of apoptotic cells **Maaike Schilperoort** *(The Netherlands) - YIA*
10.00 – 10.15	Investigating inflammatory processes of plaque destabilization using proteomics **Lasse Lorentzen** *(Denmark) - YIA*
10.15 – 11.15	**Poster Walk (Session I).** Coffee in dining room.
11.15 – 11.40	*Invited speaker* Understanding cardiovascular inflammation requires neuroscience **Laura Tarnawski** *(Sweden)*
11.40 – 11.45	Discussion
11.45 – 12.00	Androgens worsen myocardial infarction via bone cells **Elin Svedlund Eriksson** *(Sweden) - YIA*
12.00 – 12.15	Clonal Hematopoiesis and Inflammation in Obesity **Helin Tercan** *(The Netherlands)*
12.15 – 12.30	Statins, but not PCSK9 inhibitors, reduce the adipokine chemerin in familial hypercholesterolemia: focus on lipoprotein subfractions. **Lunbo Tan** *(The Netherlands) - YIA*

SESSION II	**OTHER TOPICS** Chaired by **Line Bisgaard** (Denmark) and **Pirkka-Pekka Laurila** (Finland)
12.30 – 12.55	*Invited speaker* The use of lipidomics in CVD research **Federico Torta** *(Singapore)*
12.55 – 13.00	Discussion
13.00 – 14.00	Lunch
14.00 – 15.00	**General meeting of the *Scandinavian Society for Atherosclerosis Research*** Open for all participants, decision on next year's topics and chairpersons Afternoon free for the Louisiana Museum of Modern Art (5 min walk), beach (5 min walk), Kronborg, the castle of Hamlet (12 min by train) or downtown Copenhagen (50 min by train)
16.30 – 17.30	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
17.45 – 18.45	Dinner
SESSION II	**OTHER TOPICS – continued** Chaired by **Line Bisgaard** (Denmark) and **Pirkka-Pekka Laurila** (Finland)
18.45 – 19.10	*Invited speaker* Brown Adipose Tissue in adult humans - what is learned in twelve years **Pirjo Nuutila** *(Finland)*
19.10 – 19.15	Discussion
19.15 – 19.30	Sex differences of lipoprotein(a) levels and associated risk of morbidity and mortality by age: The Copenhagen General Population Study **Sofie Simony** *(Denmark) - YIA*
19.30 – 19.45	Low production of 12α-hydroxylated bile acids prevents hepatic steatosis in Cyp2c70-/- mice by reducing fat absorption **Rumei Li** *(The Netherlands) - YIA*
19.45 – 20.00	Selective inhibition of DHCR24 ameliorates hepatic steatosis and inflammation through LXRα without inducing hyperlipidemia **Patrick Rensen** *(The Netherlands)*
20.00 – 20.45	**Poster Walk (Session II).** Coffee in dining room.

20.45 – 21.00	High triglyceride metabolism and increased mortality: a population-based study of 30,000 individuals **Mia Østergaard Johansen** *(Denmark) - YIA*
21.00 – 21.15	Inhibition of sphingolipid de novo synthesis counteracts age-related loss in fitness **Pirkka-Pekka Laurila** *(Finland)*
21.15 – 21.30	Adipocyte-Nfe2l1 protects from cholesterol-induced lipodystrophy and atherosclerosis **Carolin Muley** *(Germany) - YIA*
21.30 – 21.45	Genetic risk of fatty liver disease and mortality in the general population **Helene Gellert-Kristensen** *(Denmark) - YIA*
21.45 –	Pub will be open

Friday, April 8th, 2022

07.45 – 08.45	Breakfast
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Chaired by **Ewa Ehrenborg** *(Sweden)* **and Jacob Juel Christensen** *(Norway)*
08.45 – 09.10	*Invited speaker* Cardiac lipids and their role for cardiomyocyte function **Malin Levin** *(Sweden)*
09.10 – 09.15	Discussion
09.15 – 09.30	Fibroblast growth factor 21 potently reduces atherosclerosis and NASH development **Cong Liu** *(The Netherlands) - YIA*
09.30 – 09.45	Elevated remnant cholesterol and 2-fold mortality rates from cardiovascular and other causes, but not from cancer **Benjamin Nilsson Wadström** *(Denmark) - YIA*
09.45 – 10.00	Linking cellular lipid metabolism profiles to the outcomes of cholesterol-lowering therapy in a general population cohort study **Iryna Hlushchenko** *(Finland) - YIA*
10.00 – 10.15	Mapping the low-density lipoprotein interactome in mouse atherosclerosis **Rocio Muñiz** *(Spain)- YIA*
10.15 – 11.15	**Poster Walk (Session III).** Coffee in dining room.
11.15 – 11.40	*Invited speaker* Perilipins and regulation of intracellular lipid droplets **Knut Tomas Dalen** *(Norway)*
11.40 – 11.45	Discussion
11.45 – 12.00	Lipoprotein lipase expressed by vascular endothelial cells of activated thermogenic adipose tissues is dispensable for the processing of triglyceride-rich lipoproteins **Ellen Thiemann** *(Germany) - YIA*
12.00 – 12.15	The role of hepatic melanocortin 1 receptor in the pathogenesis and therapeutics of metabolic disease **Keshav Thapa** *(Finland)*
12.15 – 12.30	Impact of preeclampsia on umbilical cord blood lipid concentrations

	Sofie Taageby Nielsen *(Denmark)*
12.30 – 12.45	Functional analysis of LDLR variants using automated systems **Mohammad Majharul Islam** *(Finland) - YIA*
12.45 – 13.45	Lunch
SESSION IV	**CARDIOVASCULAR DISEASE** Chaired by **Anders Berg Wulff** *(Denmark)* and **Sander Kooijman** *(Netherlands)*
13.45 – 14.10	*Invited speaker* Cardiovascular disease prevention: from traditional risk scores to individualized prevention **Martin Bødtker Mortensen** *(Denmark)*
14.10 – 14.15	Discussion
14.15 – 14.30	Characterization of laminin isoforms in human atherosclerotic lesions and human vascular cells **Shuqi Xu** *(Denmark) - YIA*
14.30 – 14.45	Pharmacological treatment with designer cytokine and exercise mimetic IC7Fc reduces atherosclerosis development **Milena Schönke** *(The Netherlands) - YIA*
14.45 – 15.00	Inhibition of pyruvate dehydrogenase kinase prevents abdominal aortic aneurysm formation in mice **Silke Griepke Dam Nielsen** *(Denmark) - YIA*
15.00-15.15	Endothelial integrity in intracranial atherosclerosis; the regulatory role of N6-methyladenosine (m6A) of the pro-atherogenic microRNA miR-494-3p in tight junction proteins **Tamar Woudenberg** *(The Netherlands) - YIA*
15.15 – 16.00	**Poster Walk (Session IV).** Coffee in dining room.
16.00 – 16.25	*Invited speaker* Metabolic control of macrophages in atherosclerosis **Jan van den Bossche** (*The Netherlands)*
16.25 – 16.30	Discussion
16.30 – 16.45	Genetic deficiency of indoleamine 2,3-dioxygenase (IDO)-1 aggravates vascular but not liver disease in a nonalcoholic steatohepatitis and atherosclerosis comorbidity model **Aastna Arora** *(Denmark) - YIA*

16.45 – 17.00	Atherosclerotic Plaque Progression is Associated with Increased Collagen Carbamylation: Impact on Macrophage Functioning **Valeria Saar-Kovrov** *(The Netherlands) - YIA*
17:00 – 17:15	Proteomic Characterization of Atherosclerotic Lesions In Situ Using Percutaneous Coronary Intervention Angioplasty Balloons **Michael Davies** *(Denmark)*
17.15 - 17.20	Concluding remarks **Mette Christoffersen** *(Denmark*)
17.20 – 18.30	Leisure time
18.30 – 19.00	**Cocktail**
19.00 –	**Banquet and dancing**

Saturday, April 9th, 2022

08.30 – 10.00	Breakfast
10.00	Departure
	Have a nice trip back home!!!

28th Annual Scandinavian Atherosclerosis Conference

April 6th-9th at Krogerup Højskole, Humlebæk, Denmark

2022 Posters

Thursday, April 7th, 2022

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 1	Protrudin regulates FAK activation, endothelial cell migration and angiogenesis **Vesa Olkkonen** *(Finland)*
No 5	Possible role of SPP1 in degenerative ascending aortic aneurysm **David Freiholtz** *(Sweden)*
No 6	Modification of "NETs" – a new driver of inflammation in atherosclerosis? **Clare Hawkins** *(Denmark)*
No 8	Mechanical Stretch Regulates Inflammatory Signaling in Human Vascular Smooth Muscle Cells **Lise Filt Jensen** *(Denmark)*
No 19	Exposure of human coronary artery endothelial cells to hypoxia results in extracellular matrix remodelling **Christine Chuang** *(Denmark)*
YIA Poster walk 10.15 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No 4	Run for your live(r): Exercise training at different times of day differentially modulates hepatic inflammation in early NAFLD **Artemiy Kovynev** *(The Netherlands)*
No 9	High lipoprotein(a) and systemic inflammation jointly confer additive high risk of atherosclerotic cardiovascular disease and aortic valve stenosis **Peter E. Thomas** *(Denmark)*
No 11	Leptin, inflammation and trained immunity in obesity **Daniela Flores Gomez** *(The Netherlands)*
No 14	High Plasma Levels of Sphingosine-1-Phosphate Associate with Increased Blood Cholesterol Levels and Reduced Cognitive Speed **Stefan Hajny** *(Sweden)*
No 15	Trained immunity, inflammation and subclinical cardiovascular phenotypes in children with obesity **Siroon Bekkering** *(The Netherlands)*

No 16	Leukocyte subpopulation counts and incident vascular endpoints: observational and genetic studies **Jiao Luo** *(Denmark)*
No 22	Human atherosclerotic lesions contain oxidant-modified extracellular matrix proteins **Karen Yang** *(Denmark)*
No 24	Targeting EZH2 to shift macrophages towards a less inflammatory phenotype in atherosclerosis **Rosalie Kempkes** *(The Netherlands)*
No 25	Mast cells associate with matrix calcification and reprogram smooth muscle cells in atherosclerotic plaques **Nikolaos-Taxiarchis Skenteris** *(Sweden)*
SESSION II	**OTHER TOPICS**
No 80	Non-alcoholic fatty liver disease by fat in blood and body **Lærke Kyhl** *(Denmark)*
No 81	Maternal lipid levels in early pregnancy as a predictor of childhood lipid levels **Jeanine Roeters van Lennep** *(The Netherlands)*
No 86	Association between plasma apolipoprotein M and cardiac autonomic neuropathy in type 1 diabetes **Christina Christoffersen** *(Denmark)*
No 94	Are patients with familial hypercholesterolemia at increased risk of musculoskeletal disorders due to the very long time high intensive lipid-lowering drugs? **Kjetil Retterstøl** *(Norway)*
YIA Poster walk 20.00 – 20.45	**Selected abstracts (3 min presentation + 2 min discussion)**
No 61	Impact of vegetarian and vegan diets on blood lipid and lipoprotein levels: a systematic review and meta-analysis **Caroline Amalie Koch** *(Denmark)*
No 78	Jet lag increases insulin levels and body fat in male but not female mice, as dependent on sex hormones **Wietse In het Panhuis** *(The Netherlands)*
No 87	Body mass index and risk of dementia **Liv Tybjærg Nordestgaard** *(Denmark)*
No 89	Modulation of energy metabolism by eicosapentaenoic acid in skeletal muscle cells from lean and obese individuals

Parmeshwar Katare *(Norway)*

No 93 A rare genetic variant in the manganese transporter SLC30A10 and elevated liver enzymes in the general population
Anne-Sofie Seidelin *(Denmark)*

No 95 Synthetic LXR agonist PFM018 as potential treatment for Alzheimer's Disease
Nikita Martens *(The Netherlands)*

No 96 Efficacy & Safety of PCSK9-Inhibitors: a Systematic Review and Meta-analysis of Real-World Data
Janneke Mulder *(The Netherlands)*

Friday, April 8th, 2022

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SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT**
No 60	Does HDL composition differ between pre-, peri-, and postmenopausal women? **Satu Lehti** *(Finland)*
No 64	Is adipose tissue dysfunction driving the development of pro-atherogenic dyslipidemia? **Fabiana Baganha** *(Sweden)*
No 66	A Randomized Controlled Dietary Intervention did not improved LDL aggregation in Patients with Rheumatoid Arthritis **Helen Lindqvist** *(Sweden)*
No 71	Apolipoprotein M and Retinoid Metabolism **Line Bisgaard** *(Denmark)*
YIA Poster walk 10.15 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No 55	The Ser251Pro SNP in perilipin 2 (PLIN2) affects chaperone-mediated autophagy **Alice Maestri** *(Sweden)*
No 57	Accelerated vascular ageing and retention of LDL in Type 2 Diabetes **Jennifer Härdfeldt** *(Sweden)*
No 59	Elevated LDL-triglycerides and increased risk of atherosclerotic cardiovascular disease: two complementary methods within the Copenhagen General Population Study **Mie Balling** *(Denmark)*
No 62	MicroRNA 33a controls SREBP-2 and LXR dependent regulation of the LDL receptor pathway **Melanie Modder** *(The Netherlands)*
No 63	How ANGPTL4 regulates LPL by catalyzed unfolding **Kristian Kølby Kristensen** *(Denmark)*
No 68	Icosapent ethyl supplementation rapidly and transiently alters the composition and functionality of circulating lipoproteins in humans **Lauri Äikäs** *(Finland)*

No 69	Cholesterol Efflux Capacity of High-density Lipoprotein Particles is Impaired in Age-related Macular Degeneration Patients with High Plasma HDL-c Levels **Yanlin Li** *(The Netherlands)*
No 70	Paradoxical regulation of cholesterol metabolism by fucosterol and saringosterol **Na Zhan** *(The Netherlands)*
No 73	Identification of the ApoC-II binding site on Lipoprotein lipase using HDX-MS **Anni Kumari** *(Denmark)*
No 74	Hypertension in pregnancy: Impact on high-density lipoprotein composition and function **Julia Stadler** *(Austria)*
SESSION IV	**CARDIOVASCULAR DISEASE**
No 27	Female patients with familial hypercholesterolemia have higher cholesterol burden at 19 and 30 years of age: data from 12-years follow-up. **Kirsten Holven** *(Norway)*
No 28	Elevated remnant cholesterol appropriately reclassifies individuals who develop myocardial infarction **Takahito Doi** *(Denmark/Japan)*
No 30	The Lp(a) equivalent to FH in ASCVD risk: CGPS **Berit Storgaard Hedegaard** *(Denmark)*
No 33	Familial Hypercholesterolemia prevalence among ethnicities – systematic review and meta-analysis **Frida Toft-Nielsen** *(Denmark)*
YIA Poster walk 15.15 – 16.00	**Selected abstracts (3 min presentation + 2 min discussion)**
No 29	Viral transgene delivery to endothelial cells in mouse models **Jannik Hjortshøj Larsen** *(Denmark)*
No 31	Unraveling macrophage heterogeneity in human atherosclerosis **Elias Wieland** *(The Netherlands)*
No 34	A Trojan horse in contrast-enhanced MRI? Superparamagnetic iron oxide nanoparticles enhance apoptosis in human and murine atherosclerosis **Adele Ruder** *(The Netherlands)*

No 38	Severe α1-antitrypsin deficiency associated with increased risk of heart failure in two large populations **Sine Voss Winther** *(Denmark)*
No 41	ApoB and non-HDL cholesterol versus LDL cholesterol for ischemic stroke risk **Camilla D. L. Johannesen** *(Denmark)*
No 42	ApoM is associated with prevalent cardiovascular disease in patients with chronic kidney disease **Sarunja Vijayakumar** *(Denmark)*
No 48	Interleukin-6 inhibition in ST-elevation myocardial infarction: Immune cell profile in the randomized ASSAIL-MI trial **Camilla Huse** *(Norway)*

Oral Presentations – Abstracts –

Inflammation and Vascular Biology

SESSION I

Effect of semaglutide on dyslipidaemia and aortic plaque development in the GAN diet-induced obese LDLR-KO mouse model of atherosclerosis

U. Roostalu, S. Evers, T. Porsgaard, C.G. Salinas, A.M. Arraut, H.H. Hansen, M. Feigh

1. Gubra, Hørsholm Kongevej 11B, Hørsholm, Denmark

Background
Atherosclerosis has become the leading cause of premature death and there is consequently an urgent need for new pharmaceuticals. Preclinical research has been hampered by lack of animal models that enable fast analysis of atherosclerosis progression. Here, we aimed at establishing a diet-induced obese (DIO) mouse model with accelerated atherosclerosis and assess the effects of glucagon like peptide 1 receptor agonist semaglutide on dyslipidaemia and vascular lesion formation.
Methods
Low-density lipoprotein receptor knockout mice (LDLR-KO, n=26) were fed Gubra Amylin NASH (GAN) diet for 12 weeks. During the GAN diet feeding period mice were dosed daily with vehicle (n=13) or semaglutide (30 nmol/kg; n=13). Untreated chow-fed wild-type mice (n=10) served as controls. Mice were characterized by plasma biochemistry, and 3D light sheet microscopy of plaque distribution and leukocyte content in intact arterial trees.
Results
Vehicle-dosed GAN DIO-LDLR-KO mice developed adiposity, hepatomegaly and elevated plasma levels of total cholesterol, triglycerides, and low- and high-density lipoprotein (LDL, HDL). 3D imaging revealed consistent atherosclerotic plaque formation in the aortic arch and arterial branching sites in vehicle-dosed GAN DIO-LDLR-KO mice. Compared to vehicle dosing, semaglutide significantly reduced body weight gain, normalized whole-body fat content, decreased plasma triglyceride and HDL levels but had no effect on plasma LDL level. Comparison of vehicle and semaglutide dosed mice by 3D light sheet imaging showed the impact of semaglutide on plaque formation and leukocyte content.
Conclusions
Our data establish the GAN DIO-LDLR-KO mouse model for evaluation of dyslipidaemia and accelerated atherosclerosis and demonstrate sensitive 3D imaging pipeline for preclinical drug discovery research.

CCN2 Deficiency Causes VSMC De-differentiation Leading to Severe Aortapathy in Normo- and Hyperlipidemic Mice

Larsen JH (1), Stubbe J (1), Lindegaard CA (1), Ketelhuth D (1), Wirka RC (6), Lindholt JS (1,4), Pyke C (5), Beck HC (3), Overgaard M (3), Rasmussen LM (2,3), Steffensen LB (1)

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2. Centre for Individualized Medicine in Arterial Diseases, Odense University Hospital, Odense, Denmark
3. Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark
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5. Novo Nordisk A/S, Måløv, Denmark
6. Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA, United States of America

Cellular Communication Network Factor 2 (CCN2) is a matricellular protein, which has been extensively studied in the context of fibrotic diseases and cancers, and clinical testing of anti-CCN2 therapy is ongoing. We found CCN2 to be abundant in non-diseased human artery tissues expressed primarily by vascular smooth muscle cells (VSMCs).

Hypothesising a critical role for CCN2 in artery biology, we first studied aortas of inducible CCN2 knockout (KO) mice. Analysis of aortas showed marked down-reguation of VSMC contractile proteins and thickening of the medial layer. Supporting a role for CCN2 in maintaining VSMC phenotype, Myocardin (MYOCD, master regulator of VSMC differentiation) was significantly reduced in CCN2-deficient aortas. In vitro, CCN2-deficient primary aortic VSMCs from both mice and humans had reduced expression of MYOCD and downstream differentiation markers (e.g. alpha smooth muscle actin, ACTA2). Concomitantly, pro-inflammatory cytokines were up-regulated.

To investigate the consequence of CCN2-deficiency in atherosclerosis, we induced hyperlipidemia by single intravenous injection of rAAV8-D377Y-mPcsk9 combined with western diet for 24 weeks. This resulted in an 8-fold increase in atherosclerosis-development with 80% of en face-prepared thoracic aortas being covered by atherosclerotic lesions.

