

31st Annual Scandinavian Atherosclerosis Conference
April 2-5, 2025 at Krogerup Højskole, Humlebæk, Denmark



2025 Program

Sponsored by:



SCIENTIFIC COMMITTEE

Clare Hawkins (Denmark)
Bente Halvorsen (Norway)
Ida Juul Rasmussen (Denmark)
Jacob Juel Christensen (Norway)
Paolo Parini (Sweden)
Patrick Rensen (Netherlands)
Eva Hurt-Camejo (Sweden)
Mia Ståhle (Finland)

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Organized by**SCANDINAVIAN SOCIETY
FOR ATHEROSCLEROSIS
RESEARCH**

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Wednesday, April 2nd, 2025

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| 16.00 – 18.00 | Arrival, registration, and coffee (dining room until 17.45) |
| 18.00 – 19.30 | Dinner |
| 19.30 – 19.35 | Welcome Kirsten B Holven (<i>Norway</i>) |
| THE NIKKILÄ MEMORIAL LECTURE | |
| 19.35 – 19.40 | Introduction of the 2024 Nikkilä Lecturer Minna Kaikkonen-Määttä (<i>Finland</i>) |
| 19.40 – 20.25 | <u>2025 Nikkilä Lecture</u> The promiscuous apolipoprotein M: good, bad and something in between Christina Christoffersen |
| 20.25 – 20.45 | Discussion |
| 20.45 – | Pub will be open |

Thursday, April 3rd, 2025

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| 07.30 – 08.30 | Breakfast |
| SESSION I | INFLAMMATION AND VASCULAR BIOLOGY Chaired by Bente Halvorsen (Norway) and Clare Hawkins (Denmark) |
| 08.30 – 09.00 | <i>Invited speaker</i> Sex influences atherosclerosis in TET2-related clonal hematopoiesis of indeterminate potential and on interleukin-1beta inhibition Amélie Vromman (USA) |
| 09.00 – 09.15 | T-cells enhance development of atherosclerosis in LDL receptor knock-out mice Ida Gregersen (Norway) |
| 09.15 – 09.30 | N-Terminal proteomics reveals distinct protein degradation patterns in different types of human carotid artery atherosclerotic plaques Michael Davies (Denmark) |
| 09.30 – 09.45 | The alpha 7 nicotinic acetylcholine receptor agonist PHA 568487 dampens inflammation in PBMCs from patients with newly discovered coronary artery disease Rebecka Wilhelmsson (Sweden)- YIA |
| 09.45 – 10.00 | Proteomics analysis of extracellular vesicle proteins in plasma from patients with atherosclerosis. Yibo Gao - (Denmark)- YIA. |
| 10.00 – 10.45 | Poster Walk (Session I) Coffee and tea |
| 10.45 – 11.15 | <i>Invited speaker</i> Trisha Grevenkoed (Denmark) Fatty acid-derived mediators in systemic inflammation |
| 11.15 – 11.30 | Comparative analysis of smooth muscle cell diversity in atherosclerosis across species and vascular beds Diana Sharysh (Denmark) - YIA |
| 11.30 – 11.45 | Sex differences in immunomodulating treatment in cardiovascular diseases Tuva Dahl (Norway) |
| 11.45 – 12.00 | Serum Tryptophan and Kynurenine levels and risk of heart failure among patients with chronic kidney disease. Sara Mohiti (Denmark)- YIA |
| 12.00 – 13.00 | Lunch |

SESSION II

CARDIOVASCULAR DISEASE

Chaired by **Ida Juul Rasmussen+** (Denmark) and **Jacob Juel Christensen** (Norway)

13.00 – 13.30

Invited speaker

Dispelling the Myths of dietary fats and cardiovascular risk

Julie Lovegrove (UK)

13.30 – 13.45

Lipoprotein(a) and risk of dementia: three independent cohort studies including 575,630 individuals

Peter E. Thomas (Denmark) - YIA

13.45 – 14.00

Risk reduction of ASCVD attributed to lowering of remnant cholesterol from statins, fibrates, APOC3 inhibitors, and ANGPTL3 inhibitors: a cohort study

Mie Balling (Denmark)

14.00 – 14.15

Adherence to lipid lowering therapy in genetic familial hypercholesterolemia and cardiovascular disease risk: a registry cohort study

Tone Svilaas (Norway)

14.15 – 14.30

Common genetic variants in GIP and GIPR as proxies for body weight reduction via GIP receptor targeting and risk of cardiovascular disease – observational and Mendelian randomization studies

Frida Emanuelsson (Denmark)

14.30 – 15.30

General meeting of the Scandinavian Society for Atherosclerosis Research
Open for all participants

Afternoon free for the Louisiana Museum of Modern Art (5 min walk), beach (5 min walk), Kronborg, the castle of Hamlet (12 min by train) or downtown Copenhagen (50 min by train)

16.00 – 17.00

The traditional soccer match between countries

Remember to bring sports clothing and suitable footwear

18.00 – 19.00

Dinner

SESSION II

CARDIOVASCULAR DISEASE – continued

Chaired by **Ida Juul Rasmussen** (Denmark) and **Jacob Juel Christensen** (Norway)

19.00 – 19.30

Invited speaker

Somatic mutations and cellular clones in atherosclerotic plaques

Lars Melholt Rasmussen (Denmark)

19.30 – 20.30

Poster Walk (Session II)

Coffee and tea

20.30 – 20.45

Abdominal Aortic Aneurysm Prevalence and Infrarenal Aortic Diameter are decreasing in the Population-based Regional Screening Program

Antti Siika (Sweden)

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|---------------|---|
| 20.45 – 21.00 | Cholesterol associates with risk of STEMI above NSTEMI Malene Kærslund Hansen (<i>Denmark</i>) |
| 21.00 – 21.15 | Elevated remnant cholesterol confers high risk of ASCVD without prompting lipid-lowering therapy: an unmet medical need Karen Hvid (<i>Denmark</i>) - <i>YIA</i> |
| 21.15 – 21.30 | Cardiovascular disease and compliance with lipid-lowering therapy among young individuals with Familial Hypercholesterolemia in Norway – A register study. Gisle Langslet (<i>Norway</i>) |
| 21.30 – | Pub will be open |

Friday, April 4th, 2025

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| 07.30 – 08.30 | Breakfast |
| SESSION III | LIPOPROTEINS AND LIPID TRANSPORT Chaired by Paolo Parini (Sweden) and Patrick Rensen (Netherlands) |
| 08.30 – 09.00 | <i>Invited speaker</i> The genetic background behind the clinical familial hypercholesterolemia phenotype Mafalda Bourbon (Portugal) |
| 09.00 – 09.15 | High cholesterol absorption efficiency enhances proatherogenic properties of LDL particles Katariina Öörni (Finland) |
| 09.15 – 09.30 | Cholesterol-lowering drug targets reduce risk of vascular-related dementia: Mendelian randomization of 500.000 individuals. Liv Nordestgaard (Denmark) |
| 09.30 – 09.45 | Low-Density Lipoprotein Cholesterol, Coronary Plaque burden, and Cardiovascular Risk in individuals beyond 75 years of age: The Western Denmark Heart Registry Malene Højgaard Andersen (Denmark) - YIA |
| 09.45 – 10.00 | Hepatic knockdown of ABCA6 increases atherosclerosis in APOE*3-Leiden.CETP mice Patrick Rensen (Netherlands) |
| 10.00 – 11.00 | Poster Walk (Session III) Coffee and tea |
| 11.00 – 11.30 | <i>Invited speaker</i> Role of HDL in sepsis rather than atherosclerosis Liam Brunham (Canada) |
| 11.30 – 11.45 | The Effects of Modulating HDL concentration on Sepsis Risk: A Mendelian Randomization study Mohan Li (Netherlands) - YIA |
| 11.45 – 12.00 | Angiopoietin-like 3 (ANGPTL3) deficiency alters hepatic metabolism and promotes substrate rerouting Ottavia Terenghi (Italy) |
| 12.00 – 12.15 | Association of HDL cholesterol, apolipoprotein E and A1 concentrations with fetal growth, maturation, and placental insufficiency. Rikke Mohr Lytsen (Denmark) - YIA |
| 12.15 – 13.15 | Lunch |
| SESSION IV | OTHER TOPICS Chaired by Frida Emanuelsson (Denmark) and Mia Ståhle (Finland) |
| 13.15 – 13.45 | <i>Invited speaker</i> Sex differences in atherosclerotic disease: Pre- and Post-Menopause Jeanine Roeters van Lennep (Netherlands) |

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|---------------|---|
| 13.45 – 14.00 | Statin-associated regulation of hepatic PNPLA3 in patients without known liver disease Osman Ahmed (Sweden) |
| 14.00 – 14.15 | Loss-of-function in endothelial lipase increases LDL cholesterol but decreases risk of atherosclerotic cardiovascular disease Benjamin Nilsson Wadström (Denmark) |
| 14.15 – 14.30 | Management of hypercholesterolemia in a patient with vanishing bile duct syndrome Lorenzo Luciani (Sweden) - YIA |
| 14.30 – 14.45 | Causal insights of LDL cholesterol and triglycerides-rich lipoproteins for risk of dementia Jiao Luo (Denmark) |
| 14.45 – 15.45 | Poster Walk (Session IV) Coffee and tea |
| 15.45 – 16.15 | <u>Invited speaker</u> Novel imaging approaches to diagnose CAD and their ability to monitor treatment response Sarah Bär (Switzerland) |
| 16.15 – 16.30 | Combined use of cardioprotective glucose-lowering drugs and statins for primary prevention of atherosclerotic cardiovascular disease in individuals with type 2 diabetes: A nationwide cohort study Tummas Ternhamar (Denmark)- YIA |
| 16.30 – 16.45 | Current guidelines result in extensive pregnancy-related off-treatment time in women with familial hypercholesterolemia Marianne Klevmoen (Norway) - YIA |
| 16.45 – 17.00 | Role of HDL in reproduction - the cholesterol efflux capacity of follicular fluid HDL predicts normal fertilization independent of HDL cholesterol levels. Uwe Tietge (Sweden) |
| 17.00 – 17.15 | Remnant cholesterol lowering in cardiovascular disease risk reduction in statin trials: meta-regression analyses Børge Grønne Nordestgaard (Denmark) |
| 17.15 - 17.20 | Concluding remarks Kirsten B. Holven (Norway) |
| 18.30 – 19.00 | Cocktail |
| 19.00 – | Banquet and dancing |

Saturday, April 5th, 2025

08.30 – 10.00

Breakfast and departure

Safe travels and see you next year

31st Annual Scandinavian Atherosclerosis Conference
April 2-5, 2025 at Krogerup Højskole, Humlebæk, Denmark



2025 Posters

Thursday, April 3, 2025

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. 01** Role of immune cells in the developmental programming of atherosclerosis
Clare Hawkins (Denmark)
- No. 02** Anti-inflammatory effects of a GLP-1 receptor agonist in comparison with an anti-IL-6 antibody in a mouse model of accelerated atherosclerosis
Louise Marie Voetmann (Denmark)
- No. 03** Dynamics of N6-methyladenosine (m6A) in STEMI and the Impact of IL-6 Inhibition
Camilla Huse (Norway)
- No. 04** Elevated adipose inflammation, but reduced hepatic triacylglycerol storage in diet-induced obese Plin4-null mice.
Atanaska Doncheva (Norway)

**YIA Poster walk I
10.00 – 10.45**

Selected abstracts (3 min presentation + 2 min discussion)

- No. 05** Location-dependent proteomics of the aortic arch from apolipoprotein E deficient mice
Kathrine V. Jokumsen (Denmark) - YIA
- No. 06** Uncovering the role of liver lymphatic vessels in MASLD using hyperlipidemic mouse models with disturbed lymphatic development
Krista Hokkanen (Finland) - YIA
- No. 07** The Role of Gasdermins in Atherosclerotic Plaque Destabilization: Gasdermin D and E – A Murder Mystery
Michelle Zurek (Finland) - YIA
- No. 08** Insights into the role of NPC2 in vascular inflammation and atherosclerosis
Jakob Hansen (Denmark) - YIA
- No. 09** Heterogenous phenotypic modulation of smooth muscle cells by mechanical stretch and TNF stimulation.
Lise Filt Jensen (Denmark) - YIA

SESSION II

CARDIOVASCULAR DISEASE

- No. 10** Efficacy and safety of short-term S100A8/A9 blockade in a myocardial ischemia-reperfusion injury porcine model
Minja Heikkilä (Finland)

- No. 11** | A dual MAGL/HSL inhibitor showing therapeutic efficacy in dyslipidemic APOE*3-Leiden.CETP mice
Jingxi Zhu (Netherlands)
- No 12** | Regulation of Endothelial Lipase by Angiopoietin-like Protein 3
Marina Mutas (Denmark)
- No 13** | Comprehensive lipid and metabolite profiling of long-term survivors of type 1 diabetes compared to healthy controls: cross-sectional results from the Dialong study in Norway
Jacob J. Christensen (Norway)
- YIA Poster walk II** | **Selected abstracts (3 min presentation + 2 min discussion)**
19.30 – 20.30
- No. 14** | Characterization of isocitrate dehydrogenase (IDH)-associated macrophage responses in atherosclerosis
Michelle Due Nilsson (Denmark) - YIA
- No. 15** | Taurine-conjugated fatty acid metabolites reduce atherosclerosis in LDLR^{-/-} mice
Katja Thorøe Michler (Denmark) - YIA
- No. 16** | Association of aerobic fitness and body composition with protein and major lipid class composition of high-density lipoprotein
Emilia Lähteenmäki (Finland) - YIA
- No. 17** | Impaired anti-inflammatory capacity of high-density lipoprotein is associated with high residual cardiovascular risk in coronary artery disease
Wanying Wu (China) - YIA
- No. 18** | SGLT-i Modify Exosomal Protein Composition: A potential Mechanism for Cardioprotective Effects
Jana Dudová (Czech Republic) - YIA

Friday, April 4, 2025

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

SESSION III

LIPOPROTEINS AND LIPID TRANSPORT

- No. 19** Circulating ApoC-III glycoforms identifies patients with metabolic dysfunction-associated steatotic liver disease independently of type 2 diabetes.
Gabriele Mocciano (UK)
- No. 20** Combining comprehensive cardiometabolic risk biomarker panels with machine learning to predict survival in severe COVID-19 patients admitted to the intensive care unit.
Debra Dorotea (Sweden)
- No. 21** Reduced lipid synthesis and oxidation but upregulated mitochondrial responses in cultured myotubes established from AMPK α 2^{-/-} mice
Arild C. Rustan (Norway)
- No. 22** Deciphering Atherosclerosis in Type 2 Diabetes: A Novel Exploration of Biomarkers in Interstitial Fluid
Cecilia Morgantini (Sweden)
- No. 23** Trafficking of LDL-derived cholesterol by combined live-cell imaging and lipid mass spectrometry of novel fluorescent cholesteryl esters
Daniel Wüstner (Denmark)

YIA Poster walk III
10.00 – 11.00

Selected abstracts (3 min presentation + 2 min discussion)

- No. 24** MARC1 downregulation reduces hepatocyte lipid content by increasing beta-oxidation.
Tanmoy Dutta (sweden) - YIA
- No. 25** PXR agonist rifampicin elevates serum Lp(a) levels substantially.
Topias Kotilainen (Finland) - YIA
- No. 26** Functional Impact of APOB Variants in Familial Hypercholesterolemia
Maria Ferreira (Portugal) - YIA
- No. 27** Elucidating the degradation pattern of apolipoprotein B-100 from human carotid atherosclerotic plaques by N-terminal proteomics.
Nicoline Wichmand Thorsen (Denmark) - YIA
- No. 28** The effect of SGLT2 inhibitors on human apoM levels in proximal tubular cells under LPS-induced cell damage.
Daniele Angelo Renato Villa (Denmark) - YIA

- No. 29** The impact of sex hormones and sex chromosomes on the HDL anti-inflammatory activity
Ana Vankova (Sweden) - YIA
- No 30** VLDL triglycerides and cholesterol in non-alcoholic fatty liver disease and myocardial infarction
Lærke Kristine Kyhl (Denmark) - YIA
- No 31** Lipoprotein(a) Testing Among General Practitioners in Norway: Shaping the Future of Cardiovascular Risk Stratification
Janeni Jeevanathan (Norway) - YIA
- SESSION IV** **OTHER TOPICS**
- No. 32** Dietary restriction of sulfur amino acid in humans has impact on serum free fatty acids and apelin gene expression in adipose tissue: findings from an 8-week randomized controlled trial.
Emma Stolt (Norway)
- No. 33** Competitive displacement of lipoprotein lipase from heparan sulfate is orchestrated by a disordered acidic cluster in GPIHBP1
Anamika Biswas (Denmark)
- No. 34** Supplementation of seaweed extracts to the diet reduces symptoms of Alzheimer's Disease in the APP^{swe}PS1 Δ E9 mouse model
Monique Mulder (Netherlands)
- No. 35** Levels of selected aminothiols in a group of patients with arterial hypertension and/or atherosclerosis after COVID-19
Beata Sarecka-Hujar (Poland)
- No. 36** Comprehensive lipid and metabolite profiling of youth with childhood onset type 1 diabetes compared to healthy controls: Results from the Norwegian ACD study
Jacob J Christensen (Norway)
- No 37** Analysis of arterial stiffness parameters assessed by photoplethysmography in patients with arterial hypertension and/or atherosclerosis according to gender
Danuta Łoboda (Poland)
- No 38** Dietary effects on metabolic health and ageing in liver
Marit Hjorth (Norway)
- YIA Poster walk IV** **Selected abstracts (3 min presentation + 2 min discussion)**
14.45 – 15.45
- No. 39** Mapping uptake and dissolution of ingested cholesterol crystals in macrophages using combined fluorescence and x-ray microscopy.
Vibeke Akkerman (Denmark) - YIA

- No. 40** Familial Hypercholesterolemia (FH): 25 years of findings in the Portuguese FH Study
Beatriz Miranda (Portugal) - YIA
- No. 41** Effect of cetoleic acid-enriched fish oil on atherosclerosis markers in high-risk patients with metabolic syndrome
Iselin Schjelle Holen (Norway) - YIA
- No. 42** Orthopedic events and incident amyloidosis in the general population
Søren Nicolaj Rønborg (Denmark) - YIA
- No. 43** The Regulation and Effect of m6A Modification on the atherosclerotic plaque and Vascular Smooth Muscle Cells (VSMCs)
Hamida Anwari (Norway) - YIA



**Oral Presentations – Abstracts –
Inflammation and Vascular Biology**

SESSION I

T cells enhance development of atherosclerosis in LDL receptor knock out mice

Ida Gregersen, Xiang Yi Kong, Sverre Holm, Ellen Sagen, Tuva Dahl, Bente Halvorsen

Research Institute of internal medicine, Rikshospitalet OUS, Oslo, Norway

Abstract

Background: T cells play an important role in atherosclerosis, but their direct contribution to atherogenesis is not fully understood. We have shown that mice with increased T cell responsiveness develop obesity without diet intervention [1]. Obesity is a risk factor for atherogenesis, but how T cells function at the intersection of these conditions remains unclear. The aim of this study was to enhance our understanding of these processes.

