European Lipoprotein Club

49th Annual Scientific Meeting

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Evangelische Akademie

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Table of contents

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Programme overview

	Monday Sept 7	Tuesday Sept 8	Wednesday Sept 9	Thursday Sept 10
08:00		07:30 Breakfast	07:30 Breakfast	07:30 Breakfast & departures
09:00		08:30 **Session C: Atherosclerosis and Vascular Biology**	08:30 **Session F: Adipose Tissue Biology**	
10:00		10:00 Coffee break and **Posters II (No. P15-P30)**		
			10:15 Coffee break	
11:00			11:00 **Session G: Brown Adipose Tissue and Thermogenesis**	
12:00	12:00 **Arrival, registration, & light lunch**	11:30 **Session D: Lipoprotein Metabolism and Cholesterol Homeostasis**	*12:15 Lunch*	
13:00		*12:45 Lunch*	13:30 **Session H: Metabolic Adaptation Across Organs**	
	13:45 **Welcome address**	13:45 **Networking & OC meeting**	14:15 **General Assembly**	
14:00	14:00 **Session A: Lipid Droplets, Lysosomes and Intracellular Lipid Trafficking**		15:30 **Networking**	
15:00				
	15:45 Coffee and **Posters I (No. P1-P14)**			
16:00		16:00 **Session E: From MASLD to Cardiometabolic Disease**		
17:00	17:15 **Session B: EAS Young Fellows**		17:30 **Social event & Young Investigator Awards**	
	17:55 **Industry sponsor flash talks**			
18:00	*18:00 Dinner*	*18:00 Dinner*		
19:00		19:00 **Wine & Posters III (No. P31-P49)**		
	19:30 **Keynote Lecture**			
21:00	*21:00 Bar open*	*21:00 Bar open*		
23:00			*23:00 Bar open*	

Oral presenters eligible for YIA

#	Name	City	Country
2	Cristy Verzijl	Groningen	The Netherlands
3	Anna van Tiel	Groningen	The Netherlands
4	Klevis Ndoj	Amsterdam	The Netherlands
5	Suravi Mukherjee	Graz	Austria
14	Zoé Begué-Racapé	Nantes	France
15	Haoran Li	Helsinki	Finland
16	Marijn Bult	Amsterdam	The Netherlands
17	Floris Feiner	Groningen	The Netherlands
18	Sebastian Hendrix	Amsterdam	The Netherlands
20	Bhawna Bhawna	Mumbai	India
22	Sonia Slablab	Paris	France
23	Sebastian Baer	Berlin	Germany
25	Zeynep Kocberber	Munich	Germany
29	Anna Jung	Munich	Germany
30	Theresa Auer	Munich	Germany
31	Weiwei Zhang	Munich	Germany
32	Luka Lilie	Hamburg	Germany
33	Lukas Blaas	Munich	Germany
36	Emma Louisa Lemberger	Munich	Germany
37	Luise Schlotterose	Oxford	United Kingdom
38	Ottavia Terenghi	Milan	Italy

Session A: Lipid Droplets, Lysosomes and Intracellular Lipid Trafficking

1

Seipin opening defines the architecture of the ER–lipid droplet interface

Dr. Veijo Salo[1,2,3], Dr. Yoel Klug[4], Dr. Sapia Jennifer[5], Dr. Justin Deme[6], Dr. Johanna Tocci[2], Dr. Anastasiia Babenko[2], Dr. Reeba Jacobs[2], Dr. Nils Eikmeier[2], Dr. Evgenia Zagoriy[2], Dr. Sara Goetz[2], Dr. Pablo Campomanes[5], Dr. Niccolò Banterle[2], Dr. Susan Lea[6,7], Dr. Pedro Carvalho[4], Dr. Julia Mahamid[2]

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Aim (background & objectives)

Neutral lipids synthesized in the endoplasmic reticulum (ER) are partitioned between storage in lipid droplets (LDs) and secretion in lipoproteins. How this fundamental decision is organized at the nanoscale within the ER membrane remains poorly understood. While LD and lipoprotein biogenesis originate from a shared lipid pool, the structural mechanisms that regulate lipid flux toward these divergent fates are unclear. Here, we aimed to define how ER membrane architecture and associated protein machinery control LD formation, and to establish a mechanistic framework for how lipid storage may be regulated within the broader context of ER lipid flux.

Method

We combined single-particle cryo-EM, in situ cryo-electron tomography, molecular modelling, and cell biological perturbation assays to define the structural basis of LD biogenesis at the ER.

Results

We show that the LD assembly factor seipin undergoes a pronounced conformational transition from a compact to an open state during LD formation. This transition defines a stereotyped ~20-nm ER–LD neck architecture and promotes neutral lipid accumulation at sites of LD biogenesis. Disrupting this conformational transition impairs LD formation, demonstrating that regulated seipin opening functions as a structural checkpoint in lipid storage. We further identify a liver-enriched ER protein that modulates this conformational transition, suggesting that protein-mediated control of ER architecture can tune lipid storage capacity in a tissue-relevant manner.

Conclusions

These findings identify seipin opening as a key step in lipid droplet formation and show how ER membrane architecture controls neutral lipid accumulation. This provides a basis for understanding how lipid storage is organized and how neutral lipid partitioning may be regulated in metabolic tissues.

2

Mapping sex-specific mechanisms of hepatic cholesterol handling: insights into lysosomal GTPase ARL8B

Dr. Cristy Verzijl[1], Dr. Ankia Visser[1], MSc. Marieke Smit[1], BSc. Niels Kloosterhuis[1], MSc. Berdien Maring[1], BSc. Mariska de Graaf[1], Dr. Karin Wolters[1], Dr. Jérôme Gilleron[2], Prof. Dr. Natalie Krahmer[3], Prof. Dr. Bart van de Sluis[1]

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Aim (background & objectives)

Atherosclerotic cardiovascular disease (ASCVD) remains the leading cause of morbidity and mortality