In advanced human carotid plaques, CCN2 was associated with ACTA2+ VSMCs of the fibrous cap suggesting a role in re-differentiation of modulated VSMCs into myofibroblasts. In support, fibrous cap thickness of aortic root lesions was reduced in CCN2 KO mice compared to wildtype littermates.

In conclusion, our data highlight a crucial role for CCN2 in maintenance of artery integrity and in atheroprotection. This finding contrasts the many detrimental effects reported for CCN2 in other tissue- and disease contexts, and counter-argues the use of anti-CCN2 therapy.

Efferocytosis induces a transient burst of glycolysis in macrophages to promote lactate-driven binding and continuing removal of apoptotic cells

Maaike Schilperoort (1), David Ngai (1), Ira Tabas (1,2,3)

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Aim: Resolving-type macrophages stabilize atherosclerotic plaques by clearing apoptotic cells (ACs) through efferocytosis. These macrophages are thought to rely mainly on oxidative phosphorylation, but emerging evidence suggests a possible link between efferocytosis and glycolysis. To help reconcile this issue, we investigated the role of glycolysis in macrophage-mediated efferocytosis.

Methods: Murine bone marrow-derived macrophages and human monocyte-derived macrophages were incubated with apoptotic Jurkat T-cells to study efferocytosis. The Seahorse analyzer was used to interrogate cellular energy metabolism.

Results: We found that efferocytosis promotes a time-dependent increase in macrophage glycolysis (+50% after 1h, unchanged after 24hrs), which distinguishes this process from more-prolonged glycolysis in pro-inflammatory macrophages. Immunoblotting and gene silencing causation experiments showed that efferocytosis-induced macrophage glycolysis (EIMG), but not inflammation-induced glycolysis, is mediated by post-translational regulation of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2). In terms of consequences, blocking EIMG with 2-deoxyglucose (2-DG) or siRNA-mediated downregulation of PFKFB2 decreased the uptake of subsequent ACs ("continual efferocytosis"). As predicted, blocking EIMG reduced lactate production, and we found that the 2-DG-induced defect in continual efferocytosis was rescued by adding back lactate but not by increasing ATP availability with dichloroacetate. Experiments using the actin inhibitor cytochalasin D showed that inhibition of EIMG blocks subsequent AC binding rather than internalization, suggesting that EIMG-mediated lactate production promotes the sensing and/or binding of ACs by macrophages.

Conclusion: These results indicate a role for EIMG-associated lactate production in continual efferocytosis, which has been shown to have a critically important role in promoting atherosclerotic plaque stabilization, particularly during atherosclerosis regression. Accordingly, ongoing studies are now directed to testing the role of EIMG in atherosclerosis.

Investigating inflammatory processes of plaque destabilization using proteomics

Lasse G. Lorentzen(1), Karin Yeung(2), Henrik Sillesen(2), and Michael J. Davies(1)

(1) Department of Biomedical Sciences, University of Copenhagen, Denmark
(2) Department of Vascular Surgery, Rigshospitalet, Denmark

Atherosclerosis is the major underlying cause of cardiovascular disease (CVD), and as such is a leading cause of death in humans worldwide. While atherosclerosis is often an asymptomatic for years, destabilization and rupture of atherosclerotic plaques can arise suddenly and give rise to an acute and often fatal myocardial infarction or stroke. Despite the importance of plaque stability in CVD, the mechanisms underlying plaque destabilization and rupture are poorly understood. Here we report a proteomics investigation of carotid artery lesion material obtained from endarectomies. By utilizing an efficient single-step extraction method in combination with state-of-the-art mass spectrometry, we were able to identify and quantify >3500 proteins from human carotid artery plaques, including 336 proteins of the extracellular matrix (ECM). Thus, we obtain unprecedented coverage of both the intra- and extracellular proteome across 21 samples (7 hard lesion, 7 mixed lesions, 7 hard lesions) with high reproducibility. We identified 767 proteins with altered abundances between hard and soft lesions, and observe enrichment of proteins involved in inflammatory responses and ECM remodelling. We found upregulation of a range of proteolytic enzymes with concomitant loss of matrix proteins in soft lesions, and to a similar but lesser extent in mixed lesions. This lead us to further investigate proteolytic degradation, and we conducted N-terminal proteomics which can both identify targets of proteolysis and pin-point the exact site of cleavage. We identify 531 proteolytic sites that display increased cleavage in soft lesions, and find that several structural ECM protein such as Fibronectin and Collagens are among the most cleaved proteins. The data presented offers a unique insight into the inflammatory and proteolytic mechanisms of plaque destabilization.

Androgens worsen myocardial infarction via bone cells

Elin Svedlund Eriksson1, Marta Lantero Rodriguez1, Inger Johansson1, Anna K. F. Mårtensson1, Anna S. Wilhelmson2, Andrei S. Chagin3, Malin Hagberg Thulin3, Björn Redfors1, Jan Borén1, Elmir Omerovic1, Malin C. Levin1, Åsa Tivesten1

1Wallenberg Laboratory for Cardiovascular and Metabolic Research, Dept of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
2The Finsen Laboratory, Rigshospitalet, Faculty of Health Sciences, Biotech Research and Innovation Centre (BRIC), Danish Stem Cell Centre (DanStem) Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.
3Dept of Internal Medicine and Clinical Nutrition, Centre for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden.

Background. In acute myocardial infarction (MI), neutrophils rapidly migrate from the bone marrow (BM) to the heart and modulate both the acute and long-term consequences of an MI. BM stromal cells regulate neutrophil egress from the BM. Compared to females, male mice have more neutrophils in the border zone, higher rupture rate/mortality, and increased left ventricular dilatation post-MI. Castration (removal of testes and thereby testosterone production in male mice) reverses these effects, but the underlying mechanism remains unclear.
Aim. Test the hypotheses that androgens adversely affect post-MI injury by regulating neutrophil egress from the BM and that this effect is mediated by the androgen receptor (AR) in BM stromal cells.
Results. Castration of male mice reduced (-43%) the neutrophil content in the heart 48h after experimental MI. Further, castration reduced post-MI mortality, rupture frequency and cardiac dilatation. The adverse effects of endogenous testosterone on cardiac remodelling post MI was inhibited by treatment with neutrophil-depleting antibodies. Genetic ablation of the AR in osterix-expressing osteo-lineage cells (O-ARKO model) fully mimicked the castration effect on neutrophil content in the heart 48h post-MI. O-ARKO mice also showed reduced GCSF-induced mobilization of neutrophils to blood and a strong protection from post-MI death, cardiac rupture and cardiac dilatation; 42% of control vs. 0% of O-ARKO mice died post-MI.
Conclusion. Androgens promote neutrophil egress from the BM by targeting BM stromal cells of the osteo-lineage. This egress increases post-MI neutrophil infiltration, mortality and adverse cardiac remodelling in male mice.
Our results may become important for the design of tissue-selective AR modulators and raise the question whether drugs that target androgen levels/AR action, which are used in large patient groups, may affect the consequences of an MI.

Clonal Hematopoiesis and Inflammation in Obesity

Helin Tercan1, Rosanne van Deuren1, Benjamin Cossins PhD1, Kiki Schraa1, Rob ter Horst1, Inge CL van den Munckhof MD1, Siroon Bekkering PhD1, Joost Rutten MD PhD1, Mihai G Netea MD PhD1, Leo AB Joosten PhD1, Alexander Hoischen PhD1, Niels P Riksen MD PhD1

1. Dept of Internal Medicine and Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

Introduction – Obesity is a risk factor for atherosclerotic cardiovascular disease (CVD) and is associated with chronic low-grade inflammation. Recently, clonal hematopoiesis has emerged as a novel risk factor for CVD. We hypothesize that clonal hematopoiesis is a cardinal driver of inflammatory processes in patients with obesity, especially with advancing age, which subsequently leads to development of CVD.
Methods – In a cross-sectional study, we recruited a cohort of 302 individuals with overweight or obesity (BMI>27), aged between 55 and 81, titled the 300-OB cohort. From all participants, we measured a panel of circulating inflammatory markers and leukocyte number, differentiation, and function (cytokine production capacity upon ex vivo stimulation with Toll-like receptor agonists). In addition, clonal hematopoiesis driver mutations (CHDMs) were identified in blood samples with ultrasensitive single-molecule molecular inversion probe sequencing assay.
Results –We identified 110 candidate CHDMs in 85 subjects, with VAFs ranging from 0.01% to 34.54%. 69 CHDMs were identified in DNMT3A and 8 in TET2 genes. Baseline characteristics, including age, sex, and BMI were similar in subjects with or without CHDMs. Subjects with CHDMs with a VAF ≥2%(CHIP-carriers) had higher circulating interleukin-6 (IL-6), but there was no difference in CRP. The presence of CHDMs did not affect cytokine production capacity of PBMCs.
Conclusions – The presence of clonal hematopoiesis is a risk factor for CVD. We identified that circulating IL-6 levels were increased in obese subjects with CHDMs with VAF>2. Other inflammatory markers or cytokine production capacity of isolated PBMCs did not correlate with the VAF of CHDMs. Further mechanistic studies are required to understand causal relationship between clonal hematopoiesis and CVD, particularly in obese individuals.

Statins, but not PCSK9 inhibitors, reduce the adipokine chemerin in familial hypercholesterolemia: focus on lipoprotein subfractions

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Background. Familial hypercholesterolemia (FH) is characterized by severe elevations in circulating LDL-cholesterol, and an increase in the risk of dyslipidemia-related cardiovascular disease (CVD). Chemerin, as a newly identified adipokine, is considered as an additional risk factor for CVD. Here we investigated whether it can be modified by cholesterol-lowering therapy.
Methods. Lipoprotein subfractions were isolated by density gradient ultracentrifugation. Lipids and chemerin concentrations were determined both before and after cholesterol lowering with either a statin (atorvastatin, simvastatin, rosuvastatin, or fluvastatin) or a PCSK9 inhibitor (PCSK9i; alirocumab or evolocumab).
Results. At baseline chemerin levels were 113±46 (statin group) and 95±44 (PCSK9i group) ng/ml, while triglyceride (TG) levels were 1.9±1.6 and 2.6±1.7 mmol/ml, high-density lipoprotein cholesterol (HDL-c) levels were 1.3±0.4 and 1.2±0.4 mmol/ml, and low-density lipoprotein cholesterol (LDL-c) levels were 5.7±1.5 and 4.9±1.4 mmol/ml (P=ns for difference between 2 groups). Chemerin correlated positively with triglycerides ($r=0.45$, $P<0.005$) and negatively with HDL-c ($r=-0.33$, $P<0.01$). Both statins and PCSK9i reduced LDL-c (by 41 and 62%, $P<0.0001$), triglycerides (by 13 and 19%, $P<0.01$), and increased HDL-c (by 8 and 23%, $P<0.01$), but only statins additionally reduced chemerin (by 35%, $P<0.005$). The lipoprotein subfraction profile revealed that chemerin accumulated particularly in the HDL3 fraction (containing >60% of all chemerin in lipoprotein subfractions), with around 30% being present in the HDL2 fraction, and around 3% in the LDL fraction. Statins reduced HDL3-c and HDL3-TG, and the level of chemerin bound to all subfractions. PCSK9i reduced HDL3-c but did not affect HDL3-TG or the level of chemerin bound to HDL3 and HDL2.
Conclusions. Circulating chemerin occurs in different lipoprotein subfractions, accumulating particularly in the HDL3 fraction. Statins, but not PCSK9i, lowers chemerin, possibly by interfering with its levels across lipoprotein subfractions. This may represent a novel cardiovascular protective function of statins.

Oral Presentations – Abstracts –

Other Topics

SESSION II

Sex differences of lipoprotein(a) levels and associated risk of morbidity and mortality by age: The Copenhagen General Population Study

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Background: Lipoprotein(a) is a well-known causal risk factor for cardiovascular morbidity and mortality. Little is known about the effect of age and sex on lipoprotein(a) levels, and it is largely unknown if the same elevation in lipoprotein(a) confer the same increase in risk of morbidity and mortality in women and men.
Objectives: Are lipoprotein(a) levels and lipoprotein(a) associated risk of morbidity and mortality by age similar in women and men?
Methods: We included 37,545 women and 32,497 men from the Copenhagen General Population Study.
Results: Plasma lipoprotein(a) increased with age and in women we found an additional increase around age 50 (age by sex interaction P=4x10-7). In women, levels were 22% higher after menopause (P=1x10-57) and 12% lower during hormone replacement therapy (P=2x10-19). Adjustment for eGFR (estimated Glomerular Filtration Rate) in both sexes and plasma estradiol in women resulted in attenuated sex differences in lipoprotein(a) levels. In sex and age stratified multivariable adjusted models, lipoprotein(a) >40 mg/dL(83 nmol/L) versus <10 mg/dL(18 nmol/L) was associated with increased risk of myocardial infarction, ischemic heart disease, aortic valve stenosis, and heart failure (men only), but not statistically significant with risk of ischemic stroke, cardiovascular mortality, or all-cause mortality.
Conclusion: Lipoprotein(a) levels increase around age 50 selectively in women; however, risk of morbidity and mortality for high lipoprotein(a) was similar in women and men above age 50. This implies that elevated lipoprotein(a) above age 50 is a relatively more common cardiovascular risk factor in women, pointing toward repeat measurement in women above age 50.

Low production of 12α-hydroxylated bile acids prevents hepatic steatosis in Cyp2c70-/- mice by reducing fat absorption

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Atherosclerotic cardiovascular disease (CVD) continues to be a leading cause of death and has been associated with non-alcoholic fatty liver disease (NAFLD): CVD-related events represent major causes of death in NAFLD patients. Differently structured primary and secondary bile acids (BAs) play variable roles in cholesterol and lipid metabolism and inflammation to modulate major CVD risk factors. We have generated Cyp2c70-/- mice with a human-like BA composition, lacking mouse/rat-specific muricholic acids (MCAs), to accelerate translation from mice to humans. We employed this model to assess the consequences of a human-like BA pool on diet-induced obesity and NAFLD development to investigate links between BAs and CVD risk factors. Male and female Cyp2c70-/- mice and wild-type (WT) littermates were challenged with a 12-week Western-type high-fat diet (WTD) with 0.25% cholesterol. Cyp2c70-deficiency induced a hydrophobic BA pool with high abundances of chenodeoxycholic acid, particularly in females, due to sex-dependent suppression of sterol 12α-hydroxylase (Cyp8b1). Plasma transaminases were elevated and hepatic fibrosis was present in Cyp2c70-/- mice, especially in females. Surprisingly, female Cyp2c70-/- mice were resistant to WTD-induced obesity and hepatic steatosis while male Cyp2c70-/- mice showed similar adiposity and moderately reduced steatosis compared to WT controls. Both intestinal cholesterol and fatty acid absorption were reduced in Cyp2c70-/- mice, the latter more strongly in females, despite unaffected biliary BA secretion rates. Intriguingly, the biliary ratio 12α-/non-12α-hydroxylated BAs significantly correlated with intestinal fatty acid absorption and hepatic fat content. Thus, the hydrophobic human-like BA pool in Cyp2c70-/- mice prevents WTD-induced obesity in female mice and NAFLD development in both genders, primarily due to impaired intestinal fat absorption. Our data point to a key role for 12α-hydroxylated BAs in control of intestinal fat and cholesterol absorption, processes that contribute to CVD risk.

Selective inhibition of DHCR24 ameliorates hepatic steatosis and inflammation through LXRα without inducing hyperlipidemia

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Background:
Liver X receptor (LXR) agonism has theoretical potential for treating NAFLD/NASH but synthetic agonists induce lipogenesis and hyperlipidemia in preclinical models. Desmosterol, which is converted by Δ24-dehydrocholesterol reductase (DHCR24) into cholesterol, is a potent endogenous LXR agonist with anti-inflammatory properties. We aimed to investigate the effects of selective DHCR24 inhibition by SH42 on hepatic steatosis and inflammation, two main hallmarks of NAFLD/NASH, in relation to lipidemia.
Methods:
Male APOE*3-Leiden.CETP mice, a well-established translational model for lipoprotein metabolism that develops diet-induced human-like NAFLD/NASH characteristics, were fed a high fat and high cholesterol diet to induce obesity and NAFLD, with or without simultaneous treatment with the selective DHCR24 inhibitor SH42.
Results:
After 8 weeks, SH42 treatment markedly increased liver and plasma desmosterol levels without influencing food intake, body weight or body composition. SH42 markedly reduced the hepatic steatosis score (-58%), DAG content (-20%) and TAG content (-39%), as well as the number of crown-like structures (-79%) in the liver. Flow cytometry analysis showed that SH42 decreased liver inflammation by preventing Kupffer cell activation (-71%) and monocyte infiltration (-79%). Interestingly, SH42 reduced plasma concentrations of free fatty acids (-16%) and cholesteryl esters (-24%) concentrations without concomitant changes in DAG and TAG levels. All of these beneficial effects of SH42 on liver lipids levels, steatosis score and liver inflammation were absent in LXRα-deficient mice.
Conclusions:
Together, inhibition of DHCR24 by SH42 increases desmosterol to prevent diet-induced hepatic steatosis and inflammation in a strictly LXRα–dependent manner, importantly without causing hyperlipidemia. We anticipate that pharmacological DHCR24 inhibition may represent a potential novel therapeutic strategy for the treatment of NAFLD/NASH.

High triglyceride metabolism and increased mortality: a population-based study of 30,000 individuals

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Aim: We tested the hypothesis that high triglyceride metabolism, marked by high plasma glycerol and β-hydroxybutyrate, is associated with increased all-cause mortality independently of elevated triglyceride-rich lipoproteins and body mass index (BMI).
Methods: We included 30,000 individuals nested within 109,751 individuals from the Copenhagen General Population Study. During a median follow-up of 11 years, 9,897 individuals died, while none were lost to follow-up. Two markers of triglyceride metabolism, plasma glycerol and β-hydroxybutyrate, were measured using nuclear magnetic resonance spectroscopy.
Results: In a multivariable adjusted model including triglycerides and BMI, higher plasma glycerol and β-hydroxybutyrate were each associated with higher risk of all-cause mortality. For glycerol, the multivariable adjusted hazard ratio for all-cause mortality was 1.34 (95%CI: 1.25–1.43) in individuals with glycerol>79.7 µmol/L (highest quartile) versus individuals with glycerol<52.0 µmol/L (lowest quartile). For β-hydroxybutyrate, the multivariable adjusted hazard ratio for all-cause mortality was 1.19 (1.12–1.27) in individuals with β-hydroxybutyrate >154.4 µmol/L (highest quartile) versus individuals with β-hydroxybutyrate <91.4 µmol/L (lowest quartile). For glycerol and β-hydroxybutyrate combined, individuals with both plasma glycerol and β-hydroxybutyrate above the median had the highest risk of all-cause mortality with a hazard ratio of 1.24 (1.18–1.31), when compared to individuals with both plasma glycerol and β-hydroxybutyrate below the median level.
Conclusion: We observed higher risk of all-cause mortality with higher triglyceride metabolism marked by higher levels of plasma glycerol and β-hydroxybutyrate. These novel findings implicate triglyceride metabolic rate as a risk factor for all-cause mortality independent of plasma triglyceride levels and BMI per se.