Methods: LDL receptor knockout mice (Ldlr^{-/-} mice) were irradiated and transplanted with bone marrow (1.0×10^6 cells) via tail vein injection. The donor mice were from transgenic mice with enhanced T cell responsiveness (termed RIAD) or from control littermates. The recipient mice were fed a high-fat diet for 16 weeks before atherosclerosis was quantified. Plasma and tissues were collected for analysis.

Results: Ldlr^{-/-} mice transplanted with bone marrow from RIAD mice developed increased body weight, as previously described [1]. Additionally, these mice had increased body and liver fat compared to those receiving control bone marrow. Recipients of RIAD bone marrow also displayed higher levels of plasma triglycerides, non-esterified fatty acids, and cholesterol, compared to controls. Importantly, these mice also developed significantly more atherosclerosis than those receiving control bone marrow. Furthermore, plasma level of lipoprotein binding protein was increased.

Conclusion: We demonstrate that bone marrow cells from mice with increased T cell responsiveness can accelerate the development of atherosclerosis in Ldlr^{-/-} mice. Our findings suggest several potential drivers, such as obesity and elevated blood lipids, along with gut barrier dysfunction, which may collectively contribute to the accelerated atherogenesis. Overall, our study highlights the complex interplay between immune system reactivity, metabolic factors, and atherosclerosis.

1. Gregersen, I., et al., *iScience*, 2024. **27**(4): p. 109471.

Elucidating the degradation pattern of apolipoprotein B-100 from human carotid atherosclerotic plaques by N-terminal proteomics.

Nicoline Wichmand Thorsen¹, Lasse Gøbel Lorentzen², Michael Jonathan Davies¹

¹University of Copenhagen, Copenhagen, Denmark. ²Vascular Research Unit - Rigshospitalet, Copenhagen, Denmark

Abstract

Apolipoprotein B-100 (ApoB) is the primary protein component of low-density lipoproteins (LDL). Under normal physiological conditions, LDL are taken up by cells through the tightly regulated LDL receptor. However modified LDL are taken up by scavenger receptors in macrophage, and other cells, resulting in lipid accumulation and 'foam' cell formation, a signature driver of atherosclerosis. Intracellular degradation of ApoB occurs within lysosomes, and primarily by cathepsins, though other proteases, including extracellular species may contribute. The contributions of different proteases within human plaques is however not well understood.

Here we report a novel proteomic/degradomic approach to investigate the nature of protein fragments from ApoB in human carotid atherosclerotic plaques. Plaques from 21 patients were morphologically classified and analyzed new N-termini fragments generated by proteases. 17814 N-terminal peptides were identified with 5735 unique peptides detected across all samples. 270 peptides were mapped as ApoB fragments covering 29% of the protein sequence, with the majority being significantly more abundant in soft/unstable plaques. The fragment sequences indicate a large numbers of proteolytic cleavage sites across the protein sequence with the C-terminal part of the beta-belt region contributing with the most fragments. Some fragments contained overlapping sequences consistent with multiple fragmentation events and exopeptidase activity. Sequence logo analysis of the amino acids around the cleavage sites shows Ser and Leu as the most common amino acids C-terminal of the cleavage site whereas Leu and Lys are found more frequently N-terminal. These sequences provide data on the likely proteases responsible for intra plaque ApoB cleavage.

In conclusion, we propose that N-terminal proteomics (degradomics), together with literature data can provide valuable insights into the degradation patterns of proteins within human atherosclerotic plaques.

The alpha 7 nicotinic acetylcholine receptor agonist PHA 568487 dampens inflammation in PBMCs from patients with newly discovered coronary artery disease

Filip Mjörnstedt¹, Rebecka Wilhelmsson¹, Marcus Ulleryd¹, Maria Hammarlund¹, Göran Bergström^{2,3}, Anders Gummesson^{2,4}, Maria E Johansson¹

¹Department of Physiology, Institute of Neuroscience and Physiology, University of Gothenburg, Gothenburg, Sweden. ²Department of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden. ³Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden. ⁴Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, Gothenburg, Sweden

Abstract

The alpha 7 nicotinic acetylcholine receptor ($\alpha 7$ nAChR) regulates inflammation in experimental models and is expressed in human peripheral blood mononuclear cells (PBMCs) and in human atherosclerotic plaques. However, its role in regulating inflammation in patients with cardiovascular disease is unknown. This study aims to investigate whether $\alpha 7$ nAChR stimulation can reduce the inflammatory response in PBMCs from patients with newly diagnosed coronary artery disease (CAD). Human PBMCs, extracted from patients with verified CAD ($n = 38$) and control participants with healthy vessels ($n = 38$), were challenged in vitro with lipopolysaccharide (LPS) in combination with the $\alpha 7$ nAChR agonist PHA 568487. Cytokine levels of the supernatants were analysed using a multiplex immunoassay. Patients in the CAD group were reexamined after 6 months. The immune response to LPS did not differ between PBMCs from control and CAD groups. $\alpha 7$ nAChR stimulation decreased TNF α in both control and CAD groups. The most pronounced effect of $\alpha 7$ nAChR stimulation was observed in patients with CAD at their first visit, where 15 of 17 cytokines were decreased (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12 (p70), IL-17A, G-CSF, GM-CSF, IFN- γ , MCP-1, MIP-1 β , and TNF α). In conclusion, stimulation with $\alpha 7$ nAChR agonist PHA 568487 dampens the inflammatory response in human PBMCs. This finding suggests that the anti-inflammatory properties of the $\alpha 7$ nAChR may have a role in treating CAD.

Proteomics analysis of extracellular vesicle proteins in plasma from patients with atherosclerosis

Yibo Gao, Lasse Lorentzen, Michael Davies

Department of Biomedical Sciences, Panum Institute, University of Copenhagen, Copenhagen, Denmark

Abstract

Atherosclerosis is the leading cause of mortality and is driven by a complex interplay of genetic predispositions and environmental factors. Despite significant advancements, many critical aspects of the disease remain poorly understood, from its underlying mechanisms to the identification of reliable prognostic biomarkers for effective patient stratification and clinical management. A deeper understanding of alterations in the blood proteome could facilitate the discovery of novel panels of biomarkers capable of detecting atherosclerosis before the onset of clinical symptoms, potentially improving early intervention strategies. Here, we performed proteomic analysis to identify candidate protein biomarkers in the blood of control subjects and those with established atherosclerosis. As previous findings have highlighted a link between atherosclerosis and protein composition and degradation, we isolated extracellular vesicles (EVs) by centrifugation from peripheral blood samples and subjected these to liquid chromatography-mass spectrometry (LC-MS/MS) analysis to examine if these particles could provide sensitive specific information on the presence of disease. 201 human blood samples were analysed, including 37 from people with established atherosclerosis and 164 healthy controls. The LC-MS/MS analysis identified 7471 peptides and 938 proteins reproducibly across the EVs. Subsequent analysis identified 515 proteins with significantly altered expression levels in atherosclerosis patients compared to healthy individuals. Enrichment analysis revealed that biological processes related to the extracellular matrix and the complement system were contributors to these differences. These findings provide valuable insights into the protein changes that occur during atherosclerosis and provide a foundation for future investigations aimed at identifying panels of biomarkers of vulnerable atherosclerotic lesions, to allow early diagnosis and personalized therapeutic strategies.

Comparative analysis of smooth muscle cell diversity in atherosclerosis across species and vascular beds

Diana Sharysh¹, Paula Nogales², Daniel Morales-Cano², Raul Izquierdo-Serrano², Laura Carramolino², Anton Markov¹, Carlos Torroja², Julian Albarrán-Juárez¹, Jacob Fog Bentzon¹

¹Aarhus University, Aarhus, Denmark. ²Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Abstract

Smooth muscle cells (SMCs) and their modulated progeny are important drivers of atherosclerosis. The causal mechanisms underlying their expansion and fates can be studied in genetically modified mouse and pig models of atherosclerosis. However, it is unclear whether the plaques studied in these models accurately represent the phenotypic diversity of human SMCs in atherosclerotic lesions of clinically important arteries.

Methods

Using newly generated and publicly available single-cell RNA sequencing data, we analyzed mesenchymal cells across atherosclerotic plaques from human carotid and coronary arteries, pig aorta and coronary arteries, and mouse brachiocephalic trunk. We validated and localized species- and vascular bed-specific SMCs using various staining techniques.

Results

A main axis of SMC phenotypic diversity spanning from contractile to a modulated phenotype was consistent across the studied species and vascular beds. However, several SMC populations were found to be species- or vascular bed-specific. We identified a human carotid-specific population, DLX5+ SMCs, located in the media and intima. A human coronary plaque-specific *REGL*+ SMC population was restricted to spots in the cap of atherosclerotic plaque. Pericytes were found only in pig and human lesions across all studied vascular beds. Pig coronary plaques had a specific VEGFA+ population, that was localized to the border of necrotic core. Mouse lesions contained specific SMC-derived cells with a chondrocyte-like phenotype (*Col2a1*+ cells), and localized to areas of chondroid metaplasia, closer to necrotic core. Overall, pig plaques differed less from human plaques, suggesting that pigs may be needed to study some aspects of SMC diversity in atherosclerosis.

Conclusion

The phenotypic diversity of SMCs within atherosclerotic plaques exhibits species- and vascular bed-specific variations; however, the fundamental SMC continuum remains conserved across both species and vascular beds.

Sex differences in immunomodulating treatment in cardiovascular diseases

Tuva Dahl¹, Camilla Huse², Kaspar Broch³, Geir Øystein Andersen⁴, Pål Aukrust⁵, Åsa Tivesten^{6,7}, Bente Halvorsen^{1,2}

¹Research Institute of Internal Medicine, Oslo University Hospital, Oslo, Norway. ²Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway. ³Dept of Cardiology, Oslo University Hospital Rikshospitalet, Oslo, Norway. ⁴Dept of Cardiology, Oslo University Hospital Ullevål, Oslo, Norway. ⁵Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway. ⁶Wallenberg Laboratory for Cardiovascular and Metabolic Research, Dept of Molecular and Clinical Medicine, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden. ⁷Dept of Endocrinology, Sahlgrenska University Hospital, Region Västra Götaland, Gothenburg, Sweden

Abstract

The preferred treatment for CVD is lipid-lowering therapy, such as statins. It is known that much of the damage and the risk factors for CVD are driven by inflammation. Therefore, the immune system is likely a key factor to further improve today's treatment and several clinical trials have focused on targeting inflammation in CVD, and inhibiting IL-6 signaling seems a promising candidate.

However, the immune response is not the same between men and women. Generally, adult females mount stronger innate and adaptive immune responses than males. This results in faster clearance of pathogens and greater vaccine efficacy in females than in males but also contributes to their increased susceptibility to inflammatory and autoimmune diseases. For the innate immune system, females are shown to have higher activation of macrophages and higher antigen-presenting cell efficiency, while men have higher levels of pro-inflammatory cytokine production, TLR expression, and higher levels of NK cell numbers. Regarding the adaptive immune system, females have higher CD4⁺ T cell counts and a higher CD4/CD8 T cell ratio, more activated T cells, more T cell proliferation, and cytotoxic activity, and a bias to Th2 cells, in addition to higher B cell numbers and higher antibody production¹.

Reanalyzing data from the ASSAIL-MI trial, where first-time STEMI patients received a single dose of tocilizumab, inhibiting the IL-6receptor before revascularization of the heart, showed sex-specific differences in regards to the effect of treatment on infarction size at the 6 months follow up. Further, in previous add-on studies, we have shown that IL-6R inhibition reduces the recruitment of neutrophils and monocytes from the bone marrow during STEMI and dampens the inflammatory phenotype of the innate immune response. Here we expand our understanding of anti-inflammatory treatment in STEMI and show differences in response to anti-inflammatory treatment depending on sex.

Serum Tryptophan and Kynurenine levels and risk of heart failure among patients with chronic kidney disease

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Abstract

Background and aims: Chronic kidney disease (CKD) is often complicated by heart failure (HF) leading to increased mortality. Emerging evidence suggests Tryptophan metabolites, through the Kynurenine pathway (KP), play a significant role in HF pathophysiology. Therefore, we explored the association of Tryptophan (TRP), Kynurenine (KYN), and Kynurenine to Tryptophan ratio (KTR) with HF in CKD, hypothesizing a link between KP alterations and HF occurrence in this population.

Methods: 673 non-dialysis patients aged 30 to 75 with CKD stages 1-5 were included. Incident HF data were collected through medical record reviews and the median follow-up time was 3.9 years. Serum concentrations of KYN and TRP were measured using High-Performance Liquid Chromatography (HPLC).

Results: Patients with more advanced stages of CKD had higher levels of KYN and KTR, and lower levels of TRP ($p < 0.001$). Following adjustments for age, sex, BMI, hypertension, and hypercholesterolemia, serum KYN and KTR remained significantly associated with prevalent HF in patients with CKD ($p = 0.012$, $p = 0.028$ respectively). Furthermore, Cox-regression analysis indicated that KTR concentration was associated with incident HF after adjusting for confounders such as age, sex, BMI, hypertension, and hypercholesterolemia and diabetes ($p = 0.019$).

Conclusion: In conclusion, the present analysis suggests that changes in the kynurenine pathway may be a new biomarker for HF in patients with CKD. Thus, KTR concentration might be associated with prevalent and future HF in patients with CKD. Further research is needed to understand the mechanisms and potential of these metabolites in refining HF risk prediction and prevention in CKD patients.

Keywords: Chronic kidney disease, Heart failure, Kynurenine, Tryptophan



Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION II

Lipoprotein(a) and risk of dementia: three independent cohort studies including 575,630 individuals

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Abstract

Background and Aims: Dementia is a leading cause of death and disability, shares risk factors with atherosclerotic cardiovascular disease (ASCVD), and 45% of dementia may be preventable. High lipoprotein(a) is a risk factor for ASCVD and all-cause mortality while results for dementia are conflicting. With lipoprotein(a) lowering drugs in clinical trial, we tested whether lipoprotein(a) associates with risk of Alzheimer's disease (AD) and/or vascular-related dementia (VRD).

Methods: We included 539,478 individuals with plasma lipoprotein(a) measurements from the Copenhagen General Population Study, the Copenhagen City Heart Study, and the UK Biobank. *LPA* KIV-2 genotypes were available in 117,029 participants in the Copenhagen studies. During a maximum follow-up of 30.2 years, 6,404 developed AD, and 7,866 VRD.

Results: On continuous scales, lipoprotein(a) levels did not associate with risk of AD or VRD when not accounting for competing risks. When accounting for such, absolute risks of VRD increased with higher lipoprotein(a) levels in the UK Biobank (N=458,601; events=5,132; p=0.007), but not in the Copenhagen studies (N=80,877; events=2,734; p=0.4). Absolute risks at age 80 in UK Biobank individuals with lipoprotein(a) >95th vs. ≤50th percentiles were 3.6% vs. 3.3% for VRD. In the Copenhagen studies, *LPA* KIV-2 number of repeats ≤5th vs. >50th percentile, determining lifelong high lipoprotein(a), associated with a hazard ratio for AD of 1.27(1.08-1.48).

Conclusion: Low lipoprotein(a) levels are not associated with risk of AD or VRD, indicating that pharmacological lowering of lipoprotein(a) is likely safe for risk of dementia. We cannot exclude that very high lipoprotein(a) increases risk of dementia.

Risk reduction of ASCVD attributed to lowering of remnant cholesterol from statins, fibrates, APOC3 inhibitors, and ANGPTL3 inhibitors: a cohort study

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Abstract

Background and Aims: Randomized clinical trials of remnant cholesterol lowering drugs show 50% and 80% reduction in remnant cholesterol with apolipoprotein C3(APOC3) and angiotensin-like 3(ANGPTL3) inhibitors. However, how many of atherosclerotic cardiovascular disease(ASCVD) cases that could be prevented lowering remnant cholesterol by these therapies is unknown. We estimated the potential of APOC3 and ANGPTL3 inhibitors to reduce the ASCVD burden through lowering of remnant cholesterol in the general population.

Methods: We included 98,311 individuals from the Copenhagen General Population Study without ASCVD at study entry including 8,506 statin users and 89,805 statin non-users. Cause-specific Cox regression was used to model rates of ASCVD and non-cardiovascular death conditional on remnant cholesterol and risk factors. Based on these models we derived the 10-year absolute risk reduction of ASCVD in individuals with remnant cholesterol >1mmol/L(>39mg/dL) for 15%, 30%, 50%, and 80% lower remnant cholesterol. Analyses were replicated in 16,009 individuals from the Copenhagen General Population Study with directly measured remnant cholesterol.

Results: During a median follow-up of 13-years, 10,596 individuals developed ASCVD. The 10-year absolute risk reduction of ASCVD in individuals with remnant cholesterol levels >1mmol/L(39mg/dL) for 15%, 30%, 50%, and 80% lower remnant cholesterol were 0.9%(95% confidence interval (CI): 0.7-1.0 %), 1.7%(1.4-2.0%), 2.7%(2.2-3.2%), and 4.1%(3.4-4.8%) for statin users and 0.4%(0.4-0.5%), 0.9%(0.8-0.9%), 1.4%(1.3-1.5%), and 2.1%(2.0-2.3%) for statin non-users.

Conclusions: We estimated a clinically meaningful 10-year absolute risk reduction of ASCVD for a 50% and 80% lower remnant cholesterol, corresponding to the lowering observed with APOC3 and ANGPTL3 inhibitors. These findings are adding on to the evidence motivating drug development of APOC3 and ANGPTL3 inhibitors.

Adherence to lipid lowering therapy in genetic familial hypercholesterolemia and cardiovascular disease risk: a registry cohort study

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Abstract

Background and Aims

Adherence to LLT in patients with genetically verified familial hypercholesterolemia (FH) in the population is unknown. We aimed to evaluate the adherence of lipid lowering therapy (LLT) and its association with risk of coronary heart disease (CHD) in the Norwegian FH-population.

Methods

We performed a registry-based cohort study on patients with genetically diagnosed heterozygous FH at Oslo University Hospital. The FH-population was linked to the Norwegian Prescription Database and the national registers of patients, medical birth, and cause of death. Adherence to statins were measured by the proportion of days covered (PDC) and classified as low (PDC<20%), medium (20%≤PDC≤80%) or high (PDC>80%). The effect of adherence on CHD was evaluated by cumulative incidence and adjusted hazard ratios (HR). The cumulative incidence of CHD was compared to age-matched controls without FH. Follow-up was through 2018.

Results

We included 3,032 FH-patients and 21,857 controls. Mean age at FH diagnosis was 36 years (SD 19), and 52% were female. Among the 2,750 FH-patients (91%) who collected a statin prescription during follow-up, adherence was high in 59%, medium in 35%, and low in 6%. Poor adherence was more common in females and in younger patients. During follow-up, 6% of the FH-patients experienced a CHD event. Patients with no statin treatment had a higher hazard of CHD (HR:5.08, 95%CI:2.68-9.63) compared to patients with high adherence. Patients with low/medium adherence had a higher hazard of CHD events (HR:2.19, 95%CI:1.10-4.36). Patients with high adherence had a cumulative incidence of CHD approaching that of controls.

Conclusions

In our study, high adherence to statin therapy was associated with a CHD-risk near to the risk in the unaffected population. Even low-dose statin therapy seems to be protective. The study highlights the importance of an early diagnosis and optimisation of existing treatment options in patients with FH.

Common genetic variants in *GIP* and *GIPR* as proxies for body weight reduction via GIP receptor targeting and risk of cardiovascular disease – observational and Mendelian randomization studies

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Abstract

Background and aims: Anti-obesity therapies co-targeting the glucose-dependent insulinotropic polypeptide (GIP) receptor achieve greater weight loss compared to glucagon-like peptide 1 receptor agonists. However, the implications for cardiovascular risk reduction and the mechanisms involved remain unclear. This study aimed to 1) investigate whether genetically proxied body weight reduction via the GIP receptor pathway lowers cardiovascular disease (CVD) risk and 2) compare the effect to polygenic weight reduction, excluding GIP-related genes, to assess if the observed cardiovascular benefits are attributable to weight loss *per se*, or involve additional, pleiotropic GIP-related effects.

Methods: Genetic scores were constructed using four variants in the GIP and GIP receptor genes associated with lower body mass index (BMI) and five variants linked to lower BMI in general. Observational and one-sample Mendelian randomization (MR) analyses were performed in 408,221 individuals from the UK Biobank, and two-sample MR analyses using summary statistics from 453,733 individuals in FinnGen.

Results: In one-sample MR analyses, 1 kg/m² lower BMI via the *GIP/GIPR* score associated with 21% lower risk of ischemic heart disease ($p = 0.002$), 23% lower risk of major adverse cardiovascular events ($p = 2 \times 10^{-4}$), and 45% lower risk of heart failure ($p = 2 \times 10^{-5}$). Similar results were observed using the polygenic BMI score and in two-sample MR analyses.

Conclusions: Genetic proxies for body weight reduction via GIP receptor targeting reduces CVD risk. Similar effects with polygenic weight reduction proxies suggest that the cardiovascular benefits of GIP receptor targeting are primarily attributable to the weight loss and related metabolic improvements.

Abdominal Aortic Aneurysm Prevalence and Infraarenal Aortic Diameter are decreasing in the Population-based Regional Screening Program

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Abstract

Introduction: Specific screening programs have been implemented to detect abdominal aortic aneurysms (AAAs) prior to their rupture. The aim of this study was to explore temporal trends within the regional screening program in Stockholm, and in detail describe the effects of tobacco on AAA progression.

Methods: During the years 2010-2023, 152,000 65-year-old men were invited to the screening program for AAA in Stockholm Region. Among all participants an aortic diameter, and presumed death date was extracted from the regional screening database. For those diagnosed with AAA, baseline demographics, and clinical follow-up was extracted from the screening database and electronic health-care records.

Results: A total of 117,120 persons were examined with ultrasound. The mean aortic diameter and prevalence of AAA decreased during the study period. In 2010-2013 it was 18.6 ± 3.3 mm and 1.32% (1.20%–1.45%), and in 2020-2023, 18.14 ± 2.8 and 0.69% (0.60%-0.79%), respectively. Aortic diameter at screening showed a significant, non-linear association with mortality (p for both < 0.001), with increased mortality at both small and large normal aortic diameters.

Aneurysms were similar across the time-periods of the screening with respect to diameter at screening, growth rate and progression to surgery. The number of patients reported as current and previous smokers decreased over the time period, the trend for the number of current smokers and previous smokers with AAA per year was -2.2 (95%CI: -3.2 - -1.2), and -3.0 (95% CI: -4.0 – -2.0), whereas the number of never smokers did not change over the time period (-0.1, 95%CI: -1.1 – 0.9). Further, smoking but not Swedish snus was significantly associated with AAA growth rate, progression to surgery and overall mortality.

Conclusions: AAA prevalence and aortic diameter is decreasing in the screened population. Smoking remains a strong predictor of aneurysm progression and overall mortality.

Cholesterol associates with risk of STEMI above NSTEMI

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Abstract

Background: Cholesterol is the primary causal risk factor for myocardial infarction (MI) covering the two different entities ST-segment elevation myocardial infarction (STEMI) and non-STEMI (NSTEMI). Importantly, STEMI holds greater acute mortality than NSTEMI, making it a critical concern for both patients and clinicians. This study tested the hypothesis that cholesterol levels are associated with STEMI above NSTEMI in statin-treated individuals.

Methods: We performed a cohort study of statin-treated individuals with ischemic heart disease determined by coronary angiography (CAG) from 2011-2020 registered in the Western Denmark Heart Registry. Low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein (non-HDL-C) were measured within 1 year after CAG. The risk of STEMI and NSTEMI was estimated as adjusted hazard ratios (aHR) and the comparison of STEMI vs NSTEMI and of 30-day mortality after STEMI vs NSTEMI was estimated as adjusted odds ratios (aOR).

Results: The study cohort included 36,739 statin-treated individuals with ischemic heart disease. During median 4.9 years of follow-up, 531 STEMI and 1,614 NSTEMI events were observed. Per 1 mmol/L higher LDL-C, the aHRs of STEMI and NSTEMI were 1.43 (95% CI: 1.30-1.57) and 1.23 (95% CI: 1.16-1.31). Among individuals with MI during follow-up, a 1 mmol/L higher LDL-C was associated with STEMI above NSTEMI: aOR 1.18 (95% CI: 1.04-1.32). Developing STEMI compared with NSTEMI was associated with a higher 30-day mortality: aOR 1.61 (95% CI: 1.01-2.56). Encouragingly, achieving the guideline-directed LDL-C goal (≤ 1.4 mmol/L) was associated with a lower risk of STEMI than NSTEMI. Results were similar for non-HDL-C and in clinically relevant subgroups.

Conclusion: In statin-treated individuals, cholesterol is associated with a higher risk of STEMI than NSTEMI. Low cholesterol levels promote stronger protection against STEMI than NSTEMI. These results are important when encouraging medication adherence.

Elevated remnant cholesterol confers high risk of ASCVD without prompting lipid-lowering therapy: an unmet medical need

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Abstract

Background and Aims

Initiation of lipid-lowering therapy may primarily be prompted by high low-density lipoprotein (LDL) cholesterol levels. If so, individuals with high risk of atherosclerotic cardiovascular disease (ASCVD) due to elevated remnant cholesterol may not be started on lipid-lowering therapy. We tested the hypothesis that elevated remnant cholesterol confers high risk of ASCVD but less initiation of lipid-lowering therapy compared to elevated LDL cholesterol.

Methods

From the Copenhagen General Population Study, 94 299 lipid-lowering therapy naïve adults without a history of ASCVD were included. Discordance groups were formed by median levels of remnant cholesterol, LDL cholesterol and apolipoprotein B (apoB). In the national Danish health registries, individuals were followed for a prescription of lipid-lowering therapy and for incident ASCVD or until December 2021.

Results

During a median follow-up of 12 years, 9 269 developed ASCVD. Compared to individuals with concordant low values of LDL and remnant cholesterol, those with high remnant cholesterol and apoB but low LDL cholesterol had a hazard ratio (HR) of 1.45 (95% confidence interval: 1.34-1.56) for ASCVD and an odds ratio (OR) of 3.0 (2.5-3.6) for starting lipid-lowering therapy within one year. Correspondingly, those with low remnant cholesterol but high apoB and high LDL cholesterol had a HR of 1.20 (1.11-1.30) for ASCVD and an OR of 5.1 (4.3-5.9) for starting lipid-lowering therapy.

Conclusions

In primary prevention elevated remnant cholesterol confers high risk of ASCVD but less initiation of lipid-lowering therapy compared to elevated LDL cholesterol, representing an unmet medical need.

Cardiovascular disease and compliance with lipid-lowering therapy among young individuals with Familial Hypercholesterolemia in Norway – A register study

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Abstract

Aims

Investigation of cardiovascular disease (CVD), all-cause death and use of statins and ezetimibe among young Norwegian individuals with heterozygous Familial hypercholesterolemia (FH).

Methods

We included subjects with genetically verified FH born 1988-2008 and twenty controls per FH subject, with linkage to prescription data, hospitalization data and cause of death from Norwegian registries. Subjects were followed for CHD and death during 2008-2018, and for dispensed prescriptions during 2004-2018.

Results

1351 subjects with FH and 27015 controls were included. Mean age (SD) at start of follow-up was 12.3 (5.4) years. There was one Coronary Heart Disease (CHD) event and 6 deaths in the FH-group and 3 CHD events and 53 deaths in the control group. CHD and all-cause death were non-significantly increased in the FH-group, hazard ratios (95% confidence intervals) 6.68 (0.69-64.20) and 2.26 (0.98-5.28), respectively. None of the deaths in the FH-group were related to cardiovascular disease.

83% of subjects with FH had been prescribed a statin, and 21% ezetimibe.

During the first year after the first prescription, 18.5% of subjects did not refill their prescription within 180 days after the end date of the previous prescription.

69% and 60% of subjects with a prescription had more than 80% of days covered with statins and ezetimibe, respectively. After 8 years, around 70% of subjects were covered with statins.

Conclusions

Results suggest increased risk of CHD in FH relative to controls, but measures are imprecise because of low absolute risks. Compliance with lipid lowering therapy was moderate.



**Oral Presentations – Abstracts –
Lipoproteins and Lipid Transport**

SESSION III

High cholesterol absorption efficiency enhances proatherogenic properties of LDL particles

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Abstract

Approximately 30% of individuals have high cholesterol absorption efficiency, which originates from genetic variabilities in the small intestinal sterol transporters. In these individuals, atherosclerotic cardiovascular disease (ASCVD) risk is increased compared with those with low cholesterol absorption. Serum lipid concentrations do not explain the increased risk. We evaluated the link between cholesterol absorption and proatherogenic properties of low-density lipoproteins (LDLs) in a cohort of 90 individuals without lipid lowering therapy or ASCVD.

The participants were divided into low (n=45) and high (n=45) cholesterol absorbers by the median value of serum cholestanol to cholesterol ratio, a validated biomarker of cholesterol absorption efficiency. LDL aggregation susceptibility and the binding of serum lipoproteins to proteoglycans were determined as biomarkers related to proatherogenic properties of lipoproteins. Lipoprotein subclasses were determined by NMR spectrometry, LDL lipidome by mass spectrometry, and serum cholesterol and noncholesterol sterols using gas-liquid chromatography. Dietary cholesterol and serum and lipoprotein cholesterol levels were similar between the groups. In high absorbers, LDL particles were larger and LDL aggregation susceptibility and the binding of lipoproteins to proteoglycans were higher than in the low absorbers. Of LDL surface lipids, sphingomyelin 34:1;O2, lysophosphatidylcholine 18:0, and phosphatidylcholine 32:0 correlated positively, while phosphatidylcholine 32:1 correlated negatively with serum cholestanol levels. These lipids were also associated with increased LDL aggregation and proteoglycan-binding. High cholesterol absorption was associated with a specific LDL lipidome and increased size of LDL particles that were prone to aggregate and showed increased binding to proteoglycans. These data may partly explain the high ASCVD risk associated with high cholesterol absorption efficiency.

CHOLESTEROL-LOWERING DRUG TARGETS REDUCE RISK OF VASCULAR-RELATED DEMENTIA: MENDELIAN RANDOMIZATION OF 500,000 INDIVIDUALS

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Abstract

BACKGROUND AND AIMS: Dementia is a devastating neurodegenerative disease currently affecting 60 million people worldwide, but little progress has been made in its treatment and prevention. Recent research is pointing towards a shared pathogenesis between dementia and atherosclerotic cardiovascular disease, and several shared modifiable risk factors have been suggested including atherogenic cholesterol and triglycerides. We tested whether atherogenic cholesterol- and triglyceride-lowering drug targets reduce risk of vascular-related dementia, Alzheimer's disease, all-cause dementia, and ischemic heart disease.

METHODS: We included 500,000 individuals from prospective cohorts of the general population, and selected genetic variants within *HMGCR*, *NPC1L1*, *ANGPTL4*, *LPL*, and *CETP*. Unweighted allele scores were generated, and Cox regression, Mendelian randomization, and meta-analyses performed.

RESULTS: In meta-analyses of Cox regression results, hazard ratios for risk of vascular-related dementia/ischemic heart disease per 1 mmol/L (39 mg/dL) lower low-density lipoprotein cholesterol were 0.02(95%CI:0.008-0.04)/0.19(0.13-0.28) for *HMGCR* and 0.0001(0.000-0.0007)/0.02(0.006-0.04) for *NPC1L1*. Corresponding HRs per 1 mmol/L (39 mg/dL) lower non-high-density cholesterol were 0.22(0.17-0.29)/0.42(0.36-0.48) for *CETP*. Finally, corresponding hazard ratios per halving in triglycerides were 0.70(0.38-1.28)/0.65(0.57-0.73) for *ANGPTL4* and 0.96(0.60-1.31)/0.60(0.51-0.71) for *LPL*. Results for Alzheimer's disease were null. Mendelian randomization results were directionally consistent with Cox regression results.

DISCUSSION: Genetic lowering of atherogenic cholesterol via *HMGCR*, *NPC1L1*, and *CETP* reduce the risk of vascular-related dementia, but not the risk of Alzheimer's disease. This is important because it suggests that these drug targets could be used in dementia prevention.

Low-Density Lipoprotein Cholesterol, Coronary Plaque burden, and Cardiovascular Risk in individuals beyond 75 years of age: The Western Denmark Heart Registry

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Abstract

Background: The role of low-density lipoprotein cholesterol (LDL-C) in atherosclerotic cardiovascular disease in older individuals remains controversial.

Purpose: To assess whether LDL-C is associated with plaque burden on coronary computed tomography angiography (CCTA) and future myocardial infarctions (MI) in statin-naïve symptomatic individuals across different ages with a primary focus on individuals aged >75 years.

Methods: This contemporary cohort study included individuals who underwent CCTA between 2008 and 2021, sourced from the Western Denmark Heart Registry. Outcomes and measures included 1) adjusted risk ratio (aRR) for any plaque and early revascularization (within 90 days of CCTA) and 2) adjusted hazard ratio (aHR) for MI.

Results: The study included 37,910 statin-naïve symptomatic individuals. The median age was 57 years (Q1-Q3 50-65), and 1,562 (4%) were aged >75 years. The prevalence of any plaque was 19,962 (53%). During a median follow-up of 5.1 years, 433 (1%) individuals experienced MI, including 36 (2%) of those aged >75 years. The aRR for any plaque was 1.14 (95% CI 1.07-1.22) and 3.18 (1.94-5.23) for early revascularization in individuals aged >75 years with high versus low LDL-C (>4.4 versus <2.7 mmol/L). The 90-day risk for early revascularization was 20% (95% CI 13-29%) in individuals aged >75 years with LDL-C >4.4 mmol/L. The aHR for MI was 1.57 (95% CI 1.06-2.32) per 1 mmol/L higher LDL-C for individuals aged >75 years. The 5-year risk for MI was 7% (95% CI 2-15%) in individuals aged >75 years with LDL-C >4.4 mmol/L.

Conclusion: LDL-C was strongly associated with the presence of coronary plaque and elevated cardiovascular risk in individuals >75 years of age. These findings suggest that LDL-C influences prognosis throughout life, leading to a potentially lifelong need for LDL-C management to prevent cardiovascular disease.

Hepatic knockdown of ABCA6 increases atherosclerosis in APOE*3-Leiden.CETP mice

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Abstract

Background: Previously, a rare missense variant of ABCA6 was previously found to be strongly associated with hypercholesterolemia, and we have shown that hepatic knockdown of Abca6 in mice decreases hepatic uptake of VLDL remnants. Here, we aimed to evaluate the effects of hepatic expression of ABCA6 on atherosclerosis development in APOE*3-Leiden.CETP mice, a well-established model for human-like lipoprotein metabolism and atherosclerosis development.

Methods: Female APOE*3-Leiden.CETP mice, fed a Western-type diet (16% fat and 0.15% cholesterol) received either adeno-associated virus 8 expressing the Cas9 protein and a sgRNA targeting Abca6 (Abca6 AAV-CRISPR) or an empty AAV vector expressing and a scrambled sgRNA (Control AAV-CRISPR). Following AAV-CRISPR delivery. After 16 weeks, atherosclerosis was assessed in the aortic root.

Results: Hepatic knockdown of Abca6 (-65%; p<0.001) did not affect body weight gain or organ weight. Abca6 knockdown did not affect without plasma triglycerides, phospholipids and free fatty acids, and increased plasma cholesterol (+13%; p<0.001), specific for nonHDL-C (+14%; p<0.001), accompanied with lower hepatic expression of syndecan-1 and unchanged liver lipids. Despite the relatively subtle cholesterol-increasing effect, Abca6 knockdown largely increased atherosclerotic lesion area (+65%; p<0.001) and the relative number of severe (type IV and V) lesions (+38%; p<0.05).

Conclusion: Hepatic ABCA6 protects against hypercholesterolemia and atherosclerosis development in APOE*3-Leiden.CETP mice, which suggests that improving hepatic ABCA6 function may protect against atherosclerotic cardiovascular disease in humans.

The Effects of Modulating HDL concentration on Sepsis Risk: A Mendelian Randomization study

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Abstract

Background: While high-density lipoprotein (HDL) has been clearly associated with reduced cardiovascular disease risk, recent genetic and intervention studies did not indicate this relationship is causal. However, there is increasing evidence supporting the potential impact of modifying HDL levels on sepsis outcomes. Here, we performed two-sample Mendelian Randomization (MR) to study the association between HDL and its key regulators on sepsis.

Methods: We used genetic variants independently associated ($p < 5 \times 10^{-8}$) with HDL particle concentration, levels of ApoA1 and CETP as instrumental variables from previously published genome-wide association studies (GWAS). Independent variants were harmonized and linked to GWAS summary results from 10,154 sepsis cases and 452,764 controls for the SNP-outcome associations. Inverse-variance weighted (IVW) analyses were employed as primary analyses, and additional tests were performed for potential bias by directional pleiotropy. Protein-protein interaction (PPI) network analyses were used to identify key regulators for concentration of HDL particles and ApoA1.

Results: Based on the IVW analyses, we found evidence of an association between genetically-influenced higher HDL particle concentration and lower risk of sepsis (odds ratio (OR): 0.86, 95% confidential interval (CI): 0.77-0.96). Similarly, genetically-influenced higher ApoA1 concentration was associated with a lower risk of sepsis (OR: 0.90, 95% CI: 0.85 - 0.96). No evidence was found these associations were harmed by directional pleiotropy. Through PPI network analyses, we identified cholesteryl ester transfer protein (CETP) as a key player in regulating concentration of HDL particles and ApoA1, and sepsis risk as such.

Conclusion: Our MR study provides evidence for a potential causal relationship between HDL particle concentration and the risk of sepsis. We hypothesize that increasing HDL through inhibiting CETP might serve as a therapeutic strategy for human sepsis.

Angiopietin-like 3 (ANGPTL3) deficiency alters hepatic metabolism and promotes substrate rerouting

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Abstract

ANGPTL3, a hepatokine which inhibits lipases, acts by sparing triglyceride-rich lipoproteins from hydrolyzation and controlling the postprandial triglycerides partitioning. This project aims to unravel the connection between hypolipidemia-derived metabolic alterations and possible hepatic responses according to substrate availability in ANGPTL3 deficiency.