Inhibition of sphingolipid de novo synthesis counteracts age-related loss in fitness

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Sphingolipids are important players in cardiometabolic disease. Here, we studied the role sphingolipids in healthy aging by comparing their tissue-distribution in young and aged individuals.
Results
There was a global accumulation of intermediates of the sphingolipid-de-novo-synthesis-pathway in aged skeletal muscle(SKM), accompanied by increased transcript abundance of enzymes. These transcript levels, especially serine palmitoyltransferase (SPT), the rate-limiting enzyme, were inversely correlated with fitness in BXD population.
Treatment of aged 18-month-old mice with SPT inhibitor myriocin for 17 weeks reduced sphingolipid levels in SKM, and increased muscle mass, strength, and exercise capacity. Analysis of SKM transcriptome pointed sphingolipid involvement in SKM regeneration. Mechanistically, transplantation of sphingolipid-depleted MuSCs into aged mice improved their SKM regeneration and exercise capacity.
Depletion of sphingolipids in MuSCs boosted the production of Myogenin, a master-regulator of myotube differentiation, leading to accelerated differentiation of large myotubes. While CRISPR-Cas9-mediated silencing of SPT activated myogenesis, inactivation of DEGS1, the enzyme converting dihydroceramides to ceramides, reduced myoblast differentiation. Indeed, loading SPT-deficient myoblasts with dihydroceramides reduced Myog and abrogated the promyogenic effect of SPT ablation.
To validate our findings in humans, we identified genetic loci reducing mRNA-expression of SPTLC1. In a Finnish cohort of aged individuals, the SPTLC1-mRNA-decreasing allele was associated with improved performance in force and fitness tests, consistent with myriocin treated mice. This association was replicated in aged UK-bank participants (n=26,000).
Our study identifies a novel role for sphingolipid metabolism in muscle regeration, and points the benefits of sphingolipid synthesis inhibition for healthy aging.

Adipocyte-Nfe2l1 protects from cholesterol-induced lipodystrophy and atherosclerosis

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Adipocytes have emerged as crucial regulators of cardiovascular health. During obesity as well as in lipodystrophy, adipocyte health is compromised, which is associated with inflammation and insulin resistance, major risk factors for atherosclerosis. Here we determine the role of Nfe2l1, a cholesterol-sensing transcription factor that regulates proteasome function, for adipocyte biology and cardiometabolic disease.
Nfe2l1 was ablated in adipocytes using Cre-loxP technology in apoE-deficient mice with Cre under control of the Adiponectin promoter for metabolic phenotyping and atherosclerosis studies. Mechanistic studies were performed in 3T3-L1 adipocytes as well as in primary white adipocytes using RNAi.
In primary white adipocytes, Nfe2l1 KO led to lower proteasomal activity, aggravated ER stress, and inflammation. On the tissue level, mice lacking adipocyte-Nfe2l1 displayed adipocyte hypertrophy, diminished browning and severe inflammation characterized by an upregulation of T cell response. On Western diet, knockout (KO) mice displayed lipodystrophy, insulin resistance, and hepatic steatosis compared to their wild-type (WT) littermate controls. Most notably, KO mice had 40% more aortic plaques compared to their WT controls. In 3T3-L1 adipocytes, cholesterol treatment in combination with proteasomal inhibition impaired the activation of Nfe2l1 and exacerbated ER stress and inflammation. The combined toxicity of cholesterol and proteasomal dysfunction was dependent on Atf3, an ER stress-induced transcription factor.
Our results demonstrate that Nfe2l1 protects adipocyte health from excessive lipid exposure, most notably cholesterol, through improved proteasomal protein quality control. In vivo this is linked to reduced insulin resistance and atherogenesis. Thus, these findings highlight a novel link between adipocyte lipotoxicity and atherosclerosis.

Genetic risk of fatty liver disease and mortality in the general population

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Aim: Fatty liver disease associates with increased mortality. This study aims to use genetic variants implicated in fatty liver disease to assess if fatty liver disease per se causes increased mortality.
Methods: We included participants from two prospective studies of the general Danish population, the Copenhagen City Heart Study and the Copenhagen General Population Study, totaling n=110,913. All participants were genotyped for six variants with known effects on fatty liver disease: PNPLA3 rs738409, TM6SF2 rs58542926, HSD17B13 rs72613567, MBOAT7 rs641738, MTARC1 rs2642438, and GCKR rs1260326. Hazard ratios were calculated using Cox-regression.
Results: During a median follow-up of 9.5 years, 16,119 individuals died, hereof 201 due to liver-related causes, 1,736 to ischemic heart disease (IHD), and 4,973 to extrahepatic cancer. The variants at PNPLA3, HSD17B13, TM6SF2, and MBOAT7 (but not those at MTARC1 and GCKR) associated with liver-related mortality with per-allele hazard ratios of 1.3 to 1.6 (p-values<0.06). The strongest effect was seen for the PNPLA3-variant, for which homozygous carriers had a three-fold higher risk of liver-related death compared to non-carriers. A genetic risk score comprised of the variants at PNPLA3, TM6SF2 and HSD17B13 was associated with stepwise increased liver-related mortality, with a maximum hazard ratio of 13 (95% CI: 3.8-48) for those with 5 or 6 versus 0 risk-increasing alleles (p-trend=9x10E-7). The fatty liver disease variants, individually or combined, did not associate with IHD-related, extrahepatic cancer-related, or all-cause mortality.
Conclusions: Genetic risk of fatty liver disease associated with liver-related but not IHD-related, extrahepatic cancer-related or overall mortality in the general population.

Oral Presentations – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Fibroblast growth factor 21 potently reduces atherosclerosis and NASH development

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Aims: Fibroblast growth factor 21 (FGF21), a key regulator of energy metabolism, is currently evaluated in humans for treatment of obesity. However, its effect on lipoprotein metabolism in relation to cardiometabolic diseases including NASH and atherosclerotic cardiovascular disease remains elusive.

Methods and Results: By using APOE*3-Leiden.CETP mice, a well-established mouse model mimicking human-like cardiometabolic diseases, we investigated the role of FGF21 in atherosclerosis and NASH via administration of a recombinant FGF21 and an AAV8 vector encoding murine-optimized FGF21, respectively. FGF21 largely lowered plasma cholesterol within lipoprotein remnants. Mechanistically, FGF21 promoted brown adipose tissue (BAT) activation and white adipose tissue (WAT) browning, thereby enhancing the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT and into WAT, consequently accelerating the clearance of the cholesterol-enriched remnants by the liver. Ultimately, FGF21 largely reduced atherosclerotic lesion area and severity. Moreover, FGF21 attenuated adipose tissue dysfunction, accompanied by alleviated insulin resistance. Remarkably, FGF21 abolished hepatic steatosis, and largely alleviated hepatic inflammation as evidenced by reduced crown-like structures, attenuated Kupffer cell activation and reduced monocyte infiltration into the liver. Finally, FGF21 inhibited the progression of liver fibrosis as indicated by a lowered hydroxyproline levels and COL1A1 expression.

Conclusions: FGF21 largely increases fatty acid oxidation in thermogenic tissues and in the liver, thereby markedly improving lipid metabolism, and as a consequence largely attenuates the development of atherosclerosis and all features of NASH. Our data provide a strong experimental basis for the clinical development of FGF21 to treat atherosclerotic cardiovascular disease and NASH.

Elevated remnant cholesterol and 2-fold mortality rates from cardiovascular and other causes, but not from cancer

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Background: Cholesterol held in triglyceride-rich lipoproteins, also called remnant cholesterol, is being increasingly acknowledged as an important causal risk factor for atherosclerosis, atherosclerotic cardiovascular disease risk and even all-cause mortality. The association with cause-specific mortality is however unknown.
Objectives: We tested the hypothesis that elevated remnant cholesterol is associated with increased mortality from cardiovascular disease, cancer, and other causes in a contemporary population-based cohort.
Methods: We included 87,201 individuals aged 20-69 years from the Copenhagen General Population Study 2003–2015 examination with remnant cholesterol calculated from a standard lipid-profile. During up to 13 years of follow-up, 687 individuals died from cardiovascular disease, 1,595 from cancer, and 856 from other causes. Cause of death was obtained from the national Danish Causes of Death Registry.
Results: Multivariable adjusted hazard ratios for cause-specific mortality in individuals with remnant cholesterol 1 mmol/L (39 mg/dL) compared to individuals with remnant cholesterol <0.5 mmol/L (<19 mg/dL) were 2.0 (95% confidence interval: 1.2-3.3) for cardiovascular disease, 1.0 (0.7-1.4) for cancer, and 1.9 (1.2-3.0) for other causes. Corresponding hazard ratios for the strongest associated sub-categories of cause-specific mortality were 4.1 (1.5-11) for ischemic heart disease, 8.2 (1.9-34) for infectious diseases, 18 (2.0-175) for hematologic diseases, and 10 (2.1-51) for endocrinological diseases.
Conclusions: Remnant cholesterol above 1 mmol/L (39 mg/dL) is associated with 2-fold mortality from cardiovascular and other causes, but not from cancer.

Linking cellular lipid metabolism profiles to the outcomes of cholesterol-lowering therapy in a general population cohort study

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Aims: Most cholesterol-lowering medication works by regulating the cellular mechanisms of cholesterol metabolism. For individuals bearing genetic variants that disrupt these mechanisms, effectiveness of the medication can be reduced. However, for the general population, it is not yet clear how an individual's unique cellular lipid metabolism profile influences the response to cholesterol-lowering medication.
Methods: Previously we utilised a novel multiparametric analysis platform to assess lipid metabolism in leukocytes of familial hypercholesterolemia (FH). Here we use this pipeline to analyse samples from 400 participants of the FINRISK 2012 general population cohort study, including 200 recipients of cholesterol-lowering medication. For each subject we have access to drug reimbursement, NMR metabolomics and clinical follow-up data.
Results: We observe large differences in cellular LDL uptake and lipid storage amongst individuals and show for the first time in a general population cohort study that reduced cellular LDL uptake associates with increased circulating LDL levels for subjects on statin medication. This association is dependent on the statin type and becomes even more evident when we look at cholesterol and cholesteryl esters in pro-atherogenic LDL, IDL and VLDL particles.
Conclusions: Our data suggests that cellular lipid metabolism can greatly vary amongst individuals from the general population, influencing the outcomes of their cholesterol-lowering treatment. Consequently, systematic characterization of cellular lipid uptake might open up new avenues for patient stratification in cholesterol-lowering therapy.

Mapping the low-density lipoprotein interactome in mouse atherosclerosis

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Retention of low-density lipoproteins (LDLs) in the arterial wall is essential in the initiation and progression of atherosclerosis. LDL is retained in early atherosclerosis through interactions between APOB and proteoglycans, but less is known about the mechanisms that retain LDL in advanced disease. The aim of the present study was to map LDL-interacting proteins in established atherosclerosis.
We adapted the proteomic technique "Selective Proteomic Proximity Labelling Assay Using Tyramide" (SPPLAT) to identify proteins that are in close proximity to LDL in plaque. LDL and high-density lipoprotein (HDL) were conjugated with horseradish peroxidase (HRP) and injected in LDL receptor knockout mice after 20 weeks of high-fat diet. At 6- and 22-hours post-injection, the aortas were exposed to a tyramide-biotin reagent processed by HRP to a short-lived radical that biotinylates nearby proteins that were analysed by proteomics.
At 6 and 22hr post-LDL/HDL-HRP injection, we identified 93 and 41 proteins that were significantly more abundant among LDL-labelled versus HDL-labelled proteins. These included lumican, decorin, collagen, fibrinogen and albumin at both time points. The binding affinity of LDL and lumican has not been found, but close interaction between LDL and lumican was supported by the proximity ligation assay (PLA). Lumican and decorin are collagen-binding proteoglycans which forms part of the porous structure of the extracellular matrix (ECM). Their tight proximity to LDL suggests that physical hindrance of movement through the ECM pores may contribute to retain LDL in plaques. To explore this, we studied the dynamics of Atto647 and Atto565-labelled LDL infused into mice at 22 and 6 hours before analysis and found a slow migration rate of LDL through the plaque.
LDL-SPPLAT can be used to explore protein interactions of LDL in vivo. The finding of lumican, decorin and collagen as LDL-neighbouring proteins and a slow pace of penetration of LDL into the plaque suggests that physical hindrance of movement through porous ECM plays a role for LDL retention in established plaques.

Lipoprotein lipase expressed by vascular endothelial cells of activated thermogenic adipose tissues is dispensable for the processing of triglyceride-rich lipoproteins

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Aim: Cold-induced activation of brown adipose tissue (BAT) has an important impact on systemic lipoprotein metabolism by accelerating the processing of circulating triglyceride-rich lipoproteins (TRL). Lipoprotein lipase (LPL) expressed by adipocytes is translocated via endothelial to the capillary lumen, where LPL acts as the central enzyme for the vascular lipoprotein processing. Based on preliminary data showing that LPL is not only expressed in adipocytes but also in endothelial cells of cold-activated BAT, we aim to dissect the relevance of endothelial versus adipocyte LPL for lipid and energy metabolism in the context of adaptive thermogenesis.

Methods: Transgenic mice lacking LPL in endothelial cells (Lplfl/fl-Cdh5Cre+), brown adipocytes (Lplfl/fl-Ucp1Cre+) and respective littermate controls (Lplfl/fl-Cdh5Cre-; Lplfl/fl-Ucp1Cre-) were exposed to a sustained cold environment. For cell type-specific analyses, LPL and marker gene expression was determined in adipocytes versus stromal vascular fraction (SVF). Body and fat weights were determined, metabolic turnover studies using radioactive tracers as well as gene and protein expression were performed to study lipid disposal and thermogenic responses in adipose tissues.

Results: Turnover studies showed that cold-induced triglyceride uptake into BAT was impaired in Lplfl/fl-Ucp1Cre+. These mice showed no impaired cold tolerance, which is probably explained by an increased glucose uptake into BAT, compensating the diminished lipid uptake. Similar studies performed in Lplfl/fl-Cdh5Cre+ and Lplfl/fl-Cdh5Cre-indicated that LPL expressed by vascular endothelial cells has no substantial impact on lipoprotein disposal, glucose uptake and adaptive thermogenesis.

Conclusion: LPL expressed by endothelial cells is dispensable for lipoprotein handling and adaptive thermogenesis mediated by cold-activated thermogenic adipose tissues. These surprising finding may be explained by a compensatory upregulation of adipocyte-derived LPL.

The role of hepatic melanocortin 1 receptor in the pathogenesis and therapeutics of metabolic disease

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Aim: Melanocortins, consisting of melanocyte stimulating hormones (α-, β-, and γ-MSH) mediate their functions through five different receptors named from MC1-R to MC5-R. MC1-R is known to be an integral regulator of skin pigmentation and immune responses. Studies conducted in our research group have revealed that MC1-R deficiency in mice disturbs cholesterol and glucose homeostasis through a yet unknown mechanism. The major aim of this project was to determine the role of MC1-R in hepatic cholesterol and lipid metabolism.

Methods: In vivo studies were carried out on liver-specific MC1-R knock-out mouse model, and hepatic phenotype of the novel mouse model has been characterized under the baseline condition. In vitro experiments were performed on HepG2 cells to further address the expression of MC1-R by qPCR and Western blot.

Results: Liver-specific MC1-R knockout mice showed increase liver weight as well as triglycerides and cholesterol contents. Immunohistochemistry analysis on liver tissue revealed extensive macro-vesicular steatosis and increased accumulation of lipid droplets in MC1-R knockout mice. Furthermore, genes involved in cholesterol bio-synthesis, transport and metabolisms were also altered. Experiments conducted on HepG2 cells revealed that MC1-R expression was rapidly down-regulated after fatty acid treatment. On the contrary, MC1-R activation with agonists α-MSH and LD211 induced the expression of LDLR & SR-BI which play a role in cholesterol homeostasis. Supporting these findings, we also detected reduced total cholesterol content after stimulating MC1-R on HepG2 cells.

Conclusions: The present study uncovers a novel role for MC1-R in cholesterol and lipid metabolism. Liver-specific MC1-R knockout mouse model showed increase liver weight, high cholesterol, and triglycerides levels, whereas MC1-R activation in hepatocytes reduced total cholesterol and enhanced LDL and HDL uptake, which may provide a novel therapeutic approach for metabolic disease.

Impact of preeclampsia on umbilical cord blood lipid concentrations

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Background and aim: Preeclampsia is a multifactorial disease and a cause of maternal and neonatal morbidity and mortality. It is a well-known risk factor for cardiovascular disease in later life for the mothers and is also associated with cardiovascular risk in the offspring. Lipid traits in the newborn are considered to reflect the child's own production. The aim of the present study was to investigate the impact of preeclampsia on umbilical cord blood lipid concentrations.
Methods: For this purpose, we used the Copenhagen Baby Heart Study, a prospective cohort study of newborns, comprising more than 13,000 umbilical cord blood samples. Concentrations of total cholesterol, calculated low-density lipoprotein (LDL) cholesterol, directly measured LDL cholesterol, apolipoprotein B (apoB), and triglycerides are available for 11,804 infants where 346 have mothers with a diagnosis of preeclampsia.
Results: Concentrations of total cholesterol, calculated LDL cholesterol, directly measured LDL cholesterol, apoB, and triglycerides in cord blood were higher in pregnancies with severe preeclampsia (n=105) compared to pregnancies with no preeclampsia (n=11,458) and mild/moderate preeclampsia (n=241) (p for trends <0.0001 for total cholesterol and LDL cholesterol; p for trend=0.0006 for apoB; p for trend=0.001 for triglycerides). Multivariable adjusted odds ratios (95% confidence interval) for preeclampsia versus no preeclampsia (reference) for cord blood concentrations above the 80th percentile were 1.46 (1.13-1.90) for total cholesterol, 1.53 (1.20-1.95) for calculated LDL cholesterol, 1.34 (1.04-1.73) for directly measured LDL cholesterol, 1.52 (1.16-1.92) for apoB, and 2.69 (2.07-3.50) for triglycerides.
Conclusion: Preeclampsia was associated with elevated concentrations of lipid traits in umbilical cord blood indicating that preeclampsia affects the lipid metabolism during fetal life. This may contribute to an increased risk of cardiovascular disease in later life.

Functional analysis of LDLR variants using automated systems

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Problems: Familial hypercholesterolemia (FH) is mostly caused by mutations in the low-density lipoprotein (LDL) receptor (LDLR) gene. However, only a small fraction of LDLR variants is functionally characterized, limiting the use of genetic tools for early diagnosis of FH and complicating the characterization of FH patients.
Methods: We established automated systems for large-scale LDLR variant characterization, regarding cellular LDL uptake, LDLR localization and lipid storage. For this purpose we combined high-content imaging with automated tools for cell culture and molecular biology.
Results: We designed a cell system which allows us to quantify the activity for different LDLR variants at high precision. Variants are expressed as GFP fusion proteins in a LDLR knock out (KO) HepG2 cell line through stable genome integration using CRISPR/Cas9. This enables low-level and uniform expression of LDLR-GFP constructs in 95% of the cells. Expression of wild-type LDLR-GFP restored LDL uptake activity in LDLR KO cells. We use high-content microscopy to determine LDL uptake and subcellular localization for each variant and normalize these activities to wild type LDLR-GFP. We set up an open-source robotics platform for large-scale generation of LDLR variant expression constructs, transfection into LDLR KO cells, automated cell culture and seeding into 384 well imaging plates. The activity and localization of each LDLR variant is quantified in lipid rich conditions and upon two lipid starvation challenges. So far, we generated more than 200 variant expression constructs and anlayzed more than 140 variants, demonstrating that we can perform reliable assessment of LDLR variants across a large activity range.
Conclusion: Our detailed functional analysis of LDLR variants paves the way for improved characterization of FH patients, opens new avenues for rare-variant association studies and can guide new personalized medicine approaches for lipid lowering therapy.

Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION IV

Characterization of laminin isoforms in human atherosclerotic lesions and human vascular cells

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The vascular basement membrane (BM) is composed of specialized extracellular matrix (ECM) proteins that underlie endothelial cells (ECs). The ECM is critical to the functional and mechanical properties of arteries by interacting with each other and growth factors to regulate cell activities. The BM is rich in laminin (LN), a trimeric protein consisting of α-, β- and γ chains. The C-terminus of the α chain interacts with integrins and plays a critical role in cell adhesion and signaling, while binding sites for perlecan, collagens, fibronectin and nidogen are present on specific domains. These interactions are believed to be perturbed in atherosclerosis, and may contribute to EC dysfunction, uncontrollable smooth muscle cell (SMC) infiltration and proliferation, and alteration to ECM composition. We hypothesize that specific LN isoforms from EC and SMC are vital in maintaining an intact and functional ECM in healthy arteries. Human coronary artery EC and SMC-derived ECM has been characterized by ELISA and proteomics. High ELISA reactivity for type IV collagen and heparan sulfate was detected in EC-ECM, whereas higher reactivity for LNs and chondroitin sulfate was detected in SMC-ECM. Different distributions of LN isoforms and chains have been confirmed in these matrices via immunocytochemistry and immunoblotting. Advanced human atherosclerotic lesions showed different isoforms and the presence of LN fragments consistent with ECM modification. LC-MS analyses showed slight differences in LN isoform expression between SMC donors, however the α2, α5, β1, β2 and γ1 LN chains were commonly detected. Data for EC-ECM are currently being collected. These data suggest that different LN isoforms produced by EC and SMC have specific roles in generating an intact and functional ECM environment in healthy arteries, and that this balance is perturbed in atherosclerosis.

Pharmacological treatment with designer cytokine and exercise mimetic IC7Fc reduces atherosclerosis development

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Introduction & aim: Exercise effectively prevents and treats cardiovascular diseases. Particularly, the exercise-inducible cytokine IL-6 and the structurally related ciliary neurotrophic factor (CNTF) convey anti-inflammatory properties. Pharmacological administration of these cytokines, however, triggers an unwanted immune response through alternative signaling routes and neutralizing antibodies. To overcome this hurdle, the chimera cytokine IC7Fc was designed combining only the anti-inflammatory signaling properties of IL-6 and CNTF, which was shown to ameliorate type 2 diabetes in diet-induced obese mice (Nature 2019). Here, we aimed to investigate if IC7Fc counteracts atherosclerosis development.
Methods & Results: Female atherosclerosis-prone APOE*3-Leiden.CETP mice were injected with IC7Fc (1 mg/kg) or saline twice per week for 7 weeks. Mice treated with IC7Fc gained more lean body mass and showed robustly reduced non-HDL plasma cholesterol (-26%; 19.4 vs. 26.1 mM). IC7Fc treatment furthermore markedly reduced atherosclerotic plaque size (-65%; 6.3*104 vs. 17.5*104 μm² per cross section), prevented lesion progression (30.1% vs. 58.0% severe atherosclerotic lesions) and reduced the expression of the inflammatory markers Vcam1 and Ccr2 (by 35.0% and 36.0%, respectively) in the aortic wall. Concomitantly, IC7Fc decreased hepatic gene expression of monocyte markers Ly6c (-28.0%) and Vsig4 (-31.0%) suggesting reduced liver inflammation while liver lipid content was unchanged.
Conclusion & outlook: The exercise mimetic IC7Fc improves lipoprotein metabolism and inflammation, resulting in a large reduction of atherosclerosis development and making it a promising new treatment for atherosclerotic cardiovascular disease. We currently further elucidate the mechanisms by which IC7Fc ameliorates hypercholesterolemia and atherogenesis.

Inhibition of pyruvate dehydrogenase kinase prevents abdominal aortic aneurysm formation in mice

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Background: Abdominal aortic aneurysm (AAA) is a life-threatening condition, where inflammation has been pinpointed as the major driver. Currently, AAA treatment relies exclusively on surgical interventions, with no proven pharmacological therapies to prevent growth or rupture. Immunomodulation by pharmacological reprogramming of immune cell metabolism has been demonstrated in the context of several inflammatory diseases. Whether the same strategy could be used in AAA remains allusive.

Aim: We hypothesized that inhibition of pyruvate dehydrogenase kinase (PDK), a gatekeeper enzyme connecting glycolysis and the TCA cycle, will promote anti-inflammatory responses that prevent AAA formation.

Methods: AAA was induced in male C57BL/6J mice by intraluminal infusion of porcine pancreatic elastase (PPE) of the infrarenal aorta. The PDK-inhibitor dichloroacetate (DCA; 1 mg/mL) was administered ad libitum through the drinking water starting one week before AAA induction, and through two consecutive weeks before sacrifice. In parallel, control mice received regular drinking water.

Results: DCA treatment significantly reduced by 22.6% the outer maximum diameter, and by 18.4% the luminal circumference of the infrarenal aorta vs controls. Histology of AAA specimens revealed that DCA substantially preserved the elastic laminae of the aortas, and significantly increased medial alpha-smooth muscle actin content by 41.4%. In line with the previous findings, proteomic analyses showed that aneurysms from DCA-treated mice presented significantly higher levels of Col1a1 and Col3a1. Surprisingly, DCA did not affect macrophage infiltration assessed by immunohistochemistry.

Conclusion: Inhibition of PDK with DCA is an effective therapy to limit AAA expansion induced by PPE. The current data shows that PDK inhibition by DCA preserves major extracellular matrix components of the aorta, and suggests its potential use as an effective modulator of deleterious smooth muscle cell responses in AAA.

Endothelial integrity in intracranial atherosclerosis; the regulatory role of N6-methyladenosine (m6A) of the pro-atherogenic microRNA miR-494-3p in tight junction proteins

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Intracranial atherosclerosis (ICAS) is a major cause of acute ischemic stroke. ICAS is defined by a later onset and a more stable plaque phenotype compared to its peripheral counterpart, with lower numbers of infiltrating macrophages, except for in advanced ICAS. Cerebral artery endothelium has decreased permeability compared to peripheral endothelium, due to higher number of tight junctions (i.e. the blood brain barrier), impeding non-selective crossing of solutes, but also of immune cells from the circulation.
In preliminary studies, we found that the pro-atherogenic microRNA miR-494-3p is expressed only in advanced ICAS and that miR-494-3p can target tight junction components TJP1 and PECAM1. Furthermore, we found that miR-494-3p is subject to N6-methyladenosine (m6A), which directly impacts miR-494-3p target repression.
To investigate the role of m6A-miR-494-3p in tight junctions and ICAS progression, we analyzed postmortem human intracranial arteries with different grades of ICAS via immunohistochemistry and in situ hybridization. Additionally, we are currently performing in vitro experiments using m6A-modified miR-494-3p mimics to study effects on junction protein expression and endothelial barrier function.
Although analyses are still ongoing, we observe a decrease in expression of junction proteins TJP1, β-catenin, VE-cadherin and PECAM1 in the endothelium in sections of intracranial arteries affected by intimal thickening and increased macrophage content, compared to healthy sections. Expression of junction proteins also differed between different arteries; e.g. high in middle cerebral and basilary artery, low in anterior cerebral artery. Furthermore, both miR-494-3p and m6A were abundantly present in advanced ICAS and miR-494-3p expression increased with lesion severity.
Our intermediate conclusion is that endothelial integrity is indeed reduced in progressing ICAS. These effects may be mediated by an increased expression of m6A-methylated miR-494-3p.

Genetic deficiency of indoleamine 2,3-dioxygenase (IDO)-1 aggravates vascular but not liver disease in a nonalcoholic steatohepatitis and atherosclerosis comorbidity model

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Background: NASH, a chronic liver disease and hepatic manifestation of metabolic syndrome, increases cardio-metabolic risk. IDO-mediated tryptophan (Trp) metabolism has been shown to play immunomodulatory roles in the liver and the artery wall. However, the potential of IDO1 as a link between NASH and cardiovascular disease has never been investigated.
Methods: ApoE-/-Ido1+/+(Control) & ApoE-/-Ido1-/-(IdoKO) mice were fed a high fat, high cholesterol diet (HFCD) for 3.5 & 7 weeks to simultaneously induce NASH and atherosclerosis. In vitro studies carried out with HepG2 cells and PMA-differentiated THP1 macrophages were used to elucidate the molecular mechanisms involved in the modulation of NASH by IDO-1.
Results: IdoKO mice showed significantly accelerated atherosclerosis after 3.5 and 7 weeks of HFCD. Surprisingly, IdoKO mice did not present a more aggressive NASH phenotype including hepatic lipid deposition, release of liver enzymes, histopathological parameters like steatosis and ballooning, macrophage infiltration, local cytokine production, and collagen deposition. As expected, a lower L-kynurenine/Trp (Kyn/Trp) ratio was found in the plasma and arteries of IdoKO mice compared to controls while no difference in the hepatic Kyn/Trp ratio was found between the groups. Hepatic transcript analyses revealed that HFCD induced a temporal increase in tryptophan 2,3-dioxygenase (Tdo2) mRNA, suggesting an alternative mechanism of Trp degradation during NASH progression. Using HepG2 hepatoma cells, we found that palmitic acid downregulated, while FeSO4 (a TDO2 inducer) upregulated TDO2 mRNA levels. Treatment of THP1 cells with the conditioned media of HepG2 cells treated with palmitic acid, FeSO4 with or without LM-10 (a TDO2 inhibitor) revealed that IL-1β is upregulated upon TDO2 inhibiting conditions, suggesting an important cross-communication between hepatocytes and macrophages through the expression of TDO2, that can regulate IL-1β secretion.
Conclusions: We show that IDO deficiency aggravates atherosclerosis but not liver disease in our NASH & atherosclerosis dual comorbidity model. Our data indicates that overexpression of TDO2 is a key compensatory mechanism balancing the kynurenine pathway in the liver but not in the artery wall, likely determining disease outcome in these tissues.

Atherosclerotic Plaque Progression is Associated with Increased Collagen Carbamylation: Impact on Macrophage Functioning

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Carbamylation is a post-translational protein modification increased in chronic kidney disease (CKD) patients as well as in late-stage atherosclerotic plaques in patients without CKD. Carbamylation was shown to cause pro-atherogenic alterations in plasma proteins and was significantly associated with increased mortality in CKD patients. However, the exact mechanism of protein carbamylation in atherosclerotic plaque and its implications for macrophages, a key player in plaque inflammation, are not known. Human atherosclerotic plaque tissue samples (n=27 ranging from early to advanced progression stage), was analyzed by immunohistochemistry for extent of carbamylated lysine (carb-lys). Parallel advanced plaque sections were analyzed by mass spectrometry imaging for carbamylated collagen peptides. Functional effects of carbamylated collagen on primary human peripheral-blood derived macrophages (PBMCs) were studied in vitro by assessing their morphological, functional and inflammatory status on a microscale multiassay platform. Overt carb-lys signal was observed in plaque smooth muscle cells, macrophages, and extracellular matrix. Advanced plaques showed significantly higher carb-lys positive relative area in advanced than early-stage plaques (160% increase, $p<0.01$). Mass spectrometry imaging showed increased relative intensity of carbamylated collagen type I and IV peptides compared to non-modified peptides in advanced plaque samples. PBMCs seeded on carbamylated collagen showed reduced ROS production (40% reduction, $p<0.05$) and a trend towards decreased phagocytosis (9% reduction, $p=0.097$) compared to the cells seeded on non-modified collagen. TNFα production by LPS-stimulated macrophages was not altered by collagen carbamylation. Protein carbamylation is abundant in late-stage atherosclerotic plaque both intracellularly in smooth muscle cells and macrophages, and extracellularly, of matrix components like collagens. In vitro, human macrophage adhesion to carbamylated collagen led to reduced ROS formation and phagocytosis. Studies are underway to characterize the molecular mechanism of plaque protein carbamylation and its functional repercussions in closer detail.

Proteomic Characterization of Atherosclerotic Lesions In Situ Using Percutaneous Coronary Intervention Angioplasty Balloons

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OBJECTIVE: Materials extracted from atherosclerotic arteries can disclose data about the molecular pathology of cardiovascular disease, but obtaining such samples is complex and requires invasive surgery. To overcome this barrier, this study investigated whether angioplasty balloons, inflated during standard percutaneous coronary interventions (PCI) retain proteins from treated (dilated) atherosclerotic lesions, and whether proteomic analysis of this material could provide data on individual lesion protein profiles, and distinguish between patients with stable and unstable coronary artery disease.

APPROACH: Patients with ST-segment elevation myocardial infarction (STEMI) and stable angina pectoris (SAP) were subjected to routine PCI. All angioplasty balloons inflated in a coronary artery were collected. Proteins retained on the balloons were extracted and analyzed using shotgun proteomic analysis.

RESULTS: Proteomics identified and quantified 1365 unique proteins captured on PCI balloons. Control balloons inflated in the ascending aorta showed minimal non-specific protein binding, indicating specificity to the luminal region of atherosclerotic lesions of the diseased artery wall. Clustering and principal component analyses showed that STEMI and SAP subjects could be separated by variations in protein content and abundance. We identified 206 proteins as differentially abundant between STEMI and SAP subjects. Pathway analysis indicated several enriched processes in the STEMI group involved in neutrophil-mediated immunity and platelet activation.

CONCLUSIONS AND RELEVANCE: Disease-related proteins from coronary artery lesions adhere to angioplasty balloons and constitute a source of material for proteomic analysis. This approach can identify proteins and processes occurring in coronary atherosclerotic lesions and distinguish between subjects with stable and unstable disease.

Posters – Abstracts –

Inflammation and Vascular Biology

SESSION I

No 1

Protrudin regulates FAK activation, endothelial cell migration and angiogenesis

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During angiogenesis, endothelial cells form protrusive sprouts and migrate towards the angiogenic stimulus. Protrudin/ZFYVE27 is an endoplasmic reticulum (ER)-anchored protein that plays an essential role in neurite outgrowth. It also promotes invadopodia formation and invasion of breast cancer cells. However, the role of Protrudin in endothelial cell function is not known. In the present study we investigate the role of Protrudin in endothelial cell protrusion, migration and angiogenesis. Our results demonstrate that Protrudin regulates angiogenic tube formation in primary endothelial cells, Human umbilical vein endothelial cells (HUVECs). Analysis of RNA sequencing data and its experimental validation revealed cell migration as a prominent cellular function affected in HUVECs subjected to Protrudin knockdown. Further, our results demonstrate that knockdown of Protrudin inhibits focal adhesion kinase (FAK) activation in HUVEC and human aortic endothelial cells (HAECs) under basal and VEGF-stimulated condition. This is associated with loss of polarized FAK distribution upon Protrudin knockdown. Reduction of Protrudin also results in a perinuclear accumulation of mTOR and inhibits S6K activation. Furthermore, C57Bl/6J mice with global deletion of Protrudin demonstrate reduced retinal vascular progression at post-natal day 7 ($n=5$, $^*p<0.05$). Overall, our results demonstrate that Protrudin regulates FAK and mTOR/S6K signaling pathways in endothelial cells and provide evidence for a key role of Protrudin in angiogenesis in vitro and in vivo. Besides a putative role in tumor angiogenesis, it will be intriguing to study the role of Protrudin in atherosclerotic lesions where it could impact neovessel formation and the growth of collaterals.

No 5

Possible role of SPP1 in degenerative ascending aortic aneurysm

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BACKGROUND:Ascending aortic aneurysm (AscAA) is a silent, degenerative disease and little is known of the molecular mechanisms involved in the disease development. Our objective was to characterize the cellular and molecular signature of degenerative AscAA occuring in patients with a tricuspid aortic valve (TAV) from a fibrotic and inflammatory perspective, and identify regulatory regions and proteins contributing to disease initiation and development.
MATERIAL AND METHODS: A total of 127 patients undergoing elective open-heart surgery for AscAA- and/or aortic valve repair at the Karolinska University hospital, Stockholm, Sweden, were included and global gene expression was measured in the intima-media portion of ascending aortic tissue biopsies from non-dilated and dilated aortas. Protein expression of candidate genes was assessed using immunohistochemistry. Interacting distal regions (enhancers) were identified using high-throughput chromosome conformation capture followed by targeted sequence capture (HiCap) in THP1 cells, followed by identification of predicted transcription factors binding sites (TFBS). Validation of putative transcription factorss (TF) was done by CUT&RUN analysis.
RESULTS: Bioinformatic analysis of global gene expression profiles identified osteopontin (SPP1) as a candidate gene for aortic dilatation in patients with TAV, and its protein expression co-localised with the expression of CD68. HiCap and subsequent TFBS analysis was used to identify potential TFs regulating SPP1. Further validation of these TFs binding to SPP1 in dilated aortic tissue is currently ongoing.
CONCLUSIONS: Our findings suggest that SPP1, regulated by HiCap and TFBS predicted TFs, may be of importance for AscAA development in TAV.

No 6

Modification of "NETs" – a new driver of inflammation in atherosclerosis?

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The release of neutrophil extracellular traps (NETs) is a key innate immune defense to combat infection. NETs consist of a mesh of DNA and histones, which are decorated with neutrophil granule proteins, including myeloperoxidase (MPO). Although NETs have important anti-bactericidal properties, they are also implicated in thrombosis and the development of atherosclerosis. However, the mechanisms involved in the pathological effects of NETs are not well understood. Enzymatically-active MPO is present on the DNA NET backbone, and produces the potent oxidant hypochlorous acid (HOCl). In this study, we examine whether the modification of NET-associated histones by HOCl promotes vascular cell dysfunction. Experiments were performed with a preparation of histones containing histone H1, H2A, H2B, H3 and H4, which are present in NETs. Evidence was obtained for extensive modification of the histones by HOCl, particularly Lys, Met, Arg and Tyr residues, which was accompanied by the formation of stable oxidation products, including 3-Cl-Tyr, and unstable chloramines. Exposure of primary human vascular cells, including human coronary artery endothelial cells and human coronary artery smooth muscle cells, to both native and HOCl-modified histones resulted in a dose-dependent loss of viability, consistent with the known toxicity of histones when present in the extracellular environment. The change in cell viability seen with the modified histones was dependent on the extent of oxidative modification. Interestingly, less cell death was apparent on pre-treatment of the histones with HOCl compared to the native histones. However, the HOCl-modified histones altered the redox balance in the cells and promoted inflammatory signaling to a greater extent than that seen with the native histones. These results provide new insights in the mechanisms responsible for NET-associated inflammation in atherosclerosis, and could provide new opportunities for therapeutic intervention.

No 8

Mechanical Stretch Regulates Inflammatory Signaling in Human Vascular Smooth Muscle Cells

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The phenotypic modulation of vascular smooth muscle cells (SMCs) plays a significant role in atherosclerosis and other cardiovascular diseases. SMCs are highly sensitive to mechanical changes in their microenvironment. During atherosclerosis, vascular SMCs are subjected to unregulated mechanical stretches leading to a shift in SMCs from a contractile state towards other phenotypes characterized by reduced or lost expression of contractility-associated proteins. To elucidate how mechanical stretch modulates the phenotype of human SMCs, we recently established an in vitro setup where cells are exposed to different intensities of stretch. We compared the changes in gene expression after physiological stretch (10% of elongation), supraphysiological stretch (15%), and static conditions by RNA sequencing. Our bioinformatic analysis showed that thousands of transcripts were differentially expressed during the different stretch conditions. As expected, physiological stretch increased the expression of some contractile SM marker genes (ACTG2 and TAGLN). Interestingly, several inflammatory mediators were upregulated after a supraphysiological stretch or static conditions compared to physiological stretch (CXCL1-8, CCL2, C3, IL6, ICAM1, etc.). Immunofluorescence staining showed increased activation of the NF-kB pathway by P65 nuclear translocation in cells exposed to supraphysiological stretch compared to cells exposed to physiological stretch, thus suggesting that mechanical stretch might regulate an inflammatory response through the regulation of the NF-kB pathway in SMCs. To investigate this, we are establishing a fluorescent NF-kB reporter cell line that we will use for a CRISPR-Cas9 genome-wide screening to identify genes that regulate NF-kB in response to stretch.