Angptl3-deficient (KO) mice and WT littermates were fed chow or a High-Fat Diet (HFD, 60% kcal from fat) for 16 weeks. Metabolic adaptation to different diets was assessed *in vivo* by indirect calorimetry and lipid tolerance test and lipoprotein production assay were performed. Bulk transcriptomic analyses were performed *ex vivo*.

As expected, Angptl3 KO mice were hypolipidemic when fed and at fasting, on both chow diet and HFD. After an oral oil gavage, KO mice showed lower triglycerides absorption; this was coupled with a decreased rate of hepatic lipoprotein production at 4 hours after Poloxamer injection in KO mice compared to WT, and numerically on HFD. Glucose metabolism was not impaired.

Data from indirect calorimetry in KO mice show a reduction in the Respiratory Exchange Ratio, with more oxidative metabolism when KO mice were fed a chow diet.

We assessed the different substrate utilization by activation of hepatic mTOR pathway, a sensor for caloric restriction; downstream effectors phosphorylation was assessed by western blotting and a reduction in S6K and 4E-BP1 activation was observed, suggesting a lower protein synthesis.

Liver RNA sequencing data displayed different metabolic setting of KO mice fed a chow diet when compared to WT with, higher lipid oxidation and synthesis, especially of bile acids, while hepatic signalling pathways (NR1H2/NR1H3) are shut down. In a HFD setting, KO mice showed a reduction in hepatic anabolic pathway activation.

ANGPTL3 deficiency affects the metabolic landscape by reducing circulating lipemia. Parallel changes occur in systemic metabolism, depending on the dietary setting.

Association of HDL cholesterol, apolipoprotein E and A1 concentrations with fetal growth, maturation, and placental insufficiency.

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Abstract

Background Fetal cholesterol is primarily carried by high density lipoprotein cholesterol (HDL-C) enriched with apolipoprotein E (apoE) and the fetus is highly dependent on placental transfer of cholesterol. We aimed to examine if infant HDL-C, apoE, and apolipoproteinA1 (apoA1) concentrations are associated with fetal growth/maturation, and placental insufficiency.

Methods To examine this we used data from the Copenhagen Baby Heart and COMPARE studies including more than 12,600 cord blood samples and 360 corresponding newborn venous samples, with follow-up at 2 months.

Results HDL-C concentrations correlated significantly with apoE and apoA1 at birth in cord blood (Spearman's correlation: 0.48 for apoE, 0.67 for apoA1, p-values<0.001) and venous blood (0.58 for apoE, 0.71 for apoA1, p-values<0.001), and at 2 months of age in venous blood (0.18 for apoE(p=0.003), 0.87 for apoA1(p<0.001)). From birth to 2 months of age venous blood concentrations of HDL-C and apoA1 increased while apoE decreased(p<0.001). Gestational age adjusted HDL-C and apoE concentrations in cord blood at birth were positively associated with birth weight (p<0.001), while apoA1 was only marginally associated(p=0.05). The clinical classification of fetal growth/maturation; small, appropriate, and large for gestational age, showed a significant, stepwise increase in cord blood for HDL-C and apoE concentrations, but not for apoA1, from small to large for gestational age(p for trend<0.001). For newborns exposed to placental insufficiency multifactorial adjusted odds ratios (95% CI) for cord concentrations of HDL-C, apoE, and apoA1 below the 20th percentile were 1.39 (1.17-1.66) for HDL-C, 1.47 (1.23-1.76) for apoE, and 1.19 (0.99-1.43) for apoA1.

Conclusions HDL-C and apoE at birth are positively associated with fetal growth/maturation. Placental insufficiency is associated with increased risk of low newborn HDL-C and apoE concentrations, suggesting these lipid traits as markers of placental health.



Oral Presentations – Abstracts –

Other Topics

SESSION IV

Statin-associated regulation of hepatic PNPLA3 in patients without known liver disease

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Abstract

Genetic variants of patatin-like phospholipase domain containing 3 (PNPLA3) are associated with both susceptibility to metabolic dysfunction-associated steatotic liver disease (MASLD) and response to statins treatment in MASLD patients. Access to liver biopsies before established MASLD is limited, and statins and PNPLA3 in early liver steatosis are thus difficult to study.

In this study, liver biopsies were collected from 261 patients without any known liver diseases at surgery and stratified based on statin use and criteria for the metabolic syndrome (MS). Genotypes and transcript levels were measured using Illumina and Affymetrix arrays, and metabolic and lipoprotein profiles by clinical assays. Statin effects on PNPLA3, de novo lipogenesis (DNL), and lipid accumulation were further studied in vitro.

The PNPLA3^{I148M} genetic variant was associated with significantly lower hepatic levels of cholesterol synthesis-associated transcripts. Patients with MS had significantly higher hepatic levels of MASLD and lipogenesis-associated transcripts than non-MS patients. Patients with MS on statin therapy had significantly higher hepatic levels of PNPLA3, acetyl-CoA carboxylase alpha, and ATP citrate lyase, and statin use was associated with higher plasma fasting glucose, insulin, and HbA1c. Exposure of hepatocyte-like HepG2 cells to atorvastatin promoted intracellular accumulation of triglycerides and lipogenesis-associated transcripts. Atorvastatin-exposure of HepG2, sterol O-acyltransferase (SOAT) 2-only-HepG2, primary human hepatic stellate, and hepatic stellate cell-like LX2 cells significantly increased levels of PNPLA3 and SREBF2-target genes, whereas knockdown of SREBF2 attenuated this effect.

Collectively, these observations suggest statin-associated regulation of PNPLA3 and DNL in liver. The potential interaction between PNPLA3 genotype and metabolic status should be considered in future studies in the context of statin therapy for MASLD.

Loss-of-function in endothelial lipase increases LDL cholesterol but decreases risk of atherosclerotic cardiovascular disease

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Abstract

Background: The LDL cholesterol-lowering effect of angiotensin-like 3 (ANGPTL3) inhibitors is likely explained by endothelial lipase derepression, a novel and poorly characterized mechanism. We investigated if loss-of-function in *LIPG*, encoding endothelial lipase, is associated with increased levels of LDL cholesterol and increased risk of atherosclerotic cardiovascular disease (ASCVD).

Methods: We included individual-level data on individuals of European ancestry from the UK Biobank to estimate difference in LDL cholesterol and other lipids. We then added summary-level data on individuals from FinnGen, Biobank Japan, the Million Veteran Program, and the CARDIoGRAMplusC4D consortium to estimate risk of ASCVD, coronary artery disease, ischemic stroke, and peripheral artery disease per *LIPG* loss-of-function allele.

Results: Among individuals in the UK Biobank, 116 had two *LIPG* loss-of-function alleles, 13,109 had one allele, and 380,677 were non-carriers. Per loss-of-function allele in *LIPG*, LDL cholesterol levels were 2.3% (95% confidence interval: 1.9-2.8%) higher, HDL cholesterol was 8.3% (7.8-8.8%) higher, non-HDL cholesterol was 2.6% (2.1-3.1%) higher, apolipoprotein B was 0.1% (-0.4 to 0.5%) higher, and C-reactive protein was 6.2% (6.0-6.7%) higher. In 62,009 individuals with ASCVD, 254,735 with coronary artery disease, 43,009 with ischemic stroke, and 42,293 with peripheral artery disease, versus 331,893, 995,908, 738,353, and 917,953 controls, respectively, corresponding odds ratios per *LIPG* loss-of-function allele were 0.88 (0.83-0.92), 0.92 (0.89-0.95), 0.86 (0.78-0.95), and 0.92 (0.86-0.98), respectively.

Conclusions: Genetic endothelial lipase inactivation was associated with decreased risk of ASCVD despite increased LDL cholesterol. This supports endothelial lipase repression as a strategy to reduce ASCVD risk. Additionally, these findings raise questions about pharmacological endothelial lipase derepression with ANGPTL3 inhibition.

Management of hypercholesterolemia in a patient with vanishing bile duct syndrome

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Abstract

In severe chronic cholestasis, bile acids (BAs) and unesterified cholesterol (UC) accumulate in the hepatocyte cytoplasm, leading to toxic effects. To prevent cellular damage, UC is secreted with phospholipids (PLs) into the Disse's space instead of the bile canaliculi, forming a non-canonical lipoprotein known as Lipoprotein X (LpX). LpX has a density similar to low-density lipoproteins (LDL) and interferes with laboratory measurements for LDL. Its unique biochemical structure and the absence of apolipoprotein B (ApoB) nullifies the effect of standard cholesterol-lowering therapies.

A 73-year-old female patient with severe and rare form of cholestasis due to drug-related vanishing bile duct syndrome (VBDS) exhibited what was initially believed to be a drastically altered plasma LDL cholesterol levels (29.1 mmol/L) with symptoms of extreme hypercholesterolemia, including normocytic anemia, hyponatremia, and peripheral neuropathy. The very low plasma levels of ApoB suggested the presence of circulating LpX that interfered with the clinical laboratory LDL measurements. Using size-exclusion chromatography and agarose gel electrophoresis, we could confirm high levels of LpX in plasma.

Today, a standard of care for VBDS hypercholesterolemic patients beyond the use of ursodeoxycholic acid is lacking. To disrupt the cholestatic state, we opted for bezafibrate, a pan-peroxisome-proliferator activated receptor (PPAR) agonist also targeting pregnane X receptor (PXR), and previously used to treat primary biliary cholangitis. Later an intestinal bile acid binding resin was added to reactivate bile acid synthesis which was depressed by the cholestatic state and using bezafibrate. After three months of treatment, cholestasis was almost completely resolved, LpX disappeared, and plasma cholesterol levels almost normalized. Our case gave us the possibility to define a new therapeutic approach to rapidly resolve extreme cholestatic condition associated to presence of high level LpX.

Causal insights of LDL cholesterol and triglycerides-rich lipoproteins for risk of dementia

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Abstract

Background: The 2024 *Lancet Commission report* identifies 14 modifiable risk factors that could prevent half of dementia, with low-density lipoprotein cholesterol (LDL-C) newly highlighted. Causal evidence however remains unclear due to limitations in previous two-sample Mendelian randomization (MR) studies. We aimed to establish robust causal estimates for these cardiovascular risk factors and dementia.

Methods: We analyzed individual-level data from 408,788 European participants from the UK Biobank and computed polygenetic risk scores based on independent variants derived from the largest genomic consortia for each risk factor. We conducted univariable and multivariable linear MR analyses, assessed genetic shapes by nonlinear approaches, and supplemented with sensitivity analyses including sex stratification.

Results: Genetic predisposition to high levels of LDL-C (OR, 95% CI: 1.12, 1.01-1.23), non-high-density lipoprotein cholesterol (1.30, 1.26-1.35), triglycerides (1.19, 1.01-1.41), body mass index (1.04, 1.02-1.07), systolic (1.14, 1.09-1.20) and diastolic (1.10, 1.02-1.19) blood pressure, high susceptibility of type 2 diabetes (1.04, 1.00-1.09) and smoking (1.18, 1.06-1.32), were associated with increased risk of all-cause dementia, while longer education was associated with a reduced risk (0.72, 0.65-0.79). Results for Alzheimer's disease and vascular dementia were in the same direction. Moreover, genetically-determined high physical activity level was associated with reduced risk of Alzheimer's disease (0.58, 0.33-0.99). Sensitivity analyses supported the main results, and no nonlinear shapes were detected.

Conclusion: These findings provide causal insights into cardiovascular risk factors for dementia and give hope for maintaining brain health by timely treatment of high LDL-C and triglycerides, alongside hypertensive medication, smoking cessation, and maintenance of normal weight.

Combined use of cardioprotective glucose-lowering drugs and statins for primary prevention of atherosclerotic cardiovascular disease in individuals with type 2 diabetes: A nationwide cohort study

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Abstract

Aim

Cardioprotective glucose-lowering drugs including sodium glucose cotransporter 2 inhibitors and glucagon-like peptide-1 receptor agonists, and statins are both thought to prevent atherosclerotic cardiovascular disease (ASCVD) in type 2 diabetes. They were never tested in a 2-by-2 randomized trial.

We tested the hypothesis that combination of cardioprotective glucose-lowering drug and statin is associated with lower risk of ASCVD than using either drug alone in primary prevention setting.

Methods:

Using Danish nationwide registers, we identified individuals in Denmark with type 2 diabetes treated only with metformin and without preexisting ASCVD between December 2012 through 2021. Using a new-user active comparator design, we followed 59,795 individuals initiating a cardioprotective glucose-lowering drug or dipeptidyl peptidase-4 inhibitor. We defined four treatment groups: i) both cardioprotective glucose-lowering drug and statin, ii) cardioprotective glucose-lowering drug alone, iii) statin alone, and iv) neither cardioprotective glucose-lowering drug nor statin. The primary outcome was time to first ASCVD, a composite of myocardial infarction, coronary revascularization, ischemic stroke, peripheral arterial disease, or coronary death.

Results

During mean follow-up of 3.2 years, 2,546 individuals experienced ASCVD. Compared with nonusers of cardioprotective glucose-lowering drug or statin, the multivariable adjusted hazard ratios of ASCVD were 0.87(95% confidence interval:0.76–0.98) for cardioprotective glucose-lowering drug alone, 0.69(0.63–0.77) for statin alone, and 0.64(0.56–0.73) for combined treatment with cardioprotective glucose-lowering drug and statin.

Conclusions

In individuals with type 2 diabetes and no preexisting ASCVD, treatment with statin was associated with the lowest risk of ASCVD. Combining cardioprotective glucose-lowering drug and statin was not consistently associated with lower risk of ASCVD than for statin alone.

Current guidelines result in extensive pregnancy-related off-treatment time in women with familial hypercholesterolemia

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Abstract

Background

Women with familial hypercholesterolemia (FH) lose treatment during childbearing age due to contraindication of lipid-lowering treatment (LLT) from planning of pregnancy until end of breastfeeding. We report real-life pregnancy-related off-treatment time in a case series of women with FH.

Methods

The study includes eight women with FH in Norway, who participated in the ongoing FH-FEMINA study (ClinicalTrials NCT05367310). The women were followed from gestational week 36 to 12 months post-delivery or to end of breastfeeding. Treatment history of current pregnancy was collected prospectively, and treatment history of previous pregnancies was collected retrospectively. Pregnancy-related off-treatment time was calculated for the period from stopping LLT at planning pregnancy, throughout the pregnancy, and after delivery during breastfeeding.

Results

Total pregnancy-related off-treatment time ranged from 1.6 to 11.8 years after median (min-max) 2 (1-3) child deliveries. Duration of treatment discontinuation in the planning period was median (min-max) 0.1 (0-0.8), 0.4 (0.2-0.6), 4.3 (4.3-4.3) years for women having one (n=3), two (n=4) or three (n=1) children, respectively. Median off-treatment time after delivery (breastfeeding, getting new prescription) was 1.4 (0.9-1.6), 1.7 (0.9-3.3), 5.4 (5.4-5.4) years for women having one (n=3), two (n=4) or three (n=1) children, respectively. When adding the women's untreated years in childhood or pre-diagnosis, the proportion of these women's lifetime without treatment ranged from 57 % to 84 % at ages 26 to 35, respectively.

Conclusions

To compensate for off-treatment periods later in life, early diagnosis and treatment start from the of age 8-10 is essential in girls with FH. To decrease pregnancy-related off-treatment periods, healthcare professionals should guide women with FH to restart LLT immediately after end of breastfeeding and between pregnancies, even if only for a few weeks or months.

Role of HDL in reproduction - the cholesterol efflux capacity of follicular fluid HDL predicts normal fertilization independent of HDL cholesterol levels

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Abstract

BACKGROUND: HDL is known for its protective role in cardiovascular disease, particularly its cholesterol efflux capacity, essential for removing cholesterol from cells through receptor-mediated pathways. However, since atherosclerosis typically manifests after reproductive age, it is unlikely subject to significant evolutionary pressure. Interestingly, HDL is the primary lipoprotein present in follicular fluid (FF) surrounding the developing oocyte, yet its role in reproduction remains unexplored.

METHODS: In 297 follicular fluid (FF) samples from 187 patients enrolled in an observational cohort study investigating modified natural cycle in-vitro fertilization (IVF) outcomes, cholesterol efflux capacity of HDL was determined (THP-1-derived macrophage foam cells). Nuclear magnetic resonance spectroscopy was employed to characterize HDL particles in FF and plasma in detail (sizes, particle numbers, HDL cholesterol and apolipoprotein A-I levels). Associations between HDL-related parameters and IVF outcomes (normal fertilization, embryo fragmentation, development of top-quality embryos, biochemical/clinical pregnancy) were evaluated.

RESULTS: A better follicular fluid HDL cholesterol efflux capacity was significantly associated with a higher likelihood of normal fertilization of the oocyte (adjusted odds ratio (aOR) 1.21, 95% CI 1.04 to 1.40). In contrast to plasma, small HDL particles predominated in FF, supporting entry from the general circulation via the size-selective blood-follicle barrier. HDL cholesterol levels, HDL particle numbers or apolipoprotein A-I in FF showed no association with reproductive outcomes.

SUMMARY: Our study demonstrates for the first time in humans that better cholesterol efflux properties of HDL in FF are associated with higher normal fertilization rates independent of HDL cholesterol levels. These results shed light on a potential evolutionary role of HDL beyond its traditional association with atherosclerotic cardiovascular disease.

Remnant cholesterol lowering in cardiovascular disease risk reduction in statin trials: meta-regression analyses

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Abstract

Aims

We tested the hypothesis that remnant cholesterol reduction is associated with part of the cardiovascular disease risk reduction in statin, ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor trials.

Methods

We included statin, ezetimibe, and PCSK9 inhibitor trials with more than 1,000 participants and available data on lipid levels. Baseline and follow-up low-density lipoprotein (LDL) and remnant cholesterol levels were extracted. LDL and remnant cholesterol reductions were differences between intervention and reference groups at year one. We calculated risk ratios for cardiovascular disease and death per 1 mmol/L (39 mg/dL) LDL and remnant cholesterol reductions using fixed and random effects meta-regressions.

Results

A total of 43 trials with 327,264 participants who developed 41,481 major cardiovascular events were included. On an absolute scale when LDL cholesterol was reduced 1 mmol/L (39 mg/dL) in statin trials, remnant cholesterol was reduced 0.13 mmol/L (5 mg/dL). On a relative scale when LDL cholesterol was reduced 20%, remnant cholesterol was reduced 11%. In 33 statin trials, risk ratios for major cardiovascular events per 1 mmol/L (39 mg/dL) reductions were 0.82 (95%CI:0.80-0.84) for LDL cholesterol and 0.37 (0.33-0.41) for remnant cholesterol. Corresponding risk ratios for LDL and remnant cholesterol across all 43 trials were 0.84 (0.82-0.85) and 0.36 (0.33-0.40) for major cardiovascular events, 0.79 (0.77-0.82) and 0.27 (0.23-0.32) for myocardial infarction, 0.85 (0.81-0.88) and 0.43 (0.35-0.54) for stroke, and 0.93 (0.91-0.95) and 0.71 (0.62-0.80) for death, respectively.

Conclusion

Remnant cholesterol lowering was associated with part of the cardiovascular disease risk reduction in statin trials.