No 19

Exposure of human coronary artery endothelial cells to hypoxia results in extracellular matrix remodelling

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During the development of atherosclerosis, the vascular wall accumulates lipids and becomes thickened, which results in a hypoxic environment as a result of limited O2 diffusion and increased demand. Increasing evidence suggests that hypoxia may be a driver of further extracellular matrix (ECM) modification, contributing to lesion development and instability of the lesion. This increases the risk of lesion rupture and subsequent myocardial infarction or stroke.
The aim of this study was to investigate whether exposure of human coronary artery endothelial cells (HCAEC) to 1 versus 20% O2 resulted in an altered synthesis and composition of the ECM and whether this modulated HCAEC adhesion, proliferation, gene expression of ECM proteins, production of inflammatory cytokines, and generation of reactive oxidants.
HCAEC exposure to 1% O2 compared to 20% O2 for 7 days resulted in changes in ECM mRNA expression and protein synthesis, with versican levels markedly elevated. This increase in versican was confirmed by mass spectrometry. Decreased protein expression of cell adhesion molecule, ICAM-1 as well as increased oxidant generation were detected by flow cytometry. Reduced adhesion of HCAECs to the ECM generated under 1% O2 was quantified using calcein-AM stain, although these cells demonstrated increased metabolic activity as measured by MTS.
These data indicate that exposure of HCAEC to 1 versus 20% O2, alters the ECM generated by the cells. The increased production of versican may exacerbate the progression of atherosclerosis, as this proteoglycan is a well-established binding site for lipoproteins, and therefore may contribute to lipoprotein accumulation within lesions. The concurrent increase in oxidant production may exacerbate lipoprotein modification, and contribute to the unregulated accumulation of lipids within macrophage cells in developing lesions.

YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

No 4

Run for your live(r): Exercise training at different times of day differentially modulates hepatic inflammation in early NAFLD

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Aim: Exercise effectively prevents obesity-related disorders, but it is unclear whether the beneficial health effects of exercise are restricted to unique circadian windows. We previously showed that late, but not early, exercise training over four weeks reduces atherosclerosis (-40%) and body fat mass (+0.43 g with early vs. -0.49 g with late training) in mice, suggesting a greater improvement of hyperlipidemic and inflammatory diseases with late training. Therefore, we now aimed to study whether timing of exercise training also differentially modulates NAFLD development and progression.

Methods: We used endurance-trained high fat-high cholesterol-fed NAFLD-prone male APOE*3-Leiden.CETP mice for eight weeks (5x per week, 1 hour) either in the early (ZT13) or in the late (ZT22) active phase and assessed NAFLD score (histology) and hepatic inflammation (FACS) compared to sedentary mice.

Results: Exercise training prevented an increase in body fat mass (+1.13 g with early, +1.06 g with late vs. +3.67 g with no training) and fasting plasma glucose (-0.7 mM with early, -0.8 mM with late training) as expected, but neither early nor late training affected liver triglyceride or cholesterol content compared to sedentary mice, likely due to a very early stage of hepatic steatosis. In line, hepatic expression of de novo lipogenesis genes (e.g., Fasn, Srebp1c) was similarly downregulated by early and late training. However, exercise had a distinct time-dependent effect on hepatic inflammation, as only early training promoted an influx of neutrophils and monocytes into the liver paired with increased expression of the pro-inflammatory cytokines (e.g. Tnfa, Il1b).

Conclusion: Timing of exercise is a critical factor for the positive effect in cardiometabolic disease management. We currently investigate the effect of timed training on advanced NAFLD.

No 9

High lipoprotein(a) and systemic inflammation jointly confer additive high risk of atherosclerotic cardiovascular disease and aortic valve stenosis

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Background and aims: Lipoprotein(a) and low-grade systemic inflammation are both risk factors for atherosclerotic cardiovascular disease (ASCVD) but recent evidence has indicated that the lipoprotein(a) associated ASCVD risk is observed only in individuals with low-grade systemic inflammation. We hypothesized that high lipoprotein(a) and low-grade systemic inflammation, measured as high sensitive C-reactive protein (hsCRP), jointly influence the risk of ASCVD and aortic valve stenosis (AVS).
Methods: We included 68,090 individuals from the Copenhagen General Population Study, a contemporary prospective cohort study of randomly selected individuals from the general Danish population. During a median of 8.1 years of follow-up, 5,104 developed ASCVD and 1,220 developed AVS.
Results: For individuals with hsCRP<2 mg/L, the multivariable adjusted hazard ratio for ASCVD and AVS for individuals in the 90th-100th percentiles of lipoprotein(a) levels (≥69 mg/dL, ≥147 nM) were 1.61 (95% confidence interval: 1.43-1.81) and 2.00 (1.58-2.53) compared to individuals in the 1st-32th percentiles (≤6 mg/dL, ≤10 nM) of lipoprotein(a). The corresponding hazard ratios for individuals with hsCRP≥2 mg/L were 1.59 (1.37-1.84) and 1.76 (1.34-2.32), respectively. We found no evidence of interaction of lipoprotein(a) and hsCRP on risk of ASCVD (P for interaction= 0.54) or AVS (P for interaction= 0.40). The highest absolute 10-year risk of ASCVD and AVS was found in individuals with concomitant high lipoprotein(a) and hsCRP. For men aged 70-79 with lipoprotein(a) levels in the 90th-100th percentiles and hsCRP≥2 mg/L, absolute 10-year risks of ASCVD and AVS were 36% and 14%, respectively.
Conclusion: High lipoprotein(a) and systemic inflammation jointly confer additive high risk of ASCVD and AVS.

No 11

Leptin, inflammation and trained immunity in obesity

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Leptin, an adipokine involved in energy regulation and metabolism, is associated with cardiometabolic complications of obesity, such as metabolic syndrome (MetS) and atherosclerosis. We recently showed that in obese men, MetS was associated with higher circulating levels of leptin and interleukin-6, and with increased cytokine production of isolated peripheral blood mononuclear cells (PBMCs). We now aimed to study the effect of leptin on cytokine production of innate immune cells in obese subjects and in vitro on trained immunity, the persistent hyperinflammatory phenotype of monocytes that develops after brief exposure to atherogenic stimuli.

In 302 obese subjects (BMI>27, 55-81 years), we measured circulating leptin, inflammatory markers and cytokine production upon ex-vivo stimulation of PBMCs with toll-like receptor (TLR) 2/4 agonists. To study the effects of leptin on innate immune reprogramming, we used our in vitro protocol for inducing trained innate immunity in primary human monocytes. In short, monocytes were exposed to relevant concentrations of leptin, oxLDL or both for 24h, rested for 6 days and exposed to TLR2/4 agonists, after which cytokine production is measured.

In our cohort, circulating leptin positively correlates with circulating IL1b and IL6. Leptin also associated with LPS-induced IL6 production but significance was lost after correction for multiple testing. In vitro, 24h exposure to 1 and 50 ng/ml of leptin induced trained immunity in human monocytes. We also observe that leptin potentiates oxLDL-mediated training and further enhances P3C-induced TNF production. Leptin does not alter membrane expression of TLR2/4 in trained human macrophages.

In conclusion, leptin correlates to circulating inflammatory markers in obese humans but not to cytokine production capacity. In vitro, brief exposure to leptin induces a prolonged pro-inflammatory phenotype in human monocytes. The effect of leptin on plaque macrophages warrants further investigation.

No 14

High Plasma Levels of Sphingosine-1-Phosphate Associate with Increased Blood Cholesterol Levels and Reduced Cognitive Speed

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Hypercholesterolemia, diabetes, obesity and hypertension are known contributors to progressive cognitive decline. Early detection and treatment of these specific risk profiles is thus the most promising approach to prevent, or delay, age-related cognitive impairment. Association analyses between blood lipid levels and especially cognition, however, generated mixed results in respect to the suitability of lipid-lowering therapy for protecting, or improving, cognitive function in dyslipidemic patients.
The phospholipid sphingosine-1-phosphate (S1P) has been linked to atherosclerosis, obesity, diabetes and hypertension. In addition to its regulatory role in inflammatory and vascular responses, it is also involved in neurodegenerative processes. Here, we tested S1P's association with blood lipids and cognitive function in a Swedish population-based cohort and a preclinical mouse model of chronic hypercholesterolemia.
Human plasma S1P levels associated positively with total and LDL cholesterol, and correlated negatively to a cross-sectional decrement in cognitive speed. We validated these associations in hypercholesteraemic mice and discovered that a cholesterol-lowering therapy resulted in lower plasma S1P levels, in addition to a reduced occurrence of structural and functional brain alterations. From a molecular perspective, altered ratios between the two major S1P transporters, apolipoprotein M and albumin, may significantly contribute to such phenotype, which we are currently investigating.
This translational study compellingly evidences a relationship between plasma S1P and blood cholesterol levels, in addition to cognitive function in humans and mice, thereby suggesting a vital role of S1P as an early prognostic marker for neuronal function, especially in patients suffering from hypercholesterolemia.

No 15

Trained immunity, inflammation and subclinical cardiovascular phenotypes in children with obesity

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Background and Aims - Cardiometabolic risk accrues across the lifecourse and childhood is a key period for effective prevention. Obesity, the most prevalent modifiable cardiovascular risk factor, is associated with increased inflammation in adults, but paediatric data are scarce. We investigated immune cell composition and activation in children with(out) obesity, the impact of weight loss, and correlations between innate immunophenotypes and cardiovascular phenotypes.
Methods - 33 children with severe obesity (BMIz>2.5, 12-17y) were assessed at recruitment and a median of 5-years. Anthropometric data and cardiovascular phenotypes were measured and peripheral blood mononuclear cells obtained. 22 children with normal weight (-1.5<BMIz<1.5, age-, sex- and pubertal stage-matched) were controls. The innate immunophenotype of PBMCs was characterised by flow cytometry, ex-vivo stimulation assays and RNA sequencing.
Results Children with obesity had significant changes in monocyte phenotype, including increased monocyte activation markers and cytokine production capacity. In addition, monocyte transcriptomics showed comparative upregulation of inflammatory and immunometabolic pathways and downregulation of anti-viral responses. Weight change over 5-years was not associated with cytokine production capacity nor monocyte activation. Longitudinally increased inflammatory measures correlated with adverse subclinical cardiovascular phenotypes at follow-up.
Conclusions - Children with obesity had a pro-inflammatory monocyte phenotype compared to those with normal weight. At follow-up, weight loss did not result in decreased inflammation, indicating innate immune memory. Adjunctive anti-inflammatory interventions may reverse adverse subclinical cardiovascular phenotypes in children with obesity.

No 16

Leukocyte subpopulation counts and incident vascular endpoints: observational and genetic studies

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Background: High total leukocyte and neutrophil counts and the ratio between them have been associated with high risk of cardiovascular disease. Specific vascular endpoints have however not been investigated and the causal potential of such associations remains unexplored. Our aim was to examine the associations between leukocyte subpopulation counts and risk of specific vascular endpoints using observational and genetic studies.
Methods: Observationally, we included 101,730 participants from the prospective Copenhagen General Population Study and examined the association between baseline leukocyte subpopulation counts and incident coronary artery disease (CAD), myocardial infarction (MI), ischemic cerebrovascular disease (ICVD), ischemic stroke (IS), non-Alzheimer's dementia (non-AD), and vascular dementia (VD), using Cox regression adjusting for confounding factors. Genetically, we investigated a potential causal role of cell counts and risk of CAD, MI and IS using the two-sample Mendelian randomization (MR) design based on summary level genomic consortium data, including 438,847 individuals.
Results: Multivariable adjusted hazard ratios (HR) (95% confidence interval) for participants in the 95-100th percentile group of neutrophil counts were 1.22 (1.10, 1.36) for CAD, 1.22 (1.02, 1.45) for MI, and 1.29 (1.05, 1.59) for non-AD. Odds ratios (95% confidence interval) per one-SD increase in genetically determined neutrophil counts were 1.14 (1.05, 1.23) for CAD and 1.22 (1.06, 1.41) for MI. A series of sensitivity analyses accounting for pleiotropy, weak genetic instruments, and outliers were applied and showed similar results. Genetic estimates for IS were not significant, and no genetic estimates could be generated for ICVD, non-AD and VD, due to lack of available genomic consortia.
Conclusion: High neutrophil counts were observationally and genetically associated with risk of CAD and MI, supporting a direct role for neutrophils in atherosclerosis development.

No 22

Human atherosclerotic lesions contain oxidant-modified extracellular matrix proteins

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Background and aim: Compared to stable lesions, unstable rupture-prone atherosclerotic lesions typically contain higher levels of inflammatory cells. Activation of these cells releases the enzyme myeloperoxidase (MPO) which generates potent inflammatory oxidants, including hypochlorous acid (HOCl), which can damage extracellular matrix (ECM) proteins in the lesion, potentially causing plaque destabilization. The aim of this study was therefore to examine the presence and nature of oxidant-modified ECM proteins in human carotid artery atherosclerotic lesions and to validate the detection of such species in vitro.

Methods: Proteins were extracted from human carotid artery lesions, digested with proteolytic enzymes, then subject to high pH reverse-phase fractionation. The fractions were then subject to liquid-chromatography mass spectrometry (LC-MS) to identify parent peptides and oxidation products. In vitro studies were carried out by immunoblotting, ELISA and LC-MS to validate targets.

Results: About 4900 proteins were identified in lesion samples, with fibronectin (FN) being amongst the most abundant. Multiple proteins were shown to contain modifications, including FN, which was detected as both chlorinated and nitrated species. Purified human plasma FN was shown to undergo chemical and structural changes on exposure to increasing doses of HOCl, or an MPO-enzymatic system, with formation of oxidant-derived epitopes as shown by immunoblotting, ELISA and LC-MS. In particular, the oxidation-induced modifications 3-chlorotyrosine and dityrosine was significantly increased in the modified FN.

Conclusion: These data indicate that human carotid artery atherosclerotic lesions contain oxidant-modified ECM proteins. These oxidative modifications alter protein structure, as suggested by in vitro work on the purified FN, which may in turn perturb protein function and weaken lesion structure.

No 25

Targeting EZH2 to shift macrophages towards a less inflammatory phenotype in atherosclerosis

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Atherosclerosis is a lipid-driven inflammatory disorder in which macrophages are key players. Considering that epigenetic processes are important modulators of macrophage pro- and anti-inflammatory responses, we postulate that interference in the epigenetic machinery of macrophages might offer novel approaches to combat atherosclerosis. Here, we investigate the repressive histone mark H3K27Me3 deposited by the Polycomb Repressive Complex 2 (PRC2) with its catalytic component EZH2. We previously published that myeloid deficiency of Ezh2 results in reduced atherosclerosis and inflammatory responses of foam cells in a mouse model for atherosclerosis. Currently, we are translating these findings to a human clinically relevant setting by exploring the impact of the pharmacological EZH2-inhibitor GSK126 on human macrophages.
Primary human monocytes were isolated from buffycoats and differentiated into macrophages using M-CSF. Macrophages were treated with GSK126 (5μM) and subsequently activated with LPS. Following GSK126 treatment and stimulation with LPS, we measured reduced inflammatory IL1β, IL6, IL12 and TNF cytokine levels, which matched reductions in their mRNA expression. Furthermore, membrane marker expression of the inflammatory markers CD40, CD80 and CD86 was significantly decreased. Thus, we observe a shift towards a less pro-inflammatory phenotype following immunological stimulation in EZH2-inhibited human macrophages.
Considering these outcomes and those of our murine atherosclerosis model, we are now further exploring the role of EZH2-inhibition in macrophages in the context of atherosclerosis, such as the impact on subset polarization, foam cell formation, and lipid uptake. Furthermore, we aim to explain the mechanism by which EZH2 regulates inflammation by combining sequencing data on gene regulation at the epigenetic and transcriptional levels following EZH2 inhibition. By doing this, we hope to identify novel regulatory mechanisms of inflammation in macrophages in the context of atherosclerosis.

Mast cells associate with matrix calcification and reprogram smooth muscle cells in atherosclerotic plaques

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Rationale: Calcification, a key feature of advanced human atherosclerosis, is positively associated with vascular disease burden and adverse events. However, recent studies from our group have shown that macrocalcification can be a stabilizing feature for the molecular biology of carotid plaques, based on its inverse association with various immune cell processes. Mast cells (MCs) are considered important contributors to plaque instability, but their relationship with macrocalcification remains elusive.
Objective: Our aim was to investigate the link between MC activation and key features of human plaque vulnerability, and study MC role in plaque calcification via smooth muscle cells (SMCs).
Methods and Results: Pre-operative computed tomography angiographies of 40 patients undergoing surgery for carotid stenosis were used to characterize plaque morphology. Bulk plaque transcriptomes were used for bioinformatic deconvolution of immune cell fractions. Tissue microarrays were used to histologically evaluate the contribution of degranulated and resting MCs in plaques. Activated MC fraction and their typical markers were abundant in low-calcified plaques, while they negatively correlated with macrocalcification. Bioinformatic analyses also showed associations of MCs with NK cells and other immune cell types in plaques, confirmed by immunohistochemistry. In vitro experiments showed that SMC calcification attenuated MC activation, while both active and resting MCs stimulated SMC calcification and induced their dedifferentiation towards a pro-inflammatory- and osteochondrocyte-like phenotype.
Conclusions: Integrative analyses of human plaques showed that MCs activation is inversely associated with macrocalcification and positively with morhological parameters of plaque vulnerability, immune cell fractions and clinical symptoms. Mechanistically, MCs induce SMC osteogenic reprograming, while subsequent matrix calcification attenuates MC activation, offering a new therapeutic avenue for further studies.

No 80

Posters – Abstracts –

Other Topics

SESSION II

Non-alcoholic fatty liver disease by fat in blood and body

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INTRODUCTION:
High caloric diets rich in fat and carbohydrates lead to increased fat accumulation in adipose tissue and blood. We tested the hypothesis that baseline high levels of plasma triglycerides, body mass index, and waist circumference individually and combined are associated with non-alcoholic fatty liver disease during follow-up.
METHODS:
We examined 105,981 white Danish individuals with baseline measurements of plasma triglycerides, body mass index, and waist circumference who were without diagnosed non-alcoholic fatty liver disease at baseline. During up to 15 years (median 9.4 years) of follow-up, 418 individuals were diagnosed with non-alcoholic fatty liver disease.
RESULTS:
Compared to individuals with plasma triglycerides <1 mmol/L, multivariable adjusted hazard ratios for non-alcoholic fatty liver disease were 1.48 (95% confidence interval: 1.14-1.93) for plasma triglycerides of 1-2.9 mmol/L, 2.57 (1.81-3.65) for 3-4.9 mmol/L, and 3.61 (2.32-4.88) for plasma triglycerides ≥5 mmol/L, (p-value for trend=3×10-10). Corresponding hazard ratios for higher body mass index were 1.52 (1.20-1.92), 2.22 (1.67-2.94), and 3.36 (2.32-4.88) (p=5×10-14), and for higher waist circumference 1.22 (0.93-1.59) and 2.14 (1.70-2.69) (p=3×10-11), respectively. Absolute 10-year risk of non-alcoholic fatty liver disease increased additively with higher plasma triglycerides and higher body mass index combined, and with higher plasma triglycerides and higher waist circumference combined. For plasma triglycerides <1 mmol/L and body mass index <25 kg/m2 the risk was 0.27% increasing to a maximal 1.90% at plasma triglycerides ≥5 mmol/L and body mass index ≥35 kg/m2. Corresponding values from both lowest plasma triglycerides and lowest waist circumference to highest plasma triglycerides and highest waist circumference were 0.26% and 1.39%, respectively.
DISCUSSION:
Elevated plasma triglycerides, body mass index, and waist circumference individually and especially combined are associated with increased risk of future development of non-alcoholic fatty liver disease in asymptomatic individuals.

No 81

Maternal lipid levels in early pregnancy as a predictor of childhood lipid levels

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Background
Maternal lipid levels in early pregnancy are associated with maternal health and foetal growth. It is however unclear if the maternal lipids in early pregnancy can be used to predict childhood lipid levels. The aim of this study was to assess the associations between maternal lipid levels and the lipid levels of their children, and to investigate the influence of lifestyle factors on these associations.

Methods
In 2692 women participating in the Generation R study total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-c) were measured in early pregnancy (median 13·2 weeks [90% range 10·6; 17·1]). Low-density lipoprotein-cholesterol (LDL-c), remnant cholesterol and non-HDL-c were calculated. For outcome measures, corresponding lipid measurements were determined in children at the ages of six (median 6·0 years [90% range 5·7; 7·5]) and ten years (median 9.8 years [90% range 9·5; 10·3]).

Results
Maternal lipid levels in early pregnancy are positively associated with corresponding childhood lipid levels six and ten years after pregnancy, independent of lifestyle factors such as maternal body mass index (BMI), diet and physical activity.

Conclusions
Maternal lipid levels in early pregnancy provide an insight in the lipid profile of children years later. Gestational lipid levels may therefore be used as an early predictor of children's long-term health.

No 86

Association between plasma apolipoprotein M and cardiac autonomic neuropathy in type 1 diabetes

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Background and aim: Diabetes may lead to severe complications e.g. cardiac autonomic neuropathy (CAN) characterized by increased risk of cardiovascular mortality. CAN is diagnosed by a decreased heart rate viability (HRV). Sphingosine-1-Phosphate (S1P) carried by the HDL-associated apolipoprotein M (apoM) are linked to a reduction in the heart rate, and treatment with an S1P-agonist increases HRV. The aim of the present study was to investigate if plasma apoM was associated with increased risk of CAN.
Methods: The study includes 278 individuals with Type 1 Diabetes recruited from Steno Diabetes Center in Copenhagen from 2010 to 2012.
Results: A change of 0.1 µM plasma apoM was associated with the diagnosis of CAN (Odds ratio: 1.11 (1.02; 1.21), p=0.013). ApoM plasma levels were also positively associated with CAN when adjusted for age and gender (Odds ratio: 1.11 (1.02;1.21), p=0.013) as well as cardiovascular factors (Odds ratio: 1.14 (1.04; 1.25), p=0.005) and diabetes associated factors (Odds ratio: 1.13 (1.03; 1.25), p=0.01). Plasma apoM was also associated with a significantly lower SDNN as well as high frequency power in all adjusted models.
Conclusion: Increased plasma apoM was associated with increased risk of CAN as well as a significant reduction in HRV indices representing parasympathetic activity.

No 94

Are patients with familial hypercholesterolemia at increased risk of musculoskeletal disorders due to the very long time high intensive lipid-lowering drugs?

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Introduction: Diseases occuring frequently in a "genetic high LDL-cholesterol (LDL-C) model disease" may be caused by LDL-C itself, or by the treatment of a high LDL-C. We therefore investigated the occurrence of a limited number of pre-specified diagnosed in Norwegian Familial hypercholesterolemic (FH) patients. Approximately 90% of adult FH patients use high dose statins. Few, if any other patient groups have used more statins for a longer time than FH patients. In a large cohort of FH patients, we here present preliminary and crude number of muscle disease, fibromyalgia, inflammatory polyarthropathies and systemic connective tissue diseases in FH and controls.

Material and methods: 5635 Norwegians with an FH mutation diagnosed from 1992 and onward were compared with 112589 age and sex matched controls (ratio FH vs control 1:20) drawn from the general population. The cohorts were linked to The Norwegian Patient registry containing data on hospitalizations, outpatient visits and visits at specialist care for the entire population of Norway from 2008 and onwards.

Results and discussion: We here present crude number of unique subjects registered with hospitalization or outpatient treatment in the period 2008-2018. For muscle diseases (ICD codes M60-63), there were 45 and 688 individuals, for fibromyalgia (ICD10 code M979), there were 49 and 962 individuals, for inflammatory polyarthropathies (ICD10 codes M05-M14) there were and 166 and 3404 individuals and for systemic connective tissue diseases (M30-M36) there were 105 and 2058 individuals in the FH group and control population, respectively.

Conclusion: Data for muscular and connective tissue diseases will be presented at the meeting as number of hospitalizations, outpatient visits and visits at contracted specialist care. Our preliminary data suggests that these diseases are not overrepresented in FH patients although some have used high dose statins for more than 30 years.

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YIA Poster Walk – Abstracts –

Other Topics

SESSION II

Impact of vegetarian and vegan diets on blood lipid and lipoprotein levels: a systematic review and meta-analysis

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Background: Due to growing environmental focus, plant-based diets are increasing in popularity making it highly relevant to uncover their dietary impact on disease risk factors. Blood lipids and lipoproteins are well-established risk factors for cardiovascular diseases – the leading causes of death worldwide. We therefore conducted a systematic review and meta-analysis investigating the impact of vegetarian and vegan diets on blood levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides.

Methods: We performed a literature search through September and October 2021 using PubMed and references of previous reviews. Randomized clinical trials that quantified the effect of vegan or vegetarian diets on blood lipids and lipoproteins in adults over 18 years were included. The control groups were omnivorous. A random-effects model was used to calculate mean differences in blood lipids and lipoproteins between groups. Heterogeneity was estimated with the use of I^2 statistics.

Results: 27 RCTs were included in the study. We found that compared with the omnivorous groups the vegetarian and vegan diets significantly reduced total cholesterol (TC) and LDL cholesterol (LDL-C) levels with mean differences of –0.33 mmol/L (95% confidence interval –0.43, –0,22; P=2x10-9; I^2=64.1%) and –0.31 mmol/L (–0.41, –0.21; P=3x10-10; I^2=60.7%), respectively. Triglyceride (TG) levels increased marginally in the plant-based groups with a mean difference of 0.07 mmol/L (0.00, 0.13; P=0.05; I^2=39.2%) compared with the omnivorous.

Conclusion: Vegan and vegetarian diets were associated with reduced plasma concentrations of TC and LDL-C while TG concentrations were slightly increased. Plant-based diets, therefore, have the potential to lessen the atherosclerotic burden and thereby reduce the risk of cardiovascular disease. Furthermore, these diets have the potential to markedly reduce greenhouse gas emissions and thereby improve global sustainability goals.

No 78

Jet lag increases insulin levels and body fat in male but not female mice, as dependent on sex hormones

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Aim:
Circadian disturbances, regularly occurring by shift work or social jet lag, are associated and causally linked to cardiometabolic disease. It is however unclear if sex plays a causal role in the adverse effects of circadian disturbances. Therefore, we aimed to elucidate in mice whether the effects of circadian disturbances in the form of jet lag on energy metabolism are sex-dependent and study underlying mechanisms.

Methods & results:
We exposed chow-fed male and female C57BL/6J mice to 6h phase advances every 3 days to induce jet lag for 10 weeks. Jet lag increased insulin levels and initially increased body fat mass specifically in males and increased glucose levels specifically in females, without altering food intake. To study the involvement of sex hormones in these sex-specific differences, we exposed mice to jet lags after chemical castration by injecting GnRH antagonist Degarelix. Depletion of sex hormones by Degarelix treatment prevented jet lag-induced increases in insulin levels and body fat mass specifically in males, whereas Degarelix treatment during jet lag in females further elevated glucose levels.

Conclusions:
Male mice are more prone to the effects of jet lag on cardiometabolic health than female mice, which is likely explained by sex hormones, as depletion of testosterone in male mice protects from the effects of jet lag on metabolic health, while estradiol depletion in female mice aggravates these effects. Future studies on circadian disturbances in mice and humans should consider sex as potentially important factors, which may eventually contribute to personalized advice for shift workers.

No 87

Body mass index and risk of dementia

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Background and aims:
Midlife obesity and underweight in the elderly have been associated with a high risk of dementia. However, the association between body mass (BMI) and risk of dementia depends on the type of dementia investigated. Whether there is a causal association between BMI and the vascular part of dementia called “non-Alzheimer dementia” remains unknown. We aimed to investigate the association between BMI and risk of “non-Alzheimer dementia”, Alzheimer’s disease, and all-cause dementia.

Methods:
In a prospective cohort of the Danish general population including 95,000 individuals we investigated the observational and genetically determined association between BMI and risk of “non-Alzheimer dementia”, Alzheimer’s disease, and all-cause dementia. For the genetic analyses we created a weighted allele score and divided it into four groups from lowest to highest BMI.

Results:
The observational association between BMI and risk of “non-Alzheimer dementia” and all-cause dementia was u-shaped with nadir at a BMI of 26 kg/m2. For Alzheimer’s disease the association was linear with low BMI associated with high risk. The hazard ratios (95% confidence intervals) for the highest versus the lowest group of genetically determined BMI were 1.22 (1.01- 1.47) for non-Alzheimer dementia, 1.04 (0.90-1.20) for Alzheimer’s disease, and 1.10 (0.98-1.23) for all-cause dementia.

Conclusions:
Genetically determined high BMI is associated with high risk of the vascular part of dementia in the general population. BMI is thus a potentially modifiable risk factor for dementia that could be targeted in the strive to prevent this devastating disease.

No 89

Modulation of energy metabolism by eicosapentaenoic acid in skeletal muscle cells from lean and obese individuals

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Skeletal muscle represents a major organ for metabolism of fatty acids and glucose. Various forms of lifestyle interventions that influence energy conversion and energy consumption in muscle may have an impact on counteracting metabolic diseases. With this regard, studies have shown that obesity could have negative effects on the body's energy conversion by affecting muscle function, metabolism, and impairing insulin signaling. In the present work, we have compared energy metabolism in primary skeletal muscle cells established from subjects with and without obesity, and investigated effects of in vitro exposure to the n-3 fatty acid, eicosapentaenoic acid (EPA). Energy metabolism in myotubes was studied using radiolabeled substrates. Protein expression was investigated using proteomic analysis. Our results show that skeletal muscle cells from obese donors tended to take up more glucose, fatty acid and amino acids than in cells from lean. Furthermore, cells from obese individuals showed lower fractional oxidation of these substrates than lean cells, indicating a tendency towards storage. However, in presence of the mitochondrial uncoupler carbonyl cyanide 4-(tri-fluoromethoxy) phenylhydrazone (FCCP), glucose uptake and oxidation were higher in myotubes from obese individuals than in cells from lean. Moreover, treatment with EPA increased glucose uptake and oxidation in cells from obese individuals indicating enhanced mitochondrial function. In addition, EPA increased oleic acid uptake in myotubes from obese individuals and decreased leucine fractional oxidation in skeletal muscle cells from both lean and obese individuals. Proteomics analysis showed downregulation of fatty acid metabolic pathways and upregulation of apoptotic and hypoxic pathway in myotubes from obese individuals compared to cells from lean. Downregulation of fatty acid metabolic pathway and upregulated hypoxic pathway in cells from obese individuals justifies more reliance of these cells on glycolytic pathway for energy generation. In conclusion, these results indicate that cells from obese have a higher glycolytic pathway reserve than lean cells. EPA treatment enhances energy metabolism in human myotubes from obese individuals suggesting that n-3 fatty acid supplementation in vivo may be beneficial to improve metabolic and mitochondrial function in subjects with obesity.

No 93

A rare genetic variant in the manganese transporter SLC30A10 and elevated liver enzymes in the general population.

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Aim: SLC30A0 encodes a manganese transporter which effluxes manganese from hepatocytes to the bile. Individuals homozygous for loss-of-function variants in SLC30A10 accumulate manganese in the liver and have severe phenotypes including liver cirrhosis.
A genetic variant in SLC30A10 (rs188273166, p.Thr95Ile) associated with increased plasma alanine transaminase (ALT) in a recent GWAS in the UK Biobank (UKB). The aims of the present study were to validate the association of rs188273166 with ALT in an independent cohort, and to test the clinical, hepatic and biochemical phenotypes associated with the variant.
Method: We included n=334,886 white participants from the UKB, including 14,462 with hepatic magnetic resonance imaging (MRI), and n=113,612 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study combined.
Associations with continuous and categorical outcomes were tested using linear and logistic regression, respectively.
Results: Genotyping SLC30A10 p.Thr95Ile identified 816 heterozygotes in the UKB and 111 heterozygotes in the Copenhagen cohort. Compared to non-carriers, heterozygotes had 4 U/L and 5 U/L higher levels of plasma ALT in the UKB (P=9×10-19) and Copenhagen cohort (P=0.02), respectively, and 3 U/L higher plasma aspartate transaminase (P=1×10-13) and gamma glutamyl-transferase (P=3×10-4) in the UKB. Heterozygotes also had higher corrected T1 on liver MRI, a marker of hepatic inflammation (P=4×10-7), but no change in MRI-quantified steatosis (P=0.57).
Conclusions: SLC30A10 p.Thr95Ile was associated with elevated liver enzymes in two large general population cohorts, and with MRI-quantified hepatic inflammation. We hypothesize that Thr95Ile heterozygosity associates with a mild form of hepatic manganese accumulation leading to liver damage.

No 95

Synthetic LXR agonist PFM018 as potential treatment for Alzheimer's Disease

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Liver X receptors (LXR-alpha/beta) are key regulators of cholesterol homeostasis and inflammatory processes. LXRs are a therapeutic target for cardiovascular and neurodegenerative diseases, such as Alzheimer's Disease (AD). Accumulating evidence indicates a key role for a disturbed cholesterol transport in the brain in the development and progression of AD. We showed that memory of AD mice improves significantly upon activation of brain cholesterol turnover by synthetic activators of liver X receptors (LXR-alpha/beta). However, serious side effects including hepatic steatosis and hypertriglyceridemia render these pan LXR-alpha/beta activators unsuitable for patients. Because LXR-alpha activation is held responsible for the adverse effects observed upon pan LXR-alpha/beta activation, we tested PFM018, a semi-synthetic compound that preferentially activates LXR-beta in vitro. PFM018 upregulated the gene expression of LXR target genes ABCA1, ABCG1 and APOE and protein expression of ApoE and ABCG1. In CCF cells, PFM018 decreased the concentration of cholesterol and cholesterol precursors lanosterol, lathosterol, and desmosterol, indicating a reduction in cholesterol synthesis. No effect on cholesterol synthesis was observed in SH-SY5Y cells. We have demonstrated that PFM018 can cross the blood brain barrier and accumulate in the brain in wild-type C57BL/6J mice. We are currently investigating if PFM018 can prevent cognitive decline and the development of neuropathology in an AD mouse model without negatively affecting the cardiometabolic health. In case of positive results, PFM018 may be interesting to further explore for its application in the prevention of AD and potentially also in the treatment of cardiovascular disease.

No 96

Efficacy & Safety of PCSK9-Inhibitors: a Systematic Review and Meta-analysis of Real-World Data

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Aim
To investigate the real-world efficacy and safety of PCSK9-inhibitors.

Methods
A systematic search of observational studies with PCSK9-inhibitor use of >= 3 months was performed through Embase, Medline, Web of Science Core Collection, and Google Scholar up to 1 June 2021. Study selection, data extraction and risk of bias were conducted independently by 2 authors. The primary outcome for efficacy was absolute LDL-reduction and for safety the side-effects were reported. A random-effects meta-analysis and mixed-effects regression model was performed.

Results
67 studies of patients using PCSK9-inhibitors in clinical setting were included, involving 28,266 patients, of which 44% women, mean age 60±4 years, 71% with cardiovascular disease (CVD), 74% Familial Hypercholesterolemia (FH), 59%/41% using Evolocumab/Alirocumab, 59% statin co-medication, and 57% ezetimibe. Median follow up was 6 [3; 12] months and mean adherence was 87%.
Efficacy: The absolute reduction in LDL-cholesterol after initiation of PCSK9 inhibition was -2.25 mmol/L (p= <.0001; 40 studies; 13,416 participants) corresponding to a 54.6% LDL-cholesterol reduction. In the meta-regression, no influence of sex, statin-use, FH, or follow-up duration of PCSK9-inhibitor use was found for efficacy. Whereas baseline LDL-cholesterol was correlated with absolute LDL-reduction (B 0.5257, p<.0001).
Safety: Side-effects were experienced by 27.5% of patients. The most common side-effects were flu-like symptoms (11.5%), any pain or discomfort (7.3%), myalgia (7.0%), non-response (5.9%), and injection site reactions (5.2%). At the end of follow-up, 10% of the total population discontinued their PCSK9-inhibitor of which 49% due to side-effects.

Conclusions
Real-world data of PCSK9-inhibitors show comparable efficacy and safety to trial data.

Posters – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

No 60

Does HDL composition differ between pre-, peri-, and postmenopausal women?

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High levels of High-Density Lipoprotein (HDL) have been linked to low risk of cardiovascular diseases. In menopause, levels of HDL-cholesterol rise, but the cardioprotective effect seems to be decreased. To understand this phenomenon, we studied HDL in different stages of menopausal change.
We collected serum from 216 women in pre-, peri- and postmenopausal stages (PRE, PERI, POST). The menopausal status was determined by follicle-stimulating hormone levels. We combined the serums into 12 body mass index-matched pools representing each menopausal stages and analyzed lipoprotein particle sizes, concentrations of total lipoprotein particles, ApoA1, and XL-, L-, M-, and S-sized HDL particles with nuclear magnetic resonance (NMR). With NMR we also measured concentrations of the major lipid species in each size class of HDL. From the same pools we also isolated total HDL with stepwise density ultracentrifugation. HDL lipid compositions were analyzed with mass spectrometry.
The average sizes of HDL particles were smaller in PERI (mean 9.7±0.06 nm) than in PRE (9.8±0.05 nm; p=0.014) or POST (9.8±0.08 nm; p=0.029). Concentrations of ApoA1 were lowest in PERI (mean 1.6±0.07 mM) compared to PRE (1.7±0.08 mM; p=0.037) and POST (1.7±0.09 mM; p=0.0013). Also concentrations of XL, L, and M-sized but not S-sized HDL-particles, were significantly lower in PERI (0.3, 1.8, 4.3, 10.7 µM, respectively) compared to PRE (0.3, 2.2, 4.7, 10.6 µM; p=0.018, 0.004, 0.04, 0.9) or POST (0.3, 2.3, 4.9, 11.1 µM; p= 0.029, 0.009, 0.0017, 0.18). The average proportions of lipid species in PRE differed from those of PERI, but not from those in POST.
We conclude that PERI group differs from PRE and POST in HDL size distributions and proportions of lipids, indicating that menopause may cause transient changes in HDL lipidome. However, longitudinal analysis over menopausal transition needs to be done before verify this notion.

No 66

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

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Obesity and dyslipidemia are major risk factors for the development of cardiovascular diseases (CVD), but their interconnectivity remains poorly understood. While white adipose tissue (WAT) is the largest reservoir of cholesterol in our body, the consequences of increased cholesterol uptake and storage on human adipocyte function (and dysfunction) require further research.
The purpose of this projects is to elucidate: 1) cellular mechanisms regulating adipocyte uptake of cholesterol in obesity; 2) the effects of cholesterol loading on adipocyte function; and 3) how WAT impacts systemic levels of circulating lipoproteins, driving dyslipidemia and subsequent CVD.
Our preliminary data of mRNA sequenced isolated human adipocytes shows that obese patients with dysfunctional WAT are associated with higher expression of cholesterol-uptake genes (LDLR and SR-B1) and lower expression of triglyceride-uptake genes (VLDL and LPL), which could initiate the development of dyslipidemia. Currently, we are expanding our cohort and validating these findings on protein level.
By using our Human Unilocular Vascularized Adipocyte Spheroid model (HUVAS, Ioannidou et al, 2021), we are studying how cholesterol supplementation impacts adipocyte dysfunction in lean (control) and obese (fattened) adipocyte cultures. In fact, supplementation with micellar cholesterol leads to significantly enlarged lipid droplets and higher leptin secretion. We will now access adipocyte function by quantifying insulin signaling by capillary western blot, and proinflammatory adipokine secretion levels. Moreover, we are developing a new tamoxifen-inducible mouse model for adipocyte-specific LDLR overexpression to tests to what extent adipose tissue cholesterol loading induces dyslipidemia in vivo.
Together these experiments will prove if adipose tissue cholesterol can drive the development of dyslipidemia during obesity and constitutes an interesting target in CVD prevention.

A Randomized Controlled Dietary Intervention did not improved LDL aggregation in Patients with Rheumatoid Arthritis

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Background and aims: Patients with Rheumatoid Arthritis (RA) have an increased risk of cardiovascular diseases (CVD). Diet is a major modifiable risk factor for CVD and thus the study ADIRA (Anti-inflammatory Diet in Rheumatoid Arthritis) aimed to evaluate the effect of diet on disease activity and risk factors for CVD. LDL aggregation contributes to atherosclerotic plaque progression and increases the risk for adverse cardiovascular events. The objective of this work was to evaluate effects of a dietary intervention on LDL aggregation in patients with RA.