**Posters – Abstracts –
Inflammation and Vascular Biology**

SESSION I

Role of immune cells in the developmental programming of atherosclerosis

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Abstract

The hypothesis of developmental programming proposes a link between conditions in foetal and early life and risk of disease in later life. A key example is the link between low birth weight and atherosclerosis. However, the mechanisms responsible for this association are not well understood. The progression of atherosclerosis involves infiltration and over-activity of immune cells, particularly neutrophils and macrophages, in the vessel wall. Early life changes in protein availability can render neutrophils to be more sensitive to activation. Therefore, in this study, we developed an *in vitro* model to mimic gestational growth in neutrophils and macrophages to assess whether this changes the response of the cells to inflammatory stimuli, such as phorbol myristate acetate (PMA) and lipopolysaccharide (LPS). L-histidinol was used to partially inhibit translation in PLB-985 neutrophils and J774A.1 macrophages to mimic amino acid deficiency and nutrient stress. Exposure of the cells to L-histidinol (0 – 3 mM) affected cell growth in each case, shown by a decrease in metabolic activity compared to the non-treated cells, which occurred in a dose and time-dependent manner. A loss in viability was observed with high L-histidinol concentrations (< 0.5 mM), particularly on prolonged incubation (> 48 h). Activated neutrophils produce hypochlorous acid (HOCl) to kill pathogens, which also damages the vasculature in atherosclerosis. Using the dye R19-S, we showed that L-histidinol treatment significantly increased the production of HOCl in PLB-985 cells stimulated with PMA. L-histidinol treatment of J774A.1 cells increased the mRNA expression of different cytokines and chemokines, particularly interleukin 6 (IL-6), and amplified the release of IL-6 on stimulation with LPS. Together, these data support a link between growth restriction and greater immune cell activity, which could be relevant to the development of atherosclerosis in individuals with a low birth weight.

Anti-inflammatory effects of a GLP-1 receptor agonist in comparison with an anti-IL-6 antibody in a mouse model of accelerated atherosclerosis

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Abstract

Atherosclerosis, driven by inflammation, is a major cause of cardiovascular disorders. Promising treatments for cardiovascular disease include anti-interleukin-6 (anti-IL-6) and glucagon-like peptide-1 receptor agonist (GLP-1 RA), both with anti-inflammatory properties. This study explored the mechanisms of GLP-1 RA semaglutide and an anti-mouse-IL-6 monoclonal antibody (mAb) in an accelerated atherosclerosis model.

Apolipoprotein E knockout (ApoE^{-/-}) mice were divided into five groups and fed a high-fat diet. Sixty mice had surgery with incomplete ligation and cuff placement on the left carotid artery for 3 weeks. Ten mice were sham-operated. Mice were dosed with vehicle, semaglutide, isotype mAb, or anti-mouse-IL-6 mAb. Fluorescence-activated cell sorting, immuno-assays, and histology analyses were performed.

Ligation and cuff caused significant neointimal hyperplasia, increased intimal area ($p < 0.0001$), decreased lumen area ($p = 0.0373$), and increased media area ($p = 0.0107$). There was a significant upregulation of CD45⁺ leukocytes ($p = 0.0006$), CD68⁺ macrophages ($p = 0.0003$), SMA⁺ vascular smooth muscle cells (VSMCs) ($p = 0.0031$), and phenotypically switched SMA⁺CD68⁺ VSMCs ($p < 0.0001$). Circulating CD163⁺ macrophages increased significantly, while CD4⁺ T cells decreased.

Semaglutide significantly reduced circulating chemokine KC/GRO and increased CD163⁺ macrophages. Anti-mouse-IL-6 mAb reduced plasma IL-6 levels, decreased circulating CD3⁺ T cells and CD45R/B220⁺ B cells, and increased CD11b⁺ cells and CD163⁺ macrophages. Both treatments trended towards reduced intimal area, but without statistical significance.

This study shows that semaglutide and anti-IL-6 mAb exhibit anti-inflammatory effects in an accelerated atherosclerosis model. Further experiments are needed to fully understand the potential of the model and the impact of these treatments on plaque stability and progression.

Dynamics of N⁶-methyladenosine (m⁶A) in STEMI and the Impact of IL-6 Inhibition

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Abstract

Background: Epitranscriptomics, with N⁶-methyladenosine (m⁶A) as the most common mRNA modification in mammals, represents an innovative target for treating inflammatory conditions, including cardiovascular diseases. Yet, m⁶A RNA-regulation in myocardial infarction (MI) remains under-explored.

Methods: We obtained whole blood samples from acute ST-elevation MI (STEMI) patients (n=6) at admission and after 3-7 days, alongside samples from healthy controls (n=3). Three of the patients with STEMI received anti-inflammatory treatment with tocilizumab, an interleukin-6 receptor (IL-6R) inhibitor, at hospitalization, while three patients received placebo. RNA was extracted, and m⁶A sites were pinpointed using human m⁶A single nucleotide resolution microarray analysis. Additionally, mRNA levels were evaluated through RNA sequencing.

Results: Patients with STEMI exhibited distinct m⁶A deposition patterns compared to controls, with 845 m⁶A methylation sites showing hypomethylation and 36 sites demonstrating hypermethylation against controls. Among hypomethylated transcripts, 194 showed reduced expression, while 197 were more highly expressed. Initially, m⁶A patterns indicated overall hypomethylation at admission, shifting towards hypermethylation 3-7 days post-admission. IL-6R inhibition with tocilizumab further modified the m⁶A deposition.

Conclusions: Marked differences in m⁶A deposition were observed between patients with STEMI and healthy individuals. The m⁶A pattern in patients with STEMI changed over a period of 3-7 days. The IL-6R blockade seems to modulate this process to some extent. Our findings imply that this RNA post-transcriptional regulation plays a role in the immune response during STEMI, suggesting its potential as a therapeutic target for MI.

Elevated adipose inflammation, but reduced hepatic triacylglycerol storage in diet-induced obese *Plin4*-null mice

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Abstract

Plin4 is primarily expressed in white adipose tissue (WAT) expressed at low levels in heart and skeletal muscle. It has been shown previously to be transcriptionally regulated by peroxisome proliferator-activated receptor gamma (Dalen *et al.*, 2004) and the triacylglycerol content in heart of *Plin4*^{-/-} mice is significantly reduced (Chen *et al.*, 2013). We found that conditions with elevated circulating lipids, such as fasting or prolonged feeding with an obesogenic diet containing high content of fat, fructose, and cholesterol (Western diet), increase hepatic *Plin4* expression. To investigate the functional role of *Plin4* in energy metabolism, we generated *Plin4*^{-/-} mice and assessed effects upon *Plin4* removal in the liver and WAT. Lean *Plin4*^{-/-} mice fed chow diet had no clear phenotype, except for altered expression of *Plin5* in the heart, liver, and white adipose tissue. Obese female *Plin4*^{-/-} mice fed Western diet had normal metabolic rate, but elevated insulin levels and improved glucose clearance compared to *Plin4*^{+/+} mice. The livers of *Plin4*^{-/-} mice fed a Western diet had reduced triglyceride levels and showed signs of reduced PERK-mediated endoplasmic reticulum stress. Ovarian WAT of *Plin4*^{-/-} mice fed Western diet had elevated expression of macrophage markers and more crown-like structures, but normal adipocyte cell size. Our findings connect the loss of *Plin4* to disturbances in hepatic lipid accumulation and WAT inflammation, linking *Plin4* function to metabolic health and obesity-associated comorbidities.



YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

Location-dependent proteomics of the aortic arch from apolipoprotein E deficient mice

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Abstract

Atherosclerosis is a progressive inflammatory disease of the arteries characterized by endothelial dysfunction and the development of fatty and fibrous plaques. The disease affects specific sites of the vasculature, particularly branch points and curvatures, where the endothelium is subjected to blood flow disturbances. Mouse models of atherosclerosis are a valuable tool to understand disease mechanisms and test the effects of drugs but present certain challenges including limited amounts of tissue. However, recent technological advances in mass spectrometry have allowed us to carry out proteomics on specific regions of individual murine aortas.

Aortic arches were isolated from apolipoprotein E deficient (ApoE^{-/-}) mice fed either a Western diet or regular chow for 16 weeks. Atherosclerotic plaques were dissected from the three major branches and inner curvature of the aortic arch together with some healthy-looking aortic regions. Proteins were extracted, enzymatically digested, and subjected to liquid chromatography tandem mass spectrometry.

We detected ca. 4000 proteins in the plaques and 2500 proteins in the healthy aortic samples, consisting of down to only a few milligrams of tissue. Key atherosclerosis markers, such as CD68, VCAM-1, ICAM-1, collagen type III and fibrinogen, were more abundant or only detected in plaques compared to healthy aorta. Principal component analysis showed that samples cluster according to their disease status and anatomical location within the aortic arch, indicating that they have distinctly different proteomes. Aortas from mice fed a Western diet versus regular chow, reflecting different stages of atherosclerosis, also displayed significant differences in protein expression. Gene ontology term enrichment revealed that many of these proteins are involved in inflammatory processes like neutrophil activation and platelet degranulation. These studies provide a better understanding of the molecular mechanisms underlying atherosclerosis.

Uncovering the role of liver lymphatic vessels in MASLD using hyperlipidemic mouse models with disturbed lymphatic development

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most widespread global chronic liver disease. In MASLD, excessive amounts of lipids accumulate into hepatocytes due to dysregulated lipid and glucose metabolism that may lead to steatohepatitis and cirrhosis. MASLD increases morbidity and mortality, not only due to liver complications but also through the increased risk for cardiovascular diseases. In addition to the urgent need for effective therapies, the mechanisms of progressive MASLD are still unclear. Liver lymphatics have remained an understudied area, despite their clear involvement in hepatic physiology. Liver lymphatic vessels (LVs) are involved in the immune system and transport of lipids and several proteins. Increased LV density has been reported in liver pathologies like cirrhosis. Also, hyperlipidemias impair the permeability of liver sinusoidal and lymphatic endothelium. Vascular endothelial growth factor receptor 3 (VEGFR3) is mostly expressed in lymphatic endothelial cells (LECs), regulating LEC identity and lymphangiogenesis. Here, we aim to investigate the effect of VEGFR3 signaling on liver lymphatic vasculature and the development of MASLD. Mice with an inactivating point mutation of VEGFR3 (*Chy*) were crossbred with *Ldlr*^{-/-}/*ApoB*^{100/100} mice and fed regular chow or high-fat diet (HFD) for 6 weeks. We found that HFD increased the expression lymphangiogenesis-promoting genes in the liver of *Ldlr*^{-/-}/*ApoB*^{100/100} mice. *Chy* x *Ldlr*^{-/-}/*ApoB*^{100/100} mice, with mildly dilated liver LVs, developed severe hypercholesterolemia on both regular chow and HFD. Moreover, increased hepatic steatosis and massive inflammation in hepatic portal area near LVs was detected pointing to leaky LVs and impaired lipoprotein transport. To conclude, hyperlipidemia activates liver lymphangiogenic signaling, which seems to be critical for the maintenance of liver lipid and inflammatory homeostasis, also providing new perspective to the pathogenesis of MASLD.

The Role of Gasdermins in Atherosclerotic Plaque Destabilization: Gasdermin D and E – A Murder Mystery

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Abstract

Atherosclerosis is a chronic and slowly progressing vascular disorder characterized by inflammation and lipid deposition in the arterial wall. As atherosclerosis progresses, the atherosclerotic plaque may develop an unstable phenotype and rupture, resulting in acute and persistent cardiovascular events. Cell death is a prominent feature of advanced plaques that significantly impacts atherosclerosis and plaque destabilization. Morphological studies indicate that the majority of dying cells in advanced human atherosclerotic plaques undergo necrotic cell death, making necrosis an important research target to stabilize rupture-prone plaques.

Gasdermins have recently been identified as essential effector molecules in different types of programmed necrosis through the formation of plasma membrane pores. Besides the canonical caspase-1/inflammasome pathway that activates gasdermin D (GSDMD), gasdermin E (GSDME) was recently identified as an alternative key executioner of programmed necrosis after cleavage by caspase-3. Given that immunohistochemical staining of advanced human plaques showed the presence of both gasdermin D and E in the area surrounding the necrotic core, we hypothesize a crucial role of gasdermin D and E-mediated necrosis in atherosclerotic plaque destabilization.

To determine the impact of gasdermin D and E deficiency on atherosclerosis, male and female ApoE^{-/-}, ApoE^{-/-} GSDMD^{-/-}, ApoE^{-/-} GSDME^{-/-}, and ApoE^{-/-} GSDMD^{-/-} GSDME^{-/-} mice were fed a Western-type diet for 16 weeks. Atherosclerotic plaques of the proximal aorta and the brachiocephalic artery are currently examined through histological stainings and spatial transcriptomics. To further elucidate the molecular mechanisms driving gasdermin D and E-mediated necrosis in atherosclerosis, bone marrow-derived macrophages and vascular smooth muscle cells of wild type, GSDMD^{-/-}, GSDME^{-/-}, and GSDMD^{-/-} GSDME^{-/-} mice are treated with cell death triggers commonly found in the atherosclerotic plaque environment.

Insights into the role of NPC2 in vascular inflammation and atherosclerosis

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Abstract

The Niemann-Pick C2 (NPC2) protein is a key regulator of intracellular cholesterol trafficking. Mutations in this gene can cause high levels of cholesterol accumulation within cells and severe neurological symptoms. It is well known that cholesterol is a trigger for vascular inflammation, promoting the development of atherosclerosis and cardiovascular disease. Whether NPC2-mediated cholesterol trafficking plays a role in atherogenesis remains unknown.

In this study, we used publicly available transcriptome and scRNA-seq datasets from human atherosclerotic tissues to investigate associations between *NPC2* and inflammatory- and plaque stability-related genes. Moreover, we evaluated the pro-atherogenic response of *Npc2* deletion in mouse bone marrow-derived macrophages (BMDMs) and siRNA-mediated ablation in human smooth muscle cells (SMCs).

GTEX dataset analysis showed that *NPC2* is highly expressed in the human healthy aorta and coronary arteries. RNAseq datasets from human carotid plaques revealed that *NPC2* levels are positively correlated with pro-inflammatory and negatively correlated with plaque-stabilizing genes. Interestingly, an integrative analysis of scRNA-seq data from coronary and carotid plaques shows that macrophages are the primary cell population expressing *NPC2* in the arterial wall. Despite lower expression, *NPC2* was also found to be significantly regulated in SMCs. In culture experiments, we found that BMDMs from *Npc2*^{-/-} mice release increased levels of TNF, while IL-6 and IL-1b remain unchanged after LPS stimulation. *NPC2* ablation, with siRNA, in cultured human SMCs led to an increased secretion of IL-6.

We found that macrophages and modulated SMCs express *NPC2*, which is also associated with an unstable plaque gene expression profile. The fact that *NPC2* ablation induces pro-inflammatory responses in the investigated cell types suggests that they could play an important role in atherogenesis.

Heterogenous phenotypic modulation of smooth muscle cells by mechanical stretch and TNF stimulation.

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Abstract

Background

Vascular smooth muscle cells (SMCs) are highly plastic and respond to changes in their local environment by phenotypic modulation. Several of these alternative phenotypes contribute to atherosclerosis progression. Atheroprone areas, such as curvatures and bifurcations, show alterations in the mechanical forces caused by blood flow dynamics and elevated inflammation driven by lipid accumulation.

Methods

To investigate SMC responses to mechanical stretch mimicking healthy or diseased arteries, we cultured human aortic SMCs on flexible silicon membranes coated with collagen-I, fibronectin, or laminin. SMCs were subjected to static conditions (0% elongation), physiological stretch (10% elongation), or pathological stretch (15% elongation) at 1 Hz for 6 hours. Bulk RNA-sequencing was used to study the transcriptomic response, and an NF- κ B reporter was used to assess SMC inflammatory signaling in response to stretch or tumor necrosis factor (TNF).

Results

RNA-sequencing revealed that static conditions or pathological stretch promoted inflammatory gene expression (e.g. *CCL2*, *ICAM1*, *VCAM1*), while physiological stretch upregulated some contractile genes (e.g. *CNN1*, *TAGLN*) and could attenuate TNF-induced inflammation, thus suggesting a protective effect. Surprisingly, TNF only modestly increased the number of NF- κ B-positive cells, whereas stretch showed minor effects. Additionally, we observed a difference depending on the SMC size, as larger cells were more prone to NF- κ B activation compared to smaller SMCs. These findings suggest that only a subset of SMCs drives the regulation of inflammatory gene expression.

Conclusion

Our results show that mechanical stretch affects SMC phenotypic modulation, and that physiological stretch suppresses inflammatory signaling, which is a key driver in atherosclerosis. While TNF activated NF- κ B signaling in just a subset of SMCs, stretch had limited effects, suggesting that this subset may drive the overall inflammatory response.



**Posters – Abstracts –
Cardiovascular Disease**

SESSION II

Efficacy and safety of short-term S100A8/A9 blockade in a myocardial ischemia-reperfusion injury porcine model

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Abstract

Myocardial infarction is one of the leading causes of death and disability worldwide. In addition to ischemic damage to the tissue occurring during the coronary blockage, the vital re-establishment of blood flow into the myocardium also contributes to the insult, ultimately resulting in ischemia-reperfusion injury. Among the many inflammatory mediators participating in this process is S100A8/A9, secreted in large amounts mainly from neutrophils during the acute inflammatory phase post-infarction, intensifying the immune reaction and extending the preliminary damage further. Early intervention in the inflammatory period could be an efficient therapeutic avenue to limit infarction size and prevent further complications, like the onset of heart failure. To study the inflammatory environment in the heart after ischemia and reperfusion, we established an acute ischemia-reperfusion porcine model by temporarily occluding the left anterior descending coronary artery of Finnish landrace swine and treated the animals with a novel drug, a specific S100A8/A9 blocker ABR-238901. We analyzed structural and functional changes with echocardiography and CMR-imaging and studied the systemic safety of the drug with extensive blood sampling. At the 1-month endpoint, there were no significant differences in heart function between the groups, but a trend towards more preserved ejection fraction and smaller myocardial true area at risk were observed in the treatment group. A longer follow-up time could therefore be beneficial to further highlight the therapeutic efficacy of S100A8/A9 inhibition after ischemia-reperfusion injury. Measured liver and kidney parameters did not raise any safety concerns associated with the drug, and could therefore be deemed safe for further studies in large animals.

A dual MAGL/HSL inhibitor showing therapeutic efficacy in dyslipidemic APOE*3-Leiden.CETP mice

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Abstract

Background: Monoacylglycerol lipase (MAGL) and hormone sensitive lipase (HSL) catalyze lipolysis in adipose tissue through hydrolyzing monoacylglycerol (MG) and diacylglycerol (DG), respectively. In addition, MAGL is responsible for degradation of 2-arachidonoyl glycerol (2-AG) into arachidonic acid, the precursor for prostaglandins and other inflammatory mediators. Whilst HSL inhibitors show potency in lowering circulating lipid levels, less is known about the effect of MAGL inhibitors. Here we evaluated the effects of MAGL inhibition compared to dual MAGL/HSL inhibition on lipoprotein metabolism in APOE*3-Leiden.CETP mice, a well-established model for human-like lipid metabolism.

Methods: Female APOE*3-Leiden.CETP mice were fed a Western-type diet and received a daily injection with the selective MAGL inhibitor JR-045 (30 mg/kg), a dual MAGL/HSL inhibitor LEI-515 (30 mg/kg), or vehicle for 3 weeks. Food intake and body weight were measured weekly. Body composition, plasma lipid levels, and lipoprotein kinetics were measured at endpoint.