Methods: Patients (n = 50) with RA were included in the cross-over trial ADIRA. They were randomized to either a Mediterranean-style diet intervention or a Western-style control diet, for ten weeks. After a 4-month washout they switched diet. Participants received food bags and dietary guidelines. Blood was collected and analysed for inflammation markers and lipidomics. Plasma LDL was exposed to sphingomyelinase and LDL aggregate size measured via dynamic light scattering. A linear mixed model with fixed variables for dietary treatment, period, sequence as well as the baseline value for each outcome variable was used for between groups analysis. Wilcoxon signed rank test was used for within groups analysis.

Results: Samples from forty-four patients that completed at least one period was included in the presented work. Median age was 61 years and BMI 27 kg/m2 and all remained weight stable during the intervention. LDL (median (first quartile, third quartile)) concentration at baseline was 3.5 (2.9, 4.3) and the dietary intervention led to a significant improvement of triglycerides in favour of intervention ($p = 0.006$). LDL aggregation was not changed after either of the dietary periods and did not differ between the two dietary periods.

Conclusions: Although triglyceride concentrations improved, LDL aggregation was unaltered after a 10 week dietary intervention in patients with RA.

No 71

Apolipoprotein M and Retinoid Metabolism

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Background: Retinoids regulate important cellular processes such as proliferation and immunity. In circulation, retinoids are mainly transported via binding of retinol to retinol-binding-protein-4 (RBP4). More recent studies suggest that other transport pathways, including lipoproteins, may also exist. HDL bound apolipoprotein M (apoM) is a carrier of Sphingosine-1-phosphate. In vitro studies suggest that apoM may also bind retinoids, but the physiological relevance is not clear. Thus, the aim of this study was to investigate the role of apoM in retinol transport and metabolism in vivo.
Methods: Retinoids in different plasma compartments and tissues were quantified using HPLC in wild type (WT), RBP4-KO, apoM-KO and apoM-transgenic (apoM-TgH) mice (10-fold increase of human apoM). Further, histological evaluation of retinal morphology as well as RBP4 expression analysis was performed.
Results: Plasma-retinol was increased in apoM-TgH mice (mean ±SE; 1.39 umol/L ± 0.06) compared to WT mice (1.03 umol/L ±0.07, $p<0.05$). Retinol was mainly found in the plasma protein fraction (with or without RBP4), with <10 % in HDL and <5% in LDL particles in both WT, apoM-TgH and RBP4-KO mice. No difference in the morphology of the retina was found between WT, apoM-KO and apoM-TgH mice. As apoM per see could not explain the increased level of plasma retinol in apoM-TgH mice, the level of RBP4 and retinols was further analysed. The mRNA expression of RBP4 was decreased in apoM-TgH mice compared to WT mice, whereas there was a trend toward increased plasma protein levels. Finally, no difference in liver retinol or retinyl palmitate concentration between WT and apoM-TgH mice was observed.
Conclusion: Retinol is found in ApoM-containing lipoproteins, and increased apoM levels increases plasma-retinol, but is not associated to HDL fractions. Instead, apoM per see may increase RBP4 and thereby the transport capacity of retinol. Further studies are needed to elucidate the link between apoM levels and retinol metabolism.

YIA Poster Walk – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

No 55

The Ser251Pro SNP in perilipin 2 (PLIN2) affects chaperone-mediated autophagy

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PLIN2 is the most prominent lipid droplet (LD)-associated protein in foam cells: it stabilizes LDs and LDs protect PLIN2 from degradation. LD utilization and degradation during energy depletion occurs through macrolipophagy and cytosolic lipolysis; in both mechanisms, PLIN2 needs to be removed from the LD surface prior any of those can occur. LD-bound PLIN2 is degraded by chaperone-mediated autophagy (CMA).
We have shown that the Ser251Pro SNP in PLIN2 influences macroautophagy and cholesterol efflux in human macrophages and is associated with a subclinical atherosclerosis development in humans.
Since a direct crosstalk between macroautophagy and CMA has been proposed, we aim to investigate whether the Ser251Pro PLIN SNP alters CMA and the degradation of PLIN2, thereby influencing macrolipophagy.

We cultured WT, Atg5-/- and Lamp2a-/- mouse embryonic fibroblasts transiently transfected with construct carrying either variant of PLIN2 and loaded with oleate: we measured LD content by IF. Upon genetic blockage of MA (Atg5-/-), the difference in LD accumulation between the two variants was abrogated, suggesting that this difference is mediated by changes in the autophagic activity. The differences were maintained in CMA abrogation, suggesting that PLIN2-CMA interaction is required for this phenotypic difference.
We cultured HEK293 cells stably transfected with the same PLIN2 constructs and loaded with oleate: we measured LAMP2A protein and mRNA levels and measured CMA activity using a CMA reporter. HEK293 cells carrying the Pro allele showed lower LAMP2A protein and mRNA levels and less CMA activity, indicating fewer CMA-active lysosomes.

We conclude that Ser251Pro SNP in PLIN2 affects macroautophagy in a CMA-dependent manner.

No 57

Accelerated vascular ageing and retention of LDL in Type 2 Diabetes

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AIM:
Patients with Type 2 Diabetes (T2D) are disproportionately affected by atherosclerosis compared with non-diabetics. We previously reported that the interstitial fluid to serum ratio (IF:S) for atherogenic lipoproteins is reduced in T2D. Further measures related to pathophysiological functions of atherogenic lipoproteins in serum and IF may unveil novel insights in transvascular transport of cholesterol in atherosclerosis.

METHODS:
We recruited 75 T2D patients and 75 age-and sex-matched controls and obtained serum and IF after overnight fast. IF collected from abdominal skin blisters was compared with serum obtained from peripheral blood. Cholesterol, TG, proteins, and lipoprotein lipids were determined using FPLC and ELISA. The binding affinity of serum, isolated VLDL and LDL to proteoglycans (PGs) was evaluated through ex vivo binding to human aortic PGs.

RESULTS:
As anticipated, T2D displayed a significantly reduced IF:S of apoB-100 ($p<0.001$). A reduced IF:S for apoB-100 was linked to increasing age in controls ($r=-0.385$, $p<0.001$) but not in T2D. Serum and LDL from T2D patients had increased affinity for PGs ($p<0.001$), and enhanced binding was associated with a reduced IF:S for apo-B100 ($r=-0.336$ serum, $r=0.382$ LDL, $p<0.001$). VLDL binding to PGs did not differ between the groups.

CONCLUSION
Serum LDL from T2D patients have an increased binding to PGs in association with a reduced transvascular ratio of LDL. In accordance with the “response to retention” hypothesis of atherosclerosis, this indicates an increased peripheral accumulation of LDL cholesterol in T2D, which can be seen as a process of “premature ageing”.

No 59

Elevated LDL-triglycerides and increased risk of atherosclerotic cardiovascular disease: two complementary methods within the Copenhagen General Population Study

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Aims: There is limited knowledge about the association between elevated low-density lipoprotein (LDL)-triglycerides and risk of atherosclerotic cardiovascular disease (ASCVD). We tested the hypothesis that elevated LDL-triglycerides are associated with increased risk of ASCVD.

Methods and Results: We examined individuals from the Copenhagen General Population Study, where LDL-triglycerides were measured in 38,081 individuals using a direct automated assay and in 30,066 using nuclear magnetic resonance (NMR) spectroscopy. During a median follow-up of respectively 3 and 9.2 years, 752 and 5,475 individuals in the two cohorts were diagnosed with ASCVD. For direct LDL-triglycerides, versus individuals in the 1st-50th percentiles, the multivariable adjusted hazard ratios for ASCVD were 1.38 (95% confidence interval (CI): 1.18-1.62) for the 51st-95th percentiles and 1.92 (1.46-2.51) for individuals in the 96th-100th percentiles. For NMR LDL-triglycerides corresponding hazard ratios were 1.28 (1.17-1.40) and 1.52 (1.26-1.82), respectively. Per 0.1 mmol/L (9 mg/dL) higher direct LDL-triglycerides, hazard ratios were 1.26 (1.16-1.36) for ASCVD, 1.27 (1.16-1.39) for ischemic heart disease, 1.28 (1.11-1.48) for myocardial infarction, and 1.22 (1.08-1.38) for ischemic stroke, while the odds ratio was 1.40 (1.29-1.52) for peripheral artery disease measured as an ankle-brachial index <0.8. Corresponding estimates for NMR LDL-triglycerides were 1.11 (1.07-1.15) for ASCVD, 1.15 (1.11-1.20) for ischemic heart disease, 1.14 (1.09-1.20) for myocardial infarction, 1.07 (1.01-1.13) for ischemic stroke, and 1.22 (1.07-1.39) for peripheral artery disease.

Conclusion: Elevated LDL-triglycerides, using two different methods of measurement, were associated with increased risk of ASCVD and its different sub-diseases individually.

No 62

MicroRNA 33a controls SREBP-2 and LXR dependent regulation of the LDL receptor pathway

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Background and aim: Coordinated cellular mechanisms that regulate LDLR transcription and degradation remain largely obscure. We sought to assess the effect of miR-33a on the LDLR pathway given the concerted action of SREBP-2 and miR-33a in elevating cellular cholesterol levels.

Material and methods: The role of miR-33a in LDLR expression and (V)LDL-uptake was assessed in a series of gain and loss of function studies. Post-transcriptional inhibition through direct binding to target mRNAs was determined and validated by vector-based and Par-Clip assays. The effect on plasma cholesterol and triglyceride levels was evaluated in DIO, Ldlr+/- and E3L.CETP mice.

Results: MiR-33a-3p/5p modulated LDLR protein abundance and LDL uptake without a change in mRNA. Intrinsic binding sites predominantly positioned within the PCSK9 coding sequence and IDOL 3'-UTR, facilitated inhibition and interrupted sustained LDLR repression in hepatocytes. MiR-33a-3p, but not 5p, also directly inhibited ANGPTL3 expression. Liver-targeted miR-33a-3p mimic reduced hepatic and circulating PCSK9 levels and lowered LDL levels. In E3L.CETP mice, it also attenuated postprandial TG and non-HDL-C levels as a consequence of increased triglyceride-derived fatty acid uptake by white adipose tissue and subsequent hepatic uptake of lipoprotein remnants, accompanied by reduced plasma ANGPTL3.

Conclusion: Our findings reveal a compensatory control mechanism in the LDLR pathway and highlights miR-33a-3p mimics as alternative therapeutic inhibitors of LDL-cholesterol in hypercholesterolemia.

No 63

How ANGPTL4 regulates LPL by catalyzed unfolding

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Lipoprotein lipase is the rate-limiting enzyme for serum triglyceride uptake to tissues like the heart and adipose tissue. Regulation of LPL by ANGPTL3, ANGPTL4, and ANGPTL8 is essential to ensure spatial and temporal delivery of serum triglycerides. Our recognition of the interplay between these LPL-inhibitors has greatly improved over the recent years, however, our understanding of the molecular mechanism behind the regulation has been underappreciated. We have previously shown that LPL is a marginally stable enzyme that is inactivated by protein unfolding, and that ANGPTL4 regulates LPL by catalyzing this unfolding. Using Nano-differential scanning fluorimetry we have investigated the low thermal stability of LPL further and determined the melting temperature of LPL to be 34°C. The addition of ANGPTL4 decreased the stability of LPL to <15°C, while binding of the LPL-transporter, GPIHBP1, increased the stability of LPL ~20°C, also in the presence of ANGPTL4. This explains why ANGPTL4 is such a potent inhibitor of LPL, and why binding of GPIHBP1 limits ANGPTL4 suppression. Using hydrogen-deuterium exchange mass spectrometry (HDX-MS) we further showed that ANGPTL4 bind near the active site in the □/□-hydrolase domain of LPL. Using pulse-labeled HDX we were able to pinpoint the nucleation zone for ANGPTL4-catalyzed unfolding to □3-□3 in LPL, which ultimately lead to irreversible LPL-inactivation. Our studies provide a detailed understanding of this unprecedented regulation mechanism and could help the development of stabilized LPL variants, resistant to ANGPTL4-catalyzed unfolding, and serve as a fundament for ANGPTL3/ANGPTL8 studies.

No 68

Icosapent ethyl supplementation rapidly and transiently alters the composition and functionality of circulating lipoproteins in humans

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AIM: Icosapent ethyl-supplementation has postulated benefits in improving cardiovascular health. Total circulating eicosapentaenoic acid (EPA) correlates negatively with cardiovascular disease (CVD) risk and total mortality. Here we assessed how EPA-supplementation affects the lipidome and proatherogenic properties of plasma lipoproteins.
METHODS: 39 healthy, normolipidemic volunteers received a 4 g daily dose of icosapent ethyl for 4 weeks. VLDL, LDL, and HDL were isolated from blood samples collected prior, during, and after the supplementation, and their fatty acid and lipid compositions were assessed by lipidomics, LDL aggregation propensity and proteoglycan-binding of the plasma lipoproteins were also determined.
RESULTS: Total plasma EPA increased 5-fold within the first week of supplementation and returned to baseline level 1 week after the supplementation. Among the lipoprotein fractions, 53 lipid species were significantly increased and 31 significantly decreased. Lipids containing n-3 fatty acids were increased, while those containing n-6 fatty acids were decreased, resulting in a reduction of plasma n-6/n-3 ratio from 7.9 to 2.8. Changes were qualitatively similar in all lipoprotein classes. Largest changes were observed in cholesteryl esters, followed by phosphatidylcholines and triacylglycerols. Icosapent ethyl-supplementation decreased lipoprotein binding to proteoglycans, and LDL aggregation decreased in individuals having the most aggregation-prone LDL.
CONCLUSIONS: Icosapent ethyl-supplementation leads to a rapid but transient increase in total circulating EPA, a reduction of the n-6/n-3 fatty acid ratio of lipoprotein lipids, and a reduction in proteoglycan-binding of plasma lipoproteins. EPA-containing lipids fluctuate equally in all main lipoprotein classes. Reduced proteoglycan-binding of plasma lipoproteins may partly explain the known icosapent ethyl-induced reduction in CVD risk.

No 69

Cholesterol Efflux Capacity of High-density Lipoprotein Particles is Impaired in Age-related Macular Degeneration Patients with High Plasma HDL-c Levels

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Age-related macular degeneration (AMD) is a blinding disease initiated by the formation of fat globules, so-called drusen, in the retina. Epidemiological studies demonstrated an association between elevated plasma high-density lipoprotein-cholesterol (HDL-c) levels and AMD. This is in contrast to the risk of cardiovascular disease that is negatively associated with HDL-c levels. Among the genes linked with AMD, ABCA1, CETP, LIPC and APOE are related to the role of HDL in mediating cholesterol efflux from cells and consequently in reverse cholesterol transport. In accordance, we hypothesized that the cholesterol efflux capacity (CEC) of HDL is impaired in AMD patients, consequently leading to cholesterol accumulation underneath retinal pigment epithelium (RPE) cells, facilitating drusen formation. To approach this, the capacity of HDL isolated from 30 AMD patients and 30 controls to mediate cellular cholesterol efflux from [3H]-cholesterol-loaded ARPE-19 cells was assessed by measuring the percentage of [3H]-cholesterol secreted into the culture medium. We found no differences in cholesterol efflux capacity between AMD patients and controls when comparing HDL isolated from 1 ml of plasma of each individual. However, HDL from AMD patients with high, but not low plasma HDL-c levels, showed a decreased cholesterol efflux capacity per HDL particle as compared to healthy controls. Together, our data suggest that functional impairment of HDL is not causal for AMD pathogenesis but may be a result of other metabolic dysfunctions of AMD patients. Elevated plasma HDL-c levels in AMD patients might compensate for the decreased CEC of HDL per particle.

No 70

Paradoxical regulation of cholesterol metabolism by fucosterol and saringosterol

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Accumulating evidence indicates a key role for a disturbed cholesterol transport in the brain during the progression of Alzheimer's disease (AD). Liver X receptors (LXR) are promising therapeutic targets for increasing cholesterol turnover and decreasing neuroinflammation in AD. Saringosterol and fucosterol both activate LXRs, although the latter to a lesser extent. Saringosterol can traverse the blood-brain barrier and can prevent memory decline in AD mice, possibly by affecting cholesterol turnover. Neuroprotective effects of fucosterol, have also been reported. However, the in-depth biological mechanisms underlying its neuroprotective effect is poorly understood. We investigated at which regulatory points fucosterol and saringosterol affect cholesterol turnover.
We found that fucosterol and saringosterol are internalized by HepG2, SH-SY5Y and CCF-STTG1 cells. The percentage of fucosterol internalized after 24h incubation was 12-19% and twice as muchsaringosterol. Saringosterol downregulated, while fucosterol upregulated desmosterol concentration in HepG2 cells as determined by GC-MS. Incorporation of 1-13C-acetate into newly synthesized cholesterol, revealed 13C-cholesterol was oppositely regulated by fucosterol and saringosterol. Saringosterol promoted the synthesis of 27-hydroxycholesterol(27-OHC). We hypothesis that fucosterol upregulates DHCR7, resulting in the rapid accumulation of the substrate desmosterol. Possibly, saringosterol reduced desmosterol via 27-OHC acting as a negative feedback regulator of cholesterol biosynthesis. Alternatively, saringosterol may inhibit DHCR7, interrupting synthesis of cholesterol at the level of its precursor.
Our data show opposite effects of saringosterol and fucosterol on desmosterol and cholesterol, decreasing and increasing their levels, respectively. Fucosterol may indirectly activate LXR through upregulating desmosterol as an endogenous LXR-agonist. This provides novel opportunities for modulating cholesterol homeostasis of and inflammatory processes in AD and atherosclerosis.

No 73

Identification of the ApoC-II binding site on Lipoprotein lipase using HDX-MS

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Lipoprotein lipase (LPL) plays a crucial role in maintaining lipid homeostasis in the body by catalyzing triglyceride hydrolysis in capillaries. An important regulator of intravascular lipolysis is apolipoprotein C-II (ApoC-II), the cofactor of LPL. It has previously been established that the C-terminal region of ApoC-II interacts with LPL leading to its activation. However, the interaction site of ApoC-II on LPL was not identified. This lack of information is primarily rooted in the intrinsic properties of these proteins; ApoC-II requires lipids to form its native state and the native state of LPL is highly unstable at body temperature. To eliminate these roadblocks, we formulated ApoC-II in the minimal amounts of DMPC lipids required to assemble its native state, used recombinantly expressed human LPL, and carried out the binding experiments at 25°C. We employed hydrogen-deuterium exchange mass spectrometry (HDX-MS) to map the regions involved in the ApoC-II-LPL interaction. To map the imprint of ApoC-II on LPL by HDX-MS, we measured the deuterium uptake by a continuous labelling protocol at various time points.
Our HDX-MS data shows that ApoC-II-DMPC complex slows the deuterium uptake in the following regions in LPL: β2, β3–α3, β7, active site and lid region compared to the presence of a non-binding variant (ApoC-II Q70E-DMPC). In ApoC-II, the only effect of the presence of LPL was confined to the C-terminal α-helix. Moreover, both HDX-MS and nanoDSF data shows that binding of the ApoC-II-DMPC complex to LPL leads to a significant increase in thermal stability of LPL. Intriguingly, the binding site(s) for ApoC-II overlaps with the binding site we previously assigned for the inhibitor ANGPTL4, which inactivates LPL by catalyzing the unfolding of LPL's catalytic domain. Noteworthy, ApoC-II stabilizes β7 in LPL, while ANGPTL4 increases the flexibility of this region. This could thus form a potential explanation for markedly different consequences of LPL binding by ApoC-II and ANGPTL4.