Results: Food intake, body weight and body composition were not different between the groups. Treatment with the dual MAGL/HSL inhibitor LEI-515, but not by the MAGL inhibitor JR-045, strongly attenuated adipose tissue lipolysis evidenced from a significant reduction in plasma free fatty acids (-41%) and glycerol (-52%) compared to vehicle treatment. In addition, LEI-515 treatment led to lower plasma levels of triglycerides (-58%) and total cholesterol (-73%), possibly as a result of a reduced flux of fatty acids to the liver for incorporation into VLDL. Uptake of triglyceride-derived fatty acids by adipose tissues and heart was also elevated in LEI-515 treated mice.

Conclusion: Dual MAGL/HSL inhibition, but not MAGL inhibition alone, shows therapeutic potential in dyslipidemic APOE*3-Leiden.CETP mice. In future studies we will address the question the role of dual MAGL/HSL inhibition in the treatment of atherosclerosis.

Regulation of Endothelial Lipase by Angiotensin-like Protein 3

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Abstract

Cardiovascular diseases continue to be a leading cause of mortality worldwide, with dysregulated lipoprotein metabolism playing a crucial role. Efficient processing of triglyceride-rich lipoproteins (TRLs) within blood capillaries is vital for supplying dietary lipids for energy metabolism in the heart and skeletal muscle, and for storage in white adipose tissue. Endothelial lipase (EL), a member of the triglyceride-lipase family, exhibits low triglyceride lipase activity but high phospholipid lipase activity, allowing it to hydrolyze phospholipids on high-density lipoprotein (HDL). In this study, we aim to elucidate the mechanisms by which angiotensin-like protein 3 (ANGPTL3), a liver-secreted glycoprotein, regulates EL and impacts HDL metabolism and cardiovascular disease. While ANGPTL3 is known to inhibit both lipoprotein lipase (LPL) and EL activity, its mode of action on EL, including protein stability and inhibition mechanisms, remains poorly understood.

We utilized purified recombinant human EL and assessed its phospholipase activity and thermal stability with and without ANGPTL3 and we aim to investigate its spontaneous unfolding using HDX-MS). Comparing these trajectories with those of LPL will provide insights into the evolution of their unstable α/β -hydrolase folds. By investigating the mechanism through which ANGPTL3 inhibits EL activity and its subsequent effects on HDL function and metabolism, we aim to enhance our understanding of lipoprotein regulation and its implications for metabolic diseases.

Comprehensive lipid and metabolite profiling of long-term survivors of type 1 diabetes compared to healthy controls: cross-sectional results from the Dialong study in Norway

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Abstract

A subgroup of adults with type 1 diabetes (T1D) have coronary arteries free from atherosclerosis, even after living with hyperglycemia for many decades. The mechanism for this cardioprotective effect is unknown. However, it could be related to plasma metabolites. Therefore, we aimed to characterize plasma lipid and metabolite profiles among individuals with long-term T1D compared to healthy controls.

We used data from the cross-sectional Dialong study, of patients that had lived with T1D at least 45 years. Plasma metabolites were measured using a high-throughput NMR spectroscopy platform. Atherosclerosis was assessed by CT coronary angiography. We used linear regression to analyze the metabolite differences between long-term T1D patients and healthy controls, and explored mechanisms related to atherosclerosis, and glucose- and lipid-related biomarkers.

We included 163 subjects (53% females), with mean (SD) age 62 (7) years, of which 102 had T1D. Compared to healthy controls, T1D patients had lower levels of all apoB-containing lipoprotein subclass particles and associated lipids, as well as lower VLDL size, S-HDL-P, histidine, albumin and the inflammation marker GlycA; T1D patients also had higher levels of most apoA1-containing lipoprotein subclass particles and associated lipids, and HDL size, ketone bodies and glycine. These metabolic changes in T1D were predominantly associated with LDL-C, HDL-C, and TG, but not with glucose or HbA1c. Atherosclerosis was higher among T1D; however, atherosclerosis was weakly associated with the lipid and metabolic changes observed in T1D, and strongly associated with age, male sex, HbA1c, and statin and antihypertensive therapy.

Patients with long-term T1D had lower levels of atherogenic lipoprotein particles in plasma likely resulting from selection bias due to survival. Although poor glucose control was associated with atherosclerosis, our data underscore the importance of lipid lowering for long-term survival in diabetes.



YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION II

Characterization of isocitrate dehydrogenase (IDH)-associated macrophage responses in atherosclerosis

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Abstract

Macrophages play a pivotal role in all stages of atherosclerosis, impacting plaque growth and stability. Isocitrate dehydrogenase (IDH) isoenzymes have been linked to the regulation of inflammation by influencing the tricarboxylic acid cycle (TCA), which in turn influences various intracellular signaling pathways. Whether IDHs play a role in vascular inflammation and atherogenesis remains unknown.

This study investigated the role of IDH1 and IDH2 isoenzymes in macrophage pro-atherogenic responses. Publicly available datasets were analyzed to assess IDH expression profiles in relevant human vascular tissues and cells. Additionally, pharmacological inhibition of IDH1 (DS-1001b) and IDH2 (AGI-6780) was used to evaluate their effects on bone marrow-derived macrophage (BMDM) activation and polarization in vitro.

Analysis of the Human Protein Atlas and GTEx data revealed that *IDH1* is primarily expressed in macrophages, while *IDH2* can be found in both macrophages and smooth muscle cells in non-diseased arteries. Analysis of scRNA-seq data from atherosclerotic plaques showed again high *IDH1* expression in macrophages, while *IDH2* expression was also high in NK cells and T cells, with both isoenzymes being upregulated in unstable compared to stable plaques. In vitro, we found that DS-1001b and AGI-6780 significantly reduce TNF and IL-1 β release by LPS-stimulated BMDMs in a dose-dependent manner. Further analysis of BMDM cultures showed that DS-1001b and AGI-6780 increased CD206 protein expression and *Arg1* mRNA, respectively, both markers of anti-inflammatory macrophages, suggesting IDH1 and IDH2 can also play a role in regulating macrophage phenotypes.

We show that IDH1 and IDH2 are associated with macrophage-mediated responses in human atherosclerosis. Furthermore, our in vitro experiments suggest that targeting IDH-mediated metabolism could be a promising strategy for modulating vascular inflammation and combating atherosclerosis.

Taurine-conjugated fatty acid metabolites reduce atherosclerosis in LDLR^{-/-} mice

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Abstract

Aim

Omega-3 fatty acids (ω 3-FA) are recognised for their effects on cardiovascular health, such as reducing plasma triglyceride (TG) levels, hypertension and risk of ischemic heart disease. ω 3-FA give rise to beneficial molecular effects, e.g. reduced inflammation and reduced oxidative stress. The exact mechanism by which ω 3-FA exert their functions are however unknown. A potential mediator of the effects of ω 3-FA may be ω 3-FA derived N-acyl taurines (NATs). NATs are evolutionarily conserved from crayfish to humans, and are present in bile, blood and tissues. This project aims to assess the effects of ω 3-FA derived NATs (ω 3-NAT) on various cardiometabolic outcomes, e.g. atherosclerosis.

Methods

The atherogenic mouse model, low-density lipoprotein receptor knock out mouse (LDLR^{-/-}), was crossed to our FAAH S268D mouse. FAAH S268D mice are unable to hydrolyse NATs specifically, which will accumulate in plasma and bile. The NAT species produced can be manipulated by providing specific dietary lipid species. LDLR^{-/-} x FAAH WT or S268D mice were fed western diet \pm ω 3-FA to induce atherosclerosis. After 14 weeks, mice were sacrificed, and blood and organs were collected. Atherosclerosis was assessed by measuring the plaque area in H&E-stained sections of the aortic root (7 sections per animal, 100 μ m apart).

Results

In a preliminary study, FAAH S268D mice fed ω 3-FA (i.e. high levels of ω 3-NAT) had a 64.3 % reduction in atherosclerotic plaque size, compared to WT mice also fed ω 3-FA (n=3). ω 3-NAT did not affect plasma lipid levels.

Conclusion

These preliminary data suggest that ω 3-NATs can reduce atherosclerosis in mice, but not due to a reduced level of plasma lipids. Further studies are necessary to determine the robustness of the finding, and to elucidate the mechanism by which the ω 3-NATs exert their effect on atherosclerotic plaque size.

Association of aerobic fitness and body composition with protein and major lipid class composition of high-density lipoprotein

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Abstract

Although high-density lipoprotein (HDL) has cardiometabolic protecting properties, interventions to raise HDL cholesterol concentration have failed to improve cardiometabolic health. Hence, HDL composition and functionality might be key factors in its anti-atherogenic capacity. Alterations in HDL composition have been linked to pathophysiological states, whereas endurance training is known to increase HDL concentration with shift towards bigger particle sizes, but its effect on the composition is not well understood. Therefore, we compared HDL protein and lipid composition and serum metabolic profiles between two groups differing in aerobic fitness level and body composition using mass spectrometry, thin-layer chromatography and nuclear magnetic resonance spectroscopy methodologies.

High aerobic fitness and normal body composition were associated with elevated proportion of cholesteryl esters suggesting improved cholesterol metabolism. Low aerobic fitness and high body fat percentage increased the proportion of phospholipids in HDL, while elevated proportion of triacylglycerols was associated only with high body fat percentage, suggesting modulations in surface fluidity and tendency for particle catabolism. Serum metabolic profiles supported the observations highlighting differences in the concentration of large HDL₂ particles, very-low-density lipoprotein (VLDL) and serum triacylglycerols between the groups. Low-grade inflammation due to high body fat percentage increased proteins related to inflammation, immunity and hemostasis process in HDL, whereas normal body composition was linked to increased amounts of apolipoprotein A-II and C-II suggesting improved HDL structure. Results suggest that high aerobic fitness and normal body composition have positive effects on HDL composition through reduced inflammation and better serum lipid homeostasis.

Impaired anti-inflammatory capacity of high-density lipoprotein is associated with high residual cardiovascular risk in coronary artery disease

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Abstract

Background: Coronary artery disease (CAD), a leading cause of morbidity and mortality worldwide, is closely related to atherosclerosis and inflammatory processes. High-density lipoprotein (HDL) is acknowledged for its protective role against atherosclerosis. This study explored the association between HDL anti-inflammatory function and recurrent major adverse cardiovascular events (MACE) in CAD patients.

Methods: A prospective study was conducted in 466 CAD patients from South China and stratified into tertiles based on their baseline anti-inflammatory capacity of HDL, determined as the activity to suppress vascular cell adhesion molecule-1 mRNA expression stimulated by tumor necrosis factor- α in endothelial cells in vitro.

Results: Over a median follow-up of 2.34 years, 85 (18.2%) patients experienced MACE. HDL anti-inflammatory capacity was significantly reduced in participants with recurrent MACE and was inversely associated with residual cardiovascular risk in a multivariable-adjusted model (hazard ratio [HR]: 0.94, 95% CI: 0.90-0.98). Subgroup analysis revealed a notable protective effect of HDL anti-inflammatory function in females (adjusted HR: 0.70, 95% CI: 0.53-0.93). When divided into tertiles, there was a 61% reduction in recurrent MACE among patients in the highest tertile of HDL anti-inflammatory capacity compared to reference group in a fully adjusted model (HR: 0.39, 95% CI: 0.18-0.82). Moreover, HDL anti-inflammatory capacity was inversely correlated only with estimated glomerular filtration rate, but not with other cardiovascular risk factors including HDL cholesterol and high-sensitivity C-reactive protein (hsCRP). Adding HDL anti-inflammatory capacity to traditional risk factors or the Framingham risk score improved both discrimination and reclassification indices for MACE prediction.

Conclusion: Impaired HDL anti-inflammatory capacity increased residual cardiovascular risk during follow-up in CAD patients, irrespective of HDL-C levels and hsCRP.

SGLT-i Modify Exosomal Protein Composition: A potential Mechanism for Cardioprotective Effects

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Abstract

Sodium-glucose cotransporter inhibitors 2 (SGLT-2i) are widely used in diabetes treatment. Beyond their glucose-lowering effects, they provide broader benefits, including significant cardioprotective effects. SGLT-i help manage heart failure and reduce cardiovascular complications. However, the precise mechanisms underlying these additional effects remain unclear.

Exosomes, small membrane vesicles all cells produce, play a critical role in intercellular communication. We hypothesize that SGLT-i influence exosomal composition, which could be one of the mechanisms underlying their beneficial effects.

To explore this hypothesis, we conducted *in vitro* experiments on HepG2 hepatocytes and HEK293T kidney cells treated with SGLT-2i (dapagliflozin, empagliflozin) and a dual SGLT-1/2 inhibitor (sotagliflozin). Exosomes were isolated from the culture media of treated cells and analysed through proteomic profiling using mass spectrometry and Nanoparticle Tracking Analysis (NTA).

Proteomic analysis revealed significant beneficial changes in exosomal protein composition following SGLT-i treatment. Specifically, we observed an enrichment of ribosomal proteins implicated in cell cycle regulation, stress response, and processes associated with various diseases. Additionally, increased levels of mitochondrial proteins were detected, suggesting that SGLT-i promote the release of mitochondria-containing exosomes, which have been linked with reduced inflammation and improved heart function. We further noted a reduction in transforming growth factor- β content, supporting the attenuation of fibrotic processes. NTA confirmed that treatment with SGLT-i did not affect the quantity, size, or distribution of exosomes.

Our results suggest that SGLT-i treatment positively modifies exosomal protein cargo, potentially revealing a novel mechanism underlying their cardioprotective effects.

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Posters – Abstracts –
Lipoproteins and Lipid Transport

SESSION III

Circulating ApoC-III glycoforms identifies patients with metabolic dysfunction-associated steatotic liver disease independently of type 2 diabetes.

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Abstract

Background and Aims: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a spectrum of diseases ranging from simple steatosis to more aggressive forms of liver disease and is a major risk factor for cardiovascular disease (CVD). Oxidised apolipoproteins (ApoA and ApoB) are associated with CVD, yet less is known about the oxidation status of other apolipoproteins and their association with insulin resistance (IR) and MASLD. To fill this gap, we characterised the circulating serum peptidome of MASLD patients and healthy volunteers (CTRL).

Methods: We studied 87 biopsy confirmed patients with MASLD and 20 CTRL, matched for age and sex. We first employed an untargeted LC-MS peptidomics approach (9 CTRL, 32 MASLD) and then targeted peptidomics (87 MASLD and 20 CTRL) to validate the key findings.

Results: Untargeted serum peptidomics highlighted oxidised apolipoprotein peptide fragments as the key difference between MASLD and CTRL. Focusing on intact ApoC3 oxidative status by studying its major glycoforms (ApoC30, ApoC3i, and ApoC3ii), we observed that the ratios of oxidised to non-oxidised ApoC3 glycoforms were higher in MASLD vs. CTRL (irrespective of type 2 diabetes, used as a covariate), and significantly correlated with BMI, IR, TG, HDL-C, and transaminases of the patients. Lastly, these ratios displayed almost perfect discriminatory capabilities (ROC curve >0.9) between CTRL and MASLD. These results suggest that the concentration of these peptides might be associated with peripheral organ dysfunction and, if confirmed in other cohorts, might serve as a candidate biomarker for MASLD.

Combining comprehensive cardiometabolic risk biomarker panels with machine learning to predict survival in severe COVID-19 patients admitted to the intensive care unit

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Abstract

Background

Severe COVID-19 infections have been linked to alterations in lipid and lipoprotein metabolism possibly translating into increased mortality, although mechanistic insights into the metabolic pathways involved are lacking.

Objective

The current study aimed to comprehensively characterize contemporary cardiometabolic risk biomarkers and to explore their potential for predicting outcomes of severe COVID-19 in patients admitted to the intensive care unit (ICU).

Methods

Blood samples of 94 subsequent patients with severe COVID-19 (78 surviving, 16 died) were collected within 24 hours after ICU admission. An extensive panel of lipoprotein subclasses and relevant metabolites was analyzed by nuclear magnetic resonance spectroscopy, activities of cholesteryl ester transfer protein (CETP) as well as phospholipid transfer protein (PLTP) were measured, and cholesterol efflux capacity (CEC, from THP-1-derived macrophage foam cells) determined.

Results

There were no discernible changes in standard lipid profiles and lipoprotein sizes between survivors and non-survivors, however, lipoprotein-X (LPX) and -Z (LPZ) were present in a large proportion of patients. Non-survivors displayed significantly lower levels of large HDL ($p=0.02$), including H7P ($p=0.04$) and H6P ($p=0.05$), as well as small HDL ($p=0.04$), accentuated by an absence of the smallest particles (H1P) in most samples. CETP and PLTP activities as well as CEC were similar in survivors and non-survivors and significantly correlated with multiple lipoprotein parameters, supporting their perceived biological actions. Unsupervised machine learning identified a unique metabolic signature predisposing COVID-19 patients to substantially reduced survival ($p<0.05$).

Conclusion

In-depth lipoprotein profiling reveals major shifts exclusively within HDL subclasses in non-surviving COVID-19 patients. Using machine learning, comprehensive metabolic profiling can be leveraged to predict survival rates of severe COVID-19 patients.

Reduced lipid synthesis and oxidation but upregulated mitochondrial responses in cultured myotubes established from AMPK α 2^{-/-} mice

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Abstract

Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) plays a crucial role in regulation of metabolic homeostasis. To study the role of the catalytic α 2 subunit of AMPK in skeletal muscle energy metabolism, myotube cultures were established from AMPK α 2^{+/+} and AMPK α 2^{-/-} mice. Myotubes from AMPK α 2^{-/-} mice had lower basal fatty acid and glucose oxidation compared to myotubes from AMPK α 2^{+/+} mice. However, the relative response to mitochondrial uncoupling was increased for fatty acid oxidation. Acutely added glucose extinguished the difference between AMPK α 2^{+/+} and AMPK α 2^{-/-} myotubes on fatty acid metabolism. Incorporation of acetate into lipids was also lower in myotubes from AMPK α 2^{-/-} mice. Proteomics analysis revealed that AMPK α 2^{-/-} myotubes had upregulated pathways related to mitochondrial function and fatty acid oxidation, and decreased pathways related to fatty acid and cholesterol biosynthesis. Our data suggest that AMPK α 2 subunit may play important roles in skeletal muscle energy substrate utilization, mitochondrial uncoupling, metabolic flexibility and lipid synthesis.

In press: Reduced lipid and glucose oxidation and reduced lipid synthesis in AMPK α 2^{-/-} myotubes. Archives of Physiology and Biochemistry. DOI: 10.1080/13813455.2024.2449409

Deciphering Atherosclerosis in Type 2 Diabetes: A Novel Exploration of Biomarkers in Interstitial Fluid

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Abstract

Background

Interstitial fluid (IF), a dynamic medium within anatomical compartments plays a pivotal role in vessel wall health. Our previous investigations revealed relationships between IF composition and atherogenic lipoprotein retention in the vascular wall of patients with Type 2 Diabetes (T2D), a population notably burdened by atherosclerosis. Given the unique proximity of IF to cells participating in early atherogenesis, we aim to determine whether inflammatory biomarkers in this bio-fluid could elucidate mechanisms governing lipoprotein turnover and vascular health in T2D.

Methods

Serum and IF from abdominal skin blisters were collected after an overnight fast from 29 T2D patients and matched controls. Lipoprotein lipids were quantified using FPLC. LDL properties were evaluated through ex vivo binding to human aortic proteoglycans and their aggregation susceptibility. Proteomics analyses in IF were conducted employing the Olink® Target Inflammation panel. The resulting data were analyzed using a topological approach integrating network analysis with lipoprotein and physiological characteristics.

Results

In T2D patients, several inflammatory biomarkers linked to cardiovascular disease and markers associated with human atherosclerotic lesions were elevated. Network analysis revealed positive correlations between pro-inflammatory biomarkers and metabolic parameters related to T2D, including fasting blood glucose and HbA1c. In contrast, inverse correlations were found between less aggregation-prone LDL and several inflammatory biomarkers in T2D, which were not present in controls.

Conclusions

We can identify distinct pathways involved in lipoprotein retention and inflammation by analyzing data from both peripheral serum and the rarely studied interstitial fluid. This unique approach provides valuable insights into potential targets for future diagnostic and therapeutic strategies, underscoring the importance of IF in understanding atherosclerosis.

Trafficking of LDL-derived cholesterol by combined live-cell imaging and lipid mass spectrometry of novel fluorescent cholesteryl esters

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Abstract

Low density lipoprotein (LDL) is the major carrier of cholesterol to peripheral cells, but our understanding of intracellular trafficking of LDL-derived cholesterol is scarce. This is primarily due to lack of suitable probes for cholesteryl esters (CEs), whose subcellular fate can be monitored within living cells. We present a novel approach based on combined live-cell fluorescence imaging and lipid mass spectrometry of cholestatrienol (CTL) esters and ethers, differing from the natural sterol lipids only by two additional double bonds in the steroid ring system. We show that uptake of LDL particles containing CTL-oleate ester is followed by their transport into late endosomes and lysosomes (LE/LYSs). Exit of CTL from LE/LYSs is observed upon hydrolysis of CTL-esters by acid lipase, while CTL-ethers remain in endo-lysosomes. Using lipid mass spectrometry (Lipid-MS), we measure a sigmoidal time course of CTL-ester hydrolysis matching the transport kinetics of fluorescent LDL to LE/LYSs, as shown by mathematical modeling. We also find re-esterification of CTL liberated from hydrolyzed CTL-ester starting after 6h of incubation. At the same time, we observe extensive contact formation of endo-lysosomes containing LDL with the endoplasmic reticulum (ER). These results support a model of direct cholesterol transport from LDL within LE/LYSs to the ER. Our novel approach allows for direct observation of post-endocytic trafficking of LDL-derived cholesterol in various cell types.



YIA Poster Walk – Abstracts –
Lipoproteins and Lipid Transport

SESSION III

MARC1 downregulation reduces hepatocyte lipid content by increasing *beta*-oxidation.

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Abstract

Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common disorder of liver and internal medicine. It has a significant genetic component, with a common missense variant (rs2642438) in the mitochondrial amidoxime-reducing component 1 (*MARC1*) gene providing protection against the onset and severity of MASLD. This variant causes an amino acid substitution from alanine (A) to threonine (T) at position 165, leading to decreased protein stability and increased proteasomal degradation. However, the mechanisms behind this protective effect are with contrasting results, and thus needs thorough investigation.

Methods: We used short interfering RNA (siRNA) to downregulate *MARC1* in primary human hepatocytes (PHH). Neutral lipid content was measured using Oil-Red O staining, and *beta*-oxidation was measured with radiolabeled tracers. Additionally, RNA sequencing (RNA-seq) and proteomic analysis via LC-MS were performed. Data from 239,075 individuals in the UK Biobank were also analyzed.

Results: *MARC1* Downregulation reduced neutral lipid content in primary hepatocytes homozygous for the wild-type (p.A165) but not for the mutant (p.T165) protein. This reduction was linked to increased energy substrate utilization through *beta*-oxidation of fatty acids. Consistently, 3-hydroxybutyrate (a by-product of *beta*-oxidation) levels were higher in carriers of the rs2642438 protective allele from the UK Biobank, indicating higher *beta*-oxidation. Furthermore, *MARC1* p.A165 downregulation led to a more favorable phenotype by reducing ferroptosis and reactive oxygen species levels.

Conclusions: Downregulation of *MARC1* in carriers of the wild-type protein results in lower neutral lipid levels in hepatocytes due to increased *beta*-oxidation and activates beneficial pathways for cell survival. This study suggests that reducing hepatic *MARC1* expression may be beneficial for liver steatosis.

PXR agonist rifampicin elevates serum Lp(a) levels substantially

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Abstract

Aim:

Endocrine disruption may have an effect on the development of cardiovascular diseases. In this study, we investigated the effects of pregnane X receptor (PXR) agonism, a known mechanism of endocrine disruptors, on serum lipoprotein (a) [Lp(a)]. We have previously demonstrated that PXR activation induces cholesterol synthesis and elevates LDL cholesterol and PCSK9 levels.

Methods:

Data from three randomized, placebo-controlled cross-over trials, Rifa-1, Rifa-BP and Rifa-Stea, was analyzed, where 600 mg of rifampicin, an efficient PXR agonist, or placebo was administered once daily for one week, with at least a four-week wash-out period. The total number of participants included was 50. Serum Lp(a) was measured with an immunoturbidimetric assay. A two-tailed Wilcoxon's signed rank test was performed for the data regarding Lp(a), PCSK9 and 4 β -hydroxycholesterol.

Results:

Rifampicin administration increased the mean geometric ratio of serum Lp(a) as well as PCSK9 and 4 β -hydroxycholesterol, a marker of PXR activation, by 1.49-fold, 1.48-fold and 3.38-fold, respectively ($p < 0.001$). Median Lp(a) levels for the placebo and rifampicin periods were 55 mg/L (interquartile range 116.3 mg/L) and 90 mg/L (190 mg/l). The 95% confidence interval for the mean of difference between placebo and rifampicin periods for Lp(a) was 47.58 mg/L and 128.11 mg/L, respectively. Lp(a) was elevated significantly by rifampicin regardless of the baseline serum level.

Conclusions:

PXR agonism significantly increases the serum Lp(a) levels, which is a known independent risk factor for cardiovascular disease. Thus, PXR agonism and the consequently elevated serum Lp(a) may contribute to cardiovascular events and disease associated with endocrine disruptors. Our results suggest that, in addition to the genetics, that have been previously thought to be the primary Lp(a) determinant, exogenous factors affect Lp(a) levels significantly.

Functional Impact of *APOB* Variants in Familial Hypercholesterolemia

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Abstract

Familial hypercholesterolemia (FH) is an inherited condition of lipid metabolism characterized by increased levels of LDL cholesterol, and *APOB* variants are responsible for 5%-10% of FH cases. The majority of *APOB* variants are missense, but nonsense variants and small indels in exon 29 were also identified in individuals with FH phenotype and can be the cause of disease.

The aim of this project was to study functionally *APOB* variants identified in individuals clinical diagnosed with FH in our cohort.

LDL was isolated through sequential ultracentrifugation. CHO-IIdIA7 cells were transfected with wt LDLR plasmid and incubated with FITC-labeled LDL to determine LDL binding and uptake by flow cytometry. ED-LDLR fragments purified from HEK293 cells were incubated with the *APOB* variants and antibodies, to determine apoB affinity for LDLR by ELISA assay.

Recently we assessed 8 variants: p.(Gln4316*) presented reduced affinity for the LDLR, impairing the binding of apoB to LDLR; p.(Ala1393Val), p.(Asp1456Asn), p.(Met2042Thr), p.(Asp2213del), p.(Ile3374Thr), p.(Val4295Leu) and p.(Arg4519Thr) do not appear to impact apoB's binding to the LDL receptor.

Functional studies are essential for assessing the pathogenicity of genetic variants and are one of the key criteria for their classification. These analyses provide crucial data for creating personalized therapeutic strategies. Our goal is to increase the number of characterized variants, beginning with 15 more variants from the Portuguese FH Study.

Elucidating the degradation pattern of apolipoprotein B-100 from human carotid atherosclerotic plaques by N-terminal proteomics.

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Abstract

Apolipoprotein B-100 (ApoB) is the primary protein component of low-density lipoproteins (LDL). Under normal physiological conditions, LDL are taken up by cells through the tightly regulated LDL receptor. However modified LDL are taken up by scavenger receptors in macrophage, and other cells, resulting in lipid accumulation and 'foam' cell formation, a signature driver of atherosclerosis. Intracellular degradation of ApoB occurs within lysosomes, and primarily by cathepsins, though other proteases, including extracellular species may contribute. The contributions of different proteases within human plaques is however not well understood.

Here we report a novel proteomic/degradomic approach to investigate the nature of protein fragments from ApoB in human carotid atherosclerotic plaques. Plaques from 21 patients were morphologically classified and analyzed new N-termini fragments generated by proteases. 17814 N-terminal peptides were identified with 5735 unique peptides detected across all samples. 270 peptides were mapped as ApoB fragments covering 29% of the protein sequence, with the majority being significantly more abundant in soft/unstable plaques. The fragment sequences indicate a large numbers of proteolytic cleavage sites across the protein sequence with the C-terminal part of the beta-belt region contributing with the most fragments. Some fragments contained overlapping sequences consistent with multiple fragmentation events and exopeptidase activity. Sequence logo analysis of the amino acids around the cleavage sites shows Ser and Leu as the most common amino acids C-terminal of the cleavage site whereas Leu and Lys are found more frequently N-terminal. These sequences provide data on the likely proteases responsible for intra plaque ApoB cleavage.

In conclusion, we propose that N-terminal proteomics (degradomics), together with literature data can provide valuable insights into the degradation patterns of proteins within human atherosclerotic plaques.

The effect of SGLT2 inhibitors on human apoM levels in proximal tubular cells under LPS-induced cell damage.

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Abstract

Apolipoprotein M (apoM) is a member of the lipocalin protein family primarily expressed in the liver and kidneys. During sepsis, plasma apoM levels decrease. This can cause an impairment of the endothelial barrier function and increased risk of mortality.

The sodium-glucose linked transporter 2 (SGLT2) inhibitors promote glucose excretion via inhibition of the SGLT2 receptor in the kidney. In pilot studies, it has been demonstrated that SGLT2 inhibitors improve the otherwise decreased heart function seen in mice treated with lipopolysaccharide (LPS), a model for sepsis, and at the same time restore the plasma levels of apoM. Additionally, LPS treated proximal tubular epithelial (HK-2) cells showed decreased uptake of human apoM (hapoM) *in vitro*, which is restored by Dapagliflozin, suggesting that SGLT2 inhibitors might affect apoM turnover in the kidney. However, the specific effect of SGLT2 inhibitors on kidney derived apoM is elusive.

This study investigates the effects of LPS on hapoM production, secretion and uptake in HK-2 cells overexpressing hapoM (HK-2-hapoMTG), and address whether Dapagliflozin can restore the apoM dynamics in this setting. HK2-hapoMTG cells were cultured in serum-free medium on transwell membranes. LPS treatment induced an increase in the hapoM levels in the cell culture medium of both the apical and the basolateral compartment. However, the intracellular protein levels and expression of hapoM remained unchanged. Dapagliflozin treatment increased the levels of hapoM, directly produced by the cells, both intracellularly and in the basolateral compartment. Our data suggests that LPS treatment affects hapoM uptake in the kidney, and that this is modified by Dapagliflozin treatment. This could suggest that Dapagliflozin at least partly mediates enhanced plasma hapoM levels by affecting hapoM in the kidney directly. Further studies are needed to clarify this.

The impact of sex hormones and sex chromosomes on the HDL anti-inflammatory activity

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Abstract

Interest in the cardiovascular disease (CVD) field is shifting from high-density lipoprotein cholesterol (HDL-C) levels to exploring functional anti-atherogenic properties of HDL particles. Among these, the anti-inflammatory capacity of HDL has emerged as a key function metric associated with protection against incident CVD events. Notably, this association exhibits substantial sexually dimorphic effects. The present study aimed to investigate the underlying mechanisms, focusing on the roles of sex hormones and sex chromosomes in modulating the HDL anti-inflammatory capacity.

The HDL anti-inflammatory activity (HDL-mediated suppression of VCAM-1 mRNA induction in endothelial cells) was determined in 14 transmen and 17 transwomen during gender-affirming hormone therapy at T0 (baseline), T1 (hormonal castration) and T12 (following 11 months of gender-affirming hormone therapy). Extended HDL profiling was carried out using nuclear magnetic resonance spectroscopy, and a comprehensive targeted lipidomics analysis of HDL was performed using liquid chromatography coupled with mass spectrometry.

The HDL anti-inflammatory capacity adjusted for HDL-C and HDL particle numbers increased at T1 in both groups ($p < 0.05$) and remained otherwise largely unaltered. HDL-C and total HDL particle numbers did not correlate with the HDL anti-inflammatory activity but, interestingly, specific associations with distinct HDL subpopulations emerged ($p < 0.01$). Increases in HDL core lipids were related to a worse HDL anti-inflammatory function ($p < 0.05$), while increases in specific phosphatidylcholines, -serines and -ethanolamines had the opposite effect (each $p < 0.05$). In conclusion, these data indicate that (i) neither sex chromosomes nor substantial changes in sex hormones have a fundamental impact on the HDL anti-inflammatory function and (ii) specific HDL lipids associate with the anti-inflammatory function of HDL, potentially opening an avenue to therapeutically improve HDL functionality.

VLDL triglycerides and cholesterol in non-alcoholic fatty liver disease and myocardial infarction

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Abstract

Background

Myocardial infarction is a leading cause of death in individuals with non-alcoholic fatty liver disease (NAFLD). The two diseases share elevated very low-density lipoproteins (VLDL) carrying both triglycerides and cholesterol; however, in NAFLD mainly triglycerides accumulate in liver cells while in myocardial infarction mainly cholesterol accumulates in the atherosclerotic plaque. We hypothesized that VLDL triglycerides preferentially associate with risk of NAFLD, while VLDL cholesterol preferentially associates with risk of myocardial infarction.

Methods

We examined 25,428 individuals without clinically diagnosed NAFLD or myocardial infarction at baseline from the prospective Copenhagen General Population Study and followed these individuals for a mean of 10 years. VLDL triglycerides, VLDL cholesterol, and low-density lipoprotein (LDL) cholesterol were determined using nuclear magnetic resonance spectrometry.

Results

Continuously higher VLDL triglycerides were associated with continuously higher risk of NAFLD; however, this was not the case for VLDL cholesterol, LDL cholesterol, or apolipoprotein B. Continuously higher VLDL cholesterol, LDL cholesterol, and plasma apolipoprotein B were associated with continuously higher risk of myocardial infarction. Compared to individuals with both VLDL triglycerides and VLDL cholesterol $\leq 66^{\text{th}}$ percentile, the hazard ratios for NAFLD in individuals with VLDL triglycerides $>66^{\text{th}}$ percentile were 1.61(95% CI:1.25-2.06) at high VLDL cholesterol and 1.41(0.90-2.21) at low VLDL cholesterol. Hazard ratios for myocardial infarction in individuals with VLDL cholesterol $>66^{\text{th}}$ percentile were 1.51(1.36-1.67) at high VLDL triglycerides and 1.42(1.18-1.69) at low VLDL triglycerides.

Conclusions

VLDL triglycerides predominated in NAFLD while VLDL cholesterol predominated in myocardial infarction; however, VLDL cholesterol was also elevated slightly in NAFLD while VLDL triglycerides was also elevated in myocardial infarction.

Lipoprotein(a) Testing Among General Practitioners in Norway: Shaping the Future of Cardiovascular Risk Stratification

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Abstract

Background and aims: There is a growing focus on the role of lipoprotein(a) [Lp(a)] in cardiovascular disease (CVD) development. Despite its clinical relevance, Lp(a) testing remains uncommon worldwide. However, Norway stands out with a relatively high test frequency over the past 20 years, probably due to Lp(a) being discovered by the Norwegian scientist Kåre Berg in 1963, and the easiness of ordering a test. The majority of Lp(a) tests in Norway are conducted by the general practitioners (GPs). We aimed to investigate the prevalence of Lp(a) testing among Norwegian GPs, and to identify the clinical indications and consequences associated with its use.

Methods: Data were collected in May 2024 through an online questionnaire. The sample consisted of 100 Norwegian GPs.

Results: 63% of the GPs answered that they have tested for Lp(a) at least once. When asked to tick off the tests they usually order for cardiovascular risk assessment, 9% of GPs ticked off for testing Lp(a) in patients without CVD, 16% ticked off for testing in patients with established CVD, and 19% ticked off for both, while 56% did not tick off Lp(a) testing in this context. Among the 63% of GPs that had tested for Lp(a) at least once, the most common reason for testing Lp(a) was a family history of CVD (68% and 73% for patients with and without CVD, respectively) and the most common impact of an elevated Lp(a) test was intensification of preventive treatment for other risk factors (68% and 71% in patients with and without CVD).

Conclusion: A majority of the GPs test for Lp(a), primarily due to a family history of CVD, and take proactive measures for patients with elevated levels. Currently, approximately 1% of the Norwegian population undergoes Lp(a) testing annually. However, broader use of Lp(a) testing in cardiovascular assessment is needed, especially for patients with established CVD.



**Posters – Abstracts –
Other Topics**

SESSION IV

Dietary restriction of sulfur amino acid in humans has impact on serum free fatty acids and apelin gene expression in adipose tissue: findings from an 8-week randomized controlled trial

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Abstract

Dietary restriction of sulfur amino acids (SAA) such as methionine and cysteine has shown beneficial effects in rodents, particularly concerning lipid metabolism in the liver and adipose tissue, as well as improved lipoprotein profiles. However, human studies on sulfur amino acid restriction (SAAR) and its effects on lipid metabolism and lipoprotein profiles are minimal. This study aimed to assess the impact of SAAR on gene expression in adipose tissue, serum free fatty acids (FFA), and lipoproteins in a human trial. The trial was an 8-week, double-blind, randomized controlled study with 59 participants, aged 18-45 years and a body mass index (BMI) of 27-35 kg/m². Participants followed a base diet with capsules containing either maltodextrin (SAAR group) or methionine and cysteine (Control group). Blood samples and subcutaneous white adipose tissue (scWAT) biopsies were taken at baseline, week 4, and week 8. Gene expression analysis of scWAT showed a significant reduction in apelin gene expression in the SAAR group compared to controls at 8 weeks. While no significant changes were observed in lipoprotein profiles, there was a significant increase in plasma total FFA at week 4 in the SAAR group compared to control. Additionally, specific long-chained FFAs like stearic, linolenic, and α -linolenic acids increased significantly in the SAAR group by week 8. These outcomes indicate that SAAR may not alter lipoprotein profiles in a short time as 8 weeks but does lead to increase in some free fatty acids and reduced apelin gene expression in adipose tissue. Further research is highly necessary to fully understand SAAR's full impact on human metabolic health.