No 74

Hypertension in pregnancy: Impact on high-density lipoprotein composition and function

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Background/Aims:
Preeclampsia (PE) is the leading cause of death from pregnancy complications and is associated with impaired maternal and fetal lipid metabolism. In addition, this condition is linked to an overall increased cardiovascular risk for both mother and child. Characteristics of PE are hypertension, oxidative stress and dysfunction of maternal endothelium. HDL particles exert several endothelium-protective properties, partly through involvement of sphingosine-1-phosphate (S1P), which is associated with HDL through apolipoprotein M (apoM). Therefore, PE associated reduction or loss of endothelial protective activities by HDL could further aggravate the disease.
In this study, we investigated the effects of PE on maternal and fetal HDL composition and function, focusing on HDL-associated apoM/S1P. The study cohort included 32 women with normal pregnancy (NP), 18 women diagnosed with early-onset PE, and 14 women with late-onset PE. Plasma from the mothers and cord blood from the newborns were collected.

Results:
Determination of HDL-associated lipids in apoB-depleted plasma showed a profound reduction of phospholipids in early-onset and late-onset maternal PE groups. Measurements of HDL functional parameters revealed a decrease in cholesterol efflux capacity in the late-onset PE group of cord blood when compared with the NP group.
Assessment of S1P levels in plasma showed a marked reduction in late-onset maternal PE, whereas there was a tendency for less S1P in the cord blood of early-onset PE.
Interestingly, FPLC profiles of pooled cord blood showed a higher association of S1P with albumin than with HDL. These results were confirmed by measuring apoM-associated S1P and albumin-associated S1P. Of particular interest, levels of plasma apoM ($r_S=0.424$, $p<0.001$) and S1P levels ($r_S=0.278$, $p<0.05$) between mother and fetus were correlated.

Conclusion:
During PE, maternal plasma levels of S1P are profoundly affected, which may lead to decreased vascular protection and contribute to endothelial dysfunction.

Posters – Abstracts –

Cardiovascular Disease

SESSION IV

No 27

Female patients with familial hypercholesterolemia have higher cholesterol burden at 19 and 30 years of age: data from 12-years follow-up

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Background and aims: It is the cumulative exposure to low-density lipoprotein cholesterol (LDL-C) that defines the risk of cardiovascular disease (CVD). Patients with familial hypercholesterolemia (FH) have increased risk of premature CVD, and the highest excess risk is observed in young FH women. The aim of our study was to calculate lifelong LDL-C burden in FH individuals and investigate possible gender differences.
Methods: LDL-C burden was calculated based on untreated LDL-C and repeated LDL-C measurements during follow-up. Data were obtained from medical records of 438 young FH individuals <18 years at first visit to the Lipid Clinic, Oslo University hospital. Results presented as means (SD).
Results: Patients were 11.0 (3.9) years at first visit to the Lipid Clinic and had been followed up for 12.0 (7.0) years. Girls with FH had accumulated a significant higher LDL-C burden compared to boys at 19 years of age (112 [27] vs. 101 [25] mmol-years, P<0.001) and at 30 years of age (175 [36] vs. 157 [34] mmol-years, P=0.02).
Conclusion: Young women with FH had a higher accumulated cholesterol burden compared to young men, potentially explaining the excess CVD risk in this group. This underscores the importance of early treatment initiation among girls with FH and furthermore the need for careful follow-up to ensure that statin-free periods (e.g. in relation to pregnancy and breastfeeding) are limited.

No 28

Elevated remnant cholesterol appropriately reclassifies individuals who develop myocardial infarction

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Background: Elevated remnant cholesterol levels cause atherosclerotic cardiovascular disease. We tested the hypothesis that elevated remnant cholesterol will lead to appropriate reclassification of individuals who later develop atherosclerotic cardiovascular disease.
Methods: We followed 41,928 Danish individuals for more than 10 years from the Copenhagen General Population Study without a history of diabetes, coronary artery disease, or statin use. Using predefined cut points for elevated remnant cholesterol, we calculated net reclassification indices (NRI) from below to above 5%, 7.5%, or 10% separately and combined for absolute 10-year occurrence of myocardial infarction and major adverse cardiovascular events (MACE) defined as a composite of death from coronary artery disease, myocardial infarction and coronary revascularization.
Results: For individuals with remnant cholesterol levels ≥95th percentile (≥1.6 mmol/L, 61 mg/dL), 23% ($p<0.001$) of myocardial infarction events and 21% ($p<0.001$) of MACE were reclassified correctly from below to above 5% for 10-year occurrence when adding remnant cholesterol levels to models based on conventional risk factors, while no events were reclassified incorrectly for either endpoint. Consequently, addition of remnant cholesterol levels yielded NRI of respectively 10% (95% confidence interval 1–20%) for myocardial infarction and 5% (-3–13%) for MACE. Of note, when combining reclassification from below to above 5%, 7.5%, and 10% risk of events, 42% ($p<0.001$) of individuals with myocardial infarction and 41% ($p<0.001$) with MACE were reclassified appropriately, leading to NRI of respectively 20% (9–31%) and 11% (2–21%).
Conclusions: Elevated remnant cholesterol levels considerably improved myocardial infarction and MACE risk prediction.

No 30

The Lp(a) equivalent to FH in ASCVD risk: CGPS

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Background: Familial hypercholesterolemia (FH) is a common inherited condition characterized by lifelong high plasma levels of low-density-lipoprotein (LDL) cholesterol implying a high risk of myocardial infarction (MI) and atherosclerotic cardiovascular disease (ASCVD). High plasma levels of lipoprotein(a) [LP(a)] is also associated with an increased risk of MI and ASCVD.

Aims: To investigate and compare the risk of MI and ASCVD in patients with FH to the risk in patients with high plasma levels of Lp(a).

Methods: This follow-up study was based on data from the Copenhagen General Population Study on diagnostic criteria for FH and Lp(a) levels (n = 69,644). Individuals were diagnosed with FH by three commonly used clinical criteria including MEDPED, Simon-Broome and Dutch Lipid Clinic Network (DLCN) criteria or by genetics. Nationwide Danish registries were used for identification of individuals with MI and ASCVD. Results were compared by hazard ratios (HR) with 95% confidence intervals using Cox proportional hazard regression. Main results: In continuous analyses high plasma Lp(a) levels was associated with an increased risk of both MI and ASCVD. The risk of MI for high levels of Lp(a) compared to the risk in patients with FH differed among the types of criteria used for diagnosis of FH. Thus, the risk of MI in patients with FH diagnosed by MEDPED corresponded to the risk in individuals Lp(a) levels of 70 mg/dl; for FH diagnosed by Simon-Broome 110 mg/dl; for possible FH according to DLCN 255 mg/dl; for probable and definite FH 400 mg/dl and for genetic FH 180 mg/dl, respectively. The risk of ASCVD showed results in the same direction as for MI. A combination of FH and high Lp(a) was associated with the highest risk of MI and ASCVD.

Conclusions: A lipoprotein(a) level of approximately 180 mg/dL corresponded to genetic FH for risk of myocardial infarction and ASCVD. Corresponding lipoprotein(a) levels were higher for clinical FH by DLCN criteria and lower for Simon Broome and MEDPED criteria. Plasma Lp(a) should be measured in all patients with FH for better risk prediction and treatment.

No 33

Familial Hypercholesterolemia prevalence among ethnicities – systematic review and meta-analysis

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Background
Heterozygous familial Hypercholesterolemia(FH) is a common genetic disorder leading to premature cardiovascular disease and death because of lifelong high plasma low-density lipoprotein cholesterol levels, if not treated early in life. The prevalence of FH varies between countries because of founder effects, use of different diagnostic criteria, and screening strategies. However, little is known about differences in FH prevalence according to ethnicity. We aimed to investigate the ethnic distribution of FH in diverse populations and to estimate the prevalence of FH according to ethnicity.
Methods
We performed a systematic review and meta-analysis, searching PubMed and Web of Science for studies presenting data on heterozygous FH prevalence among different ethnicities in non-founder populations. Studies with more than 100 individuals, relevant data on prevalence, ethnicity, and use of relevant diagnostic criteria were considered eligible for inclusion.
Results
Eleven general population studies and two patient studies were included in a systematic review and 11 general population studies in a random-effects metaanalysis. The overall pooled FH prevalence was 0.33% or 1:303 in 1,169,879 individuals(95% confidence interval: 0.26-0:40%; 1:385-1:250). Included studies presented data on six ethnicities black, Latino, white, Asian, brown, and mixed/other. Pooled prevalence was estimated for each group. The highest prevalence was 0.52% or 1:192(0.34-0.69; 1:294-1:145) among black individuals and 0.25% or 1:400 among Asian(0.15-0.35; 1:500-1:286).
Conclusion
The estimated FH prevalence displays a variation across ethnicity, ranging from 0.25%(1:400) to 0.52%(1:192), with the highest prevalence seen among black and the lowest among Asian individuals. The differences observed suggest that targeted screening among subpopulations may increase the identification of cases and thus the opportunity for prevention.

No 29

YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION IV

Viral transgene delivery to endothelial cells in mouse models

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For decades, genetically modified mouse models have been a mainstay for atherosclerosis research to gain mechanistic insight into cell-type-specific gene function. To eliminate time-consuming and costly generation of germ-line engineered mouse lines followed by extensive breeding programs, we aimed to develop a tool for transgene delivery to endothelial cells by recombinant adeno associated viruses (rAAVs). Current rAAVs do not target the endothelium, and we used an in vivo selection system platform (CREATE) to identify rAAV variants with a mutated coat protein that transduces endothelial cells expressing Cre recombinase. We injected a rAAV library consisting of >108 unique sequences into Tie2-Cre mice and used next-generation sequencing (NGS) to analyze the coat gene sequences recovered from the aorta and other tissues. We identified a rAAV variant representing 87.6% of recovered library sequences even though this sequence was not visible in our NGS data. In preliminary experiments, we are testing this rAAV variant using nucleus-specific green fluorescent protein (GFP) as the rAAV-delivered transgene in un-challenged mice, as well as aneurysmatic and atherosclerotic mice. We believe that identifying rAAVs for efficient transgene delivery to endothelial cells will be of high value to the field of atherosclerosis.

No 31

Unraveling macrophage heterogeneity in human atherosclerosis

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Atherosclerosis is a chronic disease that underlies the most common causes of premature death worldwide. Attempts to find an efficient treatment have had only limited success. The local abundance and central role of macrophages in atherogenesis and disease progression makes them an attractive yet challenging target for future therapies. Lately, advances in single cell transcriptomics and multiplex cytometry have allowed to broaden the perspective on macrophage heterogeneity in the human plaque. However, these approaches fail to detect more fragile cell types and are very limited when it comes to understand the functions of macrophage subsets and their interaction with the cell's micro-environment in the plaque.
Mapping the complete plaque macrophage heterogeneity and linking the identified phenotypes to their spatial environment and the stage of disease progression requires a novel, integrative approach.
We combined multiplex immunofluorescent imaging and front-in-line transcriptomic approaches on an age- and gender-matched carotid endarterectomy plaques with pathological characterization ranging from thick fibrous cap atheroma to intraplaque-haemorrhage. This approach will fill the gap in current atherosclerosclerosis research by not only unraveling the multidimensional polarization states of macrophages in atherosclerosis but also to further characterize the identified subsets' tissue localization and association with plaque parameters and disease stage.

No 34

A Trojan horse in contrast-enhanced MRI? Superparamagnetic iron oxide nanoparticles enhance apoptosis in human and murine atherosclerosis

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Aims: (Ultra) Small superparamagnetic iron oxide nanoparticles, (U)SPIO, are widely used as magnetic resonance imaging contrast media and assumed safe for clinical applications in cardiovascular disease. As safety tests largely relied on normolipidemic models, not fully representative of the clinical setting, we investigated the impact of (U)SPIOs on disease-relevant endpoints in hyperlipidemic models of atherosclerosis.

Methods and results: RAW264.7 foam cells exposed in vitro to Ferumoxide (dextran-coated SPIO), Ferumoxtran (dextran-coated USPIO), or Ferumoxytol (carboxymethyl dextran-coated USPIO) showed increased apoptosis and ROS accumulation for Ferumoxide and Ferumoxtran, whereas Ferumoxytol was tolerated well. Pro-apoptotic (TUNEL+) and pro-oxidant activity of Ferumoxide and Ferumoxtran were confirmed in plaque, spleen, and liver of hyperlipidemic ApoE-/- (n=9/group) and LDLR-/- (n=9-16/group) mice that had received single IV injections compared to saline-treated controls. Again, Ferumoxytol failed to induce apoptosis or oxidative stress in these tissues. Concomitant antioxidant treatment (EUK-8/EUK-134) largely prevented these effects in vitro (-68%, $P<0.05$) and plaques from LDLR-/- mice (-60%, $p<0.001$, n=8/group). Repeated Ferumoxtran injection of LDLR-/- mice with pre-existing atherosclerosis enhanced plaque inflammation and apoptosis but did not alter plaque size. Strikingly, carotid artery plaques of endarterectomy patients who received Ferumoxtran before surgery (n=9) also showed 5-fold increased apoptosis (18.2 vs. 3.7% respectively; $p=0.004$) compared to controls who did not receive Ferumoxtran. Mechanistically, neither coating nor particle size seemed accountable for the observed cytotoxicity of Ferumoxide and Ferumoxtran.

Conclusions: Ferumoxide and Ferumoxtran, but not Ferumoxytol, induced apoptosis of lipid-laden macrophages in human and murine atherosclerosis, potentially impacting disease progression in patients with advanced atherosclerosis.

No 38

Severe α1-antitrypsin deficiency associated with increased risk of heart failure in two large populations

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Introduction: Individuals with severe α1-antitrypsin deficiency (AATD) have increased elastase activity resulting in continuous degradation of elastin and an early onset of chronic obstructive pulmonary disease. It has been suggested that the increased elastase activity over time also affects the elastic properties of other organs, for instance the heart. We tested whether severe AATD is associated with susceptibility to heart failure events in the Danish population.
Methods: We genotyped 91,429 individuals from the Copenhagen General Population Study and 187 patients from the Danish AATD registry and recorded admissions and deaths due to heart failure during 9 years of follow-up. We validated our findings in a nationwide population-based cohort study of 2,209 patients with AATD and 21,871 controls without AATD matched on birthday, sex, and municipality. There was no overlap between the two study cohorts.
Results: Persons with severe AATD had an increased risk of heart failure hospitalization during follow-up compared with individuals without AATD (HR adjusted: 1.58, 95%CI: 1.02-2.45). In the nationwide population-based cohort study patients with severe AATD also had a higher risk of heart failure hospitalization compared to individuals without this condition (HR adj.: 1.54, 1.32-1.81). The risk of heart failure hospitalization was attenuated and not statistical significant among patients with myocardial infarction (HR adj.: 1.14, 0.82-1.57) and hypertension (HR adj.: 1.23, 0.96-1.59). Risk of heart failure death or all-cause mortality during follow-up was higher in individuals with severe AATD compared with individuals without AATD (Heart failure death: HR adj.: 2.14, 1.47-3.12; All-cause mortality: HR adj.: 3.04, 2.79-3.30).
Conclusion: Individuals with severe AATD had an increased risk of heart failure in the Danish general population.

ll be of high value to the field of atherosclerosis.

No 41

ApoB and non-HDL cholesterol versus LDL cholesterol for ischemic stroke risk

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Objective: Conflicting results have been reported on the association between lipids and risk of ischemic stroke. We tested the hypothesis that the burden of ischemic stroke attributable to either elevated apolipoprotein B (apoB) or non-high-density lipoprotein (non-HDL) cholesterol is higher than that attributable to elevated low-density lipoprotein (LDL) cholesterol.
Methods: We included 104 618 individuals from an ongoing cohort study, the Copenhagen General Population Study. The associations of quintiles of apoB, non-HDL cholesterol, and LDL cholesterol with risk of ischemic stroke were estimated by Cox proportional hazards regressions with 95% confidence intervals. With 1st quintile as reference, the proportion of ischemic stroke attributable to the 2nd, 3rd, 4th, and 5th quintiles of apoB, non-HDL cholesterol, and LDL cholesterol were estimated by population attributable fractions.
Results: Higher quintiles of apoB and non-HDL cholesterol were associated with stepwise increased risk of ischemic stroke, whereas only the upper two quintiles of LDL cholesterol were associated with increased risk of ischemic stroke. A similar pattern was seen for population attributable fraction values. Compared to individuals in the 1st quintile, the combined proportion of ischemic stroke attributable to individuals in the 2nd to 5th quintiles was 16.5% for apoB (levels >82mg/dL), 14.9% for non-HDL cholesterol (>3.0mmol/L; >117mg/dL), and 7.0% for LDL cholesterol (>2.4mmol/L; >94mg/dL).
Interpretation: The proportion of ischemic stroke attributable to either elevated apoB or non-HDL cholesterol was double that attributable to elevated LDL cholesterol, indicating that the risk of ischemic stroke is better reflected by elevated apoB and non-HDL cholesterol than elevated LDL cholesterol.

No 42

ApoM is associated with prevalent cardiovascular disease in patients with chronic kidney disease

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Background and aim: Patients with CKD have an increased risk of developing cardiovascular disease (CVD) including atherosclerotic artery disease and chronic heart failure (CHF). Unfortunately, CVD is often asymptomatic in patients with CKD. Apolipoprotein M (apoM) is mainly found associated to HDL and act as a carrier of the bioactive lipid sphingosine-1-phosphate. ApoM modulates plaque formation, known to be accelerated in patients with CKD. Also, reduced apoM levels are independently associated with an increased risk of death in patients with CHF in a population without known CKD. The aim of the present study is therefore to examine the association between ApoM and CVD in patients with CKD.
Material and methods: Plasma apoM levels were analyzed with an in-house ELISA in the CPH-CKD cohort, a single-centre prospective, observational study of non-dialysis patients (741) with stage 1-5 chronic kidney disease and 174 controls.
Results: This study suggests a significant association between plasma ApoM levels and prevalent CVD as well as CHF at baseline. More specifically, patients have a 16 % reduction in odds of having CVD pr. 0.25 µM increase in plasma ApoM levels ($p < 0.002$). In addition, the study suggests a 19 % reduction in odds of having CHF pr. 0.25 µM increase in plasma ApoM levels ($p < 0.001$).
Conclusion: This study supports a role for apoM as a marker for CVD in patients with CKD. Further studies of the predictive role of apoM are needed and are
currently being conducted.

No 48

Interleukin-6 inhibition in ST-elevation myocardial infarction: Immune cell profile in the randomized ASSAIL-MI trial

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We have previously shown that interleukin (IL)-6 inhibition by tocilizumab in ST-elevation myocardial infarction (STEMI) patients improves myocardial salvage after percutaneous coronary intervention (PCI). In the same patients, we now explore the role of neutrophils and lymphocytes and how they are affected by PCI and tocilizumab treatment.

In total, 199 patients with STEMI were included in the study, and they are randomized to either tocilizumab (n=101) or placebo (n=98) before PCI. Firstly, we looked at the neutrophil levels, and their relationship to peak troponin T (TnT) and myocardial salvage to investigate whether there is a correlation between leukocyte levels and damage to the myocardial tissue. We also examined B and T cell subpopulations in a subgroup of patients (placebo n=32, tocilizumab n=37) by flow cytometry. In addition, RNA sequencing on whole blood and T cells was performed in the two treatment groups (placebo n=19, tocilizumab n=20).

Our analyses revealed several interesting findings in neutrophils. We reveal changes in neutrophil levels and function based on RNA sequencing upon tocilizumab treatment in STEMI patients. Further, we show that the altered neutrophil level might benefit the patients presenting with STEMI by a possible contribution to the myocardial salvage. Fewer effects on levels of lymphocyte subgroups were observed in tocilizumab treated patients, although we reveal an interesting effect on CD8+ T cells and their relation to myocardial salvage.

In conclusion, patients with STEMI present with an altered neutrophil level and function after tocilizumab treatment, while the effect on lymphocytes seems less pronounced. The observed differences are related to beneficial effects on myocardial salvage that is worth noticing for future attempts to develop today's treatment of acute myocardial infarction.

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