Competitive displacement of lipoprotein lipase from heparan sulfate is orchestrated by a disordered acidic cluster in GPIHBP1

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Abstract

The transport of lipoprotein lipase (LPL) to the capillary lumen from myocytes or adipocytes is essential for intravascular lipolysis and maintaining the plasma triglycerides homeostasis. Insufficient LPL activity in the capillary lumen leads to hypertriglyceridemia. Translocation of LPL across the endothelium is driven by electrostatic interactions with the disordered N-terminal tail of GPIHBP1, which has two acidic clusters at residues 5–12 and 19–30. This negatively charged tail acts as a molecular switch, facilitating the release of LPL from heparan sulfate proteoglycans (HSPGs) through electrostatic interactions. In genetically modified mice with a neutralized acidic tail, LPL gets trapped in the sub-endothelial spaces, causing hypertriglyceridemia.

Due to the disordered nature of the tail, the crystal structure of the LPL-GPIHBP1 complex does not elucidate the electrostatic interactions between LPL and GPIHBP1's acidic tail. In this study, zero-length crosslinking was implemented to map the acidic tail of GPIHBP1 onto LPL. It was found that carboxylates at positions 19–30 in GPIHBP1 interact with Lys445, Lys441, Lys414, and Lys407 near the interface of the C- and N-terminal domains of LPL. Modeling these interactions showed an extensive electrostatic interface involving both LPL domains, which explains how the acidic tail stabilizes LPL activity and conformation. Functional assays demonstrated that the acidic cluster at residues 19–30 is crucial for maintaining LPL activity, reducing ANGPTL4-mediated LPL inactivation, preventing PSCK3-induced LPL cleavage, and aiding in LPL extraction from HSPGs. These findings offer molecular insights into the electrostatic regulation and compartmentalization of LPL activity, which are vital for intravascular lipolysis and plasma triglyceride homeostasis.

Supplementation of seaweed extracts to the diet reduces symptoms of Alzheimer's Disease in the APPswePS1ΔE9 mouse model

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Abstract

We previously demonstrated that diet supplementation with seaweed *Sargassum fusiforme* (*S. fusiforme*) prevented AD-related pathology in a mouse model of Alzheimer's Disease (AD). Here, we tested a lipid extract of seaweed *Himantalia elongata* (*H. elongata*) and a supercritical fluid (SCF) extract of *S. fusiforme* that is free of excess inorganic arsenic. Diet supplementation with *H. elongata* extract prevented cognitive deterioration in APPswePS1ΔE9 mice. Similar trends were observed for *S. fusiforme* SCF extract. The cerebral amyloid-β plaque load remained unaffected. However, IHC analysis revealed that both extracts lowered glial markers in the brains of APPswePS1ΔE9 mice. While cerebellar cholesterol concentrations remained unaffected, both extracts increased desmosterol, an endogenous LXR agonist with anti-inflammatory properties. Both extracts increased cholesterol efflux and particularly *H. elongata* extract decreased the production of pro-inflammatory cytokines in LPS-stimulated THP-1-derived macrophages. Additionally, our findings suggest a reduction of AD-associated phosphorylated tau and promotion of early oligodendrocyte differentiation by *H. elongata*. RNA sequencing on the hippocampus of one-week-treated APPswePS1ΔE9 mice revealed effects of *H. elongata* on, amongst others, acetylcholine and synaptogenesis signaling pathways. In conclusion, extracts of *H. elongata* and *S. fusiforme* show potential to reduce AD-related pathology in APPswePS1ΔE9 mice. Increasing desmosterol concentrations may contribute to these effects by dampening neuroinflammation.

Levels of selected aminosulfhydryls in a group of patients with arterial hypertension and/or atherosclerosis after COVID-19

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Abstract

Background: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection may increase the risk of cardiovascular diseases (CVDs). Aminosulfhydryls are involved in antioxidant defense and in thiol redox control thus their impaired metabolism increases oxidative stress. In this study, total levels of selected aminosulfhydryls (i.e., cysteine (Cys), homocysteine (HCy), and glutathione) were assessed in COVID-19 patients with arterial hypertension (HA) and/or atherosclerosis.

Material and Methods: The study group consisted of post-COVID-19 patients (n=236, Caucasian, both sexes; aged 37-85 years), including 153 patients with HA and/or atherosclerosis, and 83 patients free from HA and/or atherosclerosis. The reference group involved 12 patients free from COVID-19, HA, and atherosclerosis. Levels of total aminosulfhydryls were assessed in the blood plasma using high-performance liquid chromatography (HPLC). In addition, the ratio of Cys/HCy was calculated for each patient.

Results: A higher HCy level was observed in COVID-19 patients with HA and/or atherosclerosis than in patients without these conditions (9.55 $\mu\text{mol/L}$ (3.96-32.40) vs 7.19 $\mu\text{mol/L}$ (3.27-11.87), respectively, $p=0.018$). The median Cys was significantly lower in the reference group compared to the COVID-19 group of patients with HA and/or atherosclerosis (123.05 $\mu\text{mol/L}$ (61.38-263.24) vs 210.22 $\mu\text{mol/L}$ (72.76-410.28) $p<0.001$). Similarly, the reference group had a lower Cys level than COVID-19 only group (123.05 $\mu\text{mol/L}$ (61.38-263.24) vs 193.84 $\mu\text{mol/L}$ (84.00-341.85), respectively, $p=0.002$). The highest glutathione level was found in patients with COVID-19 only and the lowest in the disease-free group (2.58 $\mu\text{mol/L}$ (0.40-7.49) vs 1.69 $\mu\text{mol/L}$ (1.15-3.86), respectively, $p=0.022$). A similar difference was found for the Cys/HCy ratio ($p=0.015$).

Conclusions: COVID-19 infection may affect HCy and Cys levels in patients with HA and/or atherosclerosis compared to patients free from COVID-19 disease, HA, and/or atherosclerosis.

Comprehensive lipid and metabolite profiling of youth with childhood onset type 1 diabetes compared to healthy controls: Results from the Norwegian ACD study

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Abstract

Persons with type 1 diabetes (T1D) have increased risk of early, accelerated atherosclerosis and premature cardiovascular disease. What drives this accelerated atherosclerotic process in T1D is not quite clear, although dyslipidemia and hyperglycemia seem to play an important role. Thus, we aimed to characterize the lipid-related and metabolic alterations related to hyperglycemia in youth- with childhood-onset T1D compared to healthy controls. We used data and biobank from the 5-yr follow-up of the prospective population-based Norwegian Atherosclerosis in Childhood Diabetes study. Plasma metabolites were measured using a high-throughput nuclear magnetic resonance spectroscopy platform. The differences between T1D youth and healthy controls were compared using regression models.

366 subjects, with the mean (SD) age of 18.6 (2.9) yrs, were included in the analysis, 242 (66%) of which had T1D and 122 (34%) healthy controls. Compared to healthy subjects, T1D youth had higher levels of atherogenic apoB- and apoA1-containing lipoprotein subclasses, and higher lipids and lipid species within especially the LDL and HDL subclasses. For example, they had a markedly higher triglyceride levels in their LDL particles. Most plasma fatty acids were also higher in T1D subjects, together with lactate, pyruvate, glycerol, and ketone bodies, while glutamine, histidine, creatinine and albumin were lower. Most of these metabolic alterations were linked to either glucose or HbA1c level, or both. Interestingly, while branched-chain amino acids were similar in T1D and healthy subjects, they associated strongly with glucose among T1D subjects only.

We found, in this young Norwegian cohort, that youth with T1D had more atherogenic lipid profile compared to healthy control subjects, that was highly associated with glycemic control.

Analysis of arterial stiffness parameters assessed by photoplethysmography in patients with arterial hypertension and/or atherosclerosis according to gender

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Abstract

Background: Cardiovascular risk increases in patients with hypertension and atherosclerosis. One of the early predictors of this increased risk is the change in arterial stiffness. In the study, arterial stiffness parameters using non-invasive photoplethysmography (PPG) were assessed in patients with hypertension (AH) and/or atherosclerosis (AS) in relation to gender.

Methods: The study group consisted of 333 patients (white, both sexes, aged 30–85 years), including Group I: patients without AH or AS, Group II: patients with AH, Group III: patients with AS, and Group IV: patients with AH/AS. Arterial stiffness parameters, i.e., reflection index (RI), peak-to-peak time (PPT), and stiffness index (SI), are calculated by PPG based on the analysis of the pulse wave contour.

Results: In the total group, higher mean values of RI and SI in men than in women were observed ($p < 0.001$ each). The highest mean SI value was found in patients with AS/AH ($8.90 \text{ m/s} \pm 2.00$) and the lowest in patients without AH or AS ($8.33 \text{ m/s} \pm 1.98$). The mean SI values were significantly lower in women compared to men in both group I (7.97 m/s vs 9.07 m/s , respectively, $p = 0.006$) and group II (8.05 m/s vs 9.76 m/s , respectively, $p < 0.001$). Similarly, the mean RI values were also higher in men than in women in groups I and II ($p < 0.001$ for each group).

Conclusions: This study confirmed that gender significantly affects arterial stiffness parameters. The presence of both AH and AS affects arterial stiffness.

Dietary effects on metabolic health and ageing in liver

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Abstract

The world's population is aging – by 2050 the number of people > 65 years of age will nearly double. This results in a growing number of people developing age-related diseases, which in turn has led to increased focus on promoting healthy aging. Dietary factors such as macronutrient composition, polyunsaturated fatty acids and sulphur amino acids are known to influence health span. However, few studies have examined the effects of life-long adherence to specific diets, and the biological effects of diet on aging in different tissues are unclear.

We have performed an aging study in mice that received one of five different purified diets from 16 weeks of age until middle age or old age. These diets were either low or high in fat, supplemented with polyunsaturated fatty acids or restricted in sulphur amino acids. The mice were subjected to metabolic phenotyping and glucose tolerance testing.

Two of the experimental diets (PUFA supplementation and combined methionine and cysteine restriction) protected against aging-related alterations in liver, liver damage and NASH. To further explore these effects we performed multi-omics analyses (transcriptomics, DNA methylation, lipidomics, metabolomics) and histological analysis of liver tissue. Overall we present an extensive multi-omics data resource with relevance to aging, NASH, obesity and dietary factors including PUFA and sulphur amino acids.



YIA Poster Walk – Abstracts –

Other Topics

SESSION IV

Mapping uptake and dissolution of ingested cholesterol crystals in macrophages using combined fluorescence and x-ray microscopy

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Abstract

Cholesterol crystals (CCs) are important contributors to the pathogenesis of atherosclerosis. These CCs are highly toxic to cells triggering inflammation responses in macrophages, thereby contributing to expansion and eventual rupture of the atherosclerotic plaque. CCs can also form inside foam cells when the cellular capacity to convert excess cholesterol into cholesteryl esters by acyl-CoA acyl transferase (ACAT) is exceeded. The molecular mechanisms by which CCs form in the vessel wall and inside macrophages and how they are degraded in cells are poorly understood.

Using dehydroergosterol (DHE), a naturally fluorescent analog of cholesterol that forms highly luminescent crystals, we directly observed macrophages interacting with sterol crystals through ultraviolet-sensitive fluorescence microscopy. We found that upon crystal uptake, cells became labeled in their membranes with DHE. Crystals of DHE could be distinguished from DHE in cellular membranes by the much slower photobleaching of crystalline compared to monomeric DHE. After prolonged incubation, DHE accumulated in the plasma membrane (PM) and in lipid droplets. Additionally, by using spectroscopy we have shown that cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin, can dissolve DHE crystals implicating its potential therapeutic effects on cholesterol homeostasis. By doping CCs with the fluorescent cholesterol analogue TopFluor-cholesterol, we were able to visualize uptake of CCs in macrophages using correlative fluorescence and soft X-ray microscopy. By combining these techniques, we are currently studying the engagement of CCs with lysosomes with the aim to map the pathway of intracellular cholesterol dissolution and efflux from cells.

Familial Hypercholesterolemia (FH): 25 years of findings in the Portuguese FH Study

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Abstract

Familial Hypercholesterolemia (FH) is a hereditary condition characterized by elevated LDL-C levels, which leads to increased risk of atherosclerosis and cardiovascular events. FH presents an estimated frequency of 1/300 and is expected to affect almost 33,000 Portuguese individuals. Therefore, this work summarizes the advances achieved in 25 years of diagnosis and investigation within the Portuguese FH Study (EPHF).

A lipid profile and genetic diagnosis were performed for 1291 referred index cases fulfilling FH clinical criteria (523 children, 768 adults) and 2288 relatives. In 2017, a Next Generation Sequencing panel including FH genes (LDLR, APOB, PCSK9) and FH phenocopy genes (LDLRAP1, APOE, LIPA, ABCG5, ABCG8) was implemented.

In approximately 40% (n=464) of the EPHF cohort, a genetic cause of FH was identified: 451 index cases with heterozygous FH (HeFH) and 13 with homozygous FH (HoFH). The majority of pathogenic variants were found in the LDLR gene (93%), compared with APOB (5%) and PCSK9 (2%) genes. Cascade screening allowed the identification of FH in 624 relatives (622 HeFH and 2 HoFH). Among adults with FH, 20% present cardiovascular disease (CVD) and 17% have premature CVD. Variants of uncertain significance in FH genes were identified in 63 index cases. Within 60% of the EPHF cohort (group of index cases where the genetic cause of hypercholesterolemia was not identified), 35% present hyper-Lp(a). Other monogenic causes of dyslipidemia were discovered during genetic analysis: 4 cases with lysosomal lipase deficiency (LIPA gene) and 4 cases with sitosterolemia (ABCG5 and/or ABCG8 genes).

The genetic identification of FH corresponds to 3% of the expected number of individuals affected in Portugal. Nevertheless, other rare lipid metabolism disorders were identified. To overcome FH underdiagnosis in Portugal and to promote early diagnosis and treatment to prevent CDV complications, a cost-effective screening chip array is under development.

Effect of cetoleic acid-enriched fish oil on atherosclerosis markers in high-risk patients with metabolic syndrome

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Abstract

Background and aims:

Intake of fish and oils with omega-3 fatty acids reduce the risk of cardiovascular disease, primarily through reduced blood levels of triacylglycerol (TAG). However, randomized controlled trials have shown inconsistent results regarding effect of cardiovascular disease. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can be synthesized from the essential fatty acid alpha-linolenic acid (ALA), but this conversion is shown to be inefficient in humans. When long-chain monounsaturated fatty acids as cetoleic acid are present, the synthesis of EPA and DHA from ALA has been shown to increase in animal studies. As marine omega-3 are a limited global resource and cetoleic acid is found in high concentrations in North Atlantic fish oils, it is of interest to explore the effects of cetoleic acid on health. In this study we investigated the effect of intake of cetoleic acid-enriched oils on atherosclerosis and inflammation markers in individuals with an unfavorable metabolic phenotype.

Methods:

This double-blinded randomized controlled study included 50 participants with unfavorable metabolic phenotype defined as triglycerides > 0,9 mmol/L and waist circumference > 80/94 cm (women/men). The participants were randomized to a 4 weeks intervention after a 3 weeks run-in period, receiving either marine oils with high (1716 mg/d) or low (6 mg/d) contents of cetoleic acid with similar amount of EPA and DHA. Blood samples, blood pressure and anthropometric measurements were collected at screening, baseline and end of intervention.

Results:

50 participants completed the intervention of which 11 were men and 39 were women with an age range of 25-70 years (mean 53 +/-9.5). We are currently analyzing the data including plasma concentration of EPA and DHA, as well as changes in the plasma levels of TAG, cholesterol (total, LDL and HDL), apoB, apoA, Lp(a), C-peptide, glucose and CRP. These preliminary data will be presented at the meeting.

Orthopedic events and incident amyloidosis in the general population

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Abstract

In patients diagnosed with cardiac transthyretin amyloidosis (ATTR-CA) the prevalence of certain orthopedic events is increased. However, whether orthopedic events are associated with incident amyloidosis in the general population is unclear.

We first tested which orthopedic events preceded amyloidosis in the general population, and whether an increasing number of orthopedic events at baseline was associated with a similar stepwise increase in incident amyloidosis. Second, we determined the time lag between orthopedic events and amyloidosis.

A Danish nationwide cohort (NWC) including 1,842 incident cases with amyloidosis matched 1:5 by age and sex with controls. Results were validated in a prospective cohort of 501,760 from the UK Biobank (UKB) including 491 incident cases with amyloidosis. Orthopedic events were carpal tunnel syndrome, spinal stenosis, trigger digit, hip and knee arthrosis, shoulder lesions, and biceps tendon rupture. Associations between baseline orthopedic events and risk of amyloidosis were tested using Cox proportional hazards models.

Carpal tunnel syndrome, spinal stenosis, trigger digit, and hip arthrosis were associated with hazard ratios (HRs) ranging from 2.44 and 2.57 for spinal stenosis to 7.69 and 3.33 for carpal tunnel syndrome in NWC and UKB, respectively. In individuals with two or more orthopedic events at baseline compared to individuals without events, HRs for amyloidosis increased stepwise up to 9.75 in NWC and 5.66 in UKB. Finally, the median time from diagnosis of an orthopedic event to amyloidosis ranged from 6.35 and 6.74 years for carpal tunnel syndrome to 9.19 and 11.35 years for trigger digit in NWC and UKB, respectively.

Carpal tunnel syndrome, spinal stenosis, trigger digit, and hip arthrosis are associated with incident amyloidosis in the general population, with more events associated with higher risk. Identifying these early manifestations of amyloidosis is important, as novel drugs may delay progression to ATTR-CA.

The Regulation and Effect of m⁶A Modification on the atherosclerotic plaque and Vascular Smooth Muscle Cells (VSMCs)

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Abstract

Aim: Cardiovascular diseases (CVDs) are the leading causes of death and disability globally, but their molecular mechanisms remain poorly understood. N⁶-methyladenosine (m⁶A) methylation is a key epitranscriptomic modification of mRNA, regulated by methyltransferases, demethylases, and m⁶A-binding proteins. This study investigates how m⁶A modification affects atherosclerosis and vascular smooth muscle cells (VSMCs).

Methods: We used carotid plaques from three donors and isolated RNA from the distal (early plaque) and central (late plaque) parts of the plaque. The RNA was divided into two fractions: one for RNA sequencing and one for immunoprecipitation of m⁶A-marked RNA before RNA sequencing (RipSeq). Second, we used VSMCs *in vitro* to explore the role of m⁶A methylation in VSMC phenotype switching.

Using three inhibitors for m⁶A: one inhibitor, UZH1a, targets m⁶A writers, while two target m⁶A erasers—Meclofenamic acid (MA) for FTO and ENA21 hydrochloride for ALKBH5. These inhibitors were applied to VSMCs stimulated with oxidized LDL. The inhibitors' impact was assessed using VSMC phenotype markers, foam cell markers, and inflammatory markers via RT-qPCR.

Findings: The RipSeq analysis of human plaques showed a change in pathways related to VSMC biology in the late stages of plaque formation compared to the early. *In vitro*, oxidized LDL induced a phenotypic switch in VSMCs. We detected genes unique to macrophages, indicating that smooth muscle cells may have transformed into macrophage-like foam cells. The m⁶A eraser and writer inhibitors reduced gene expression linked to the contractile phenotype.

Conclusion: m⁶A methylation is present in the atherosclerotic plaque, but its role in atherosclerosis remains unclear. Modifications to m⁶A alter the phenotype of VSMCs, which could contribute to the atherogenic process. This points to m⁶A as a potential treatment target in the future.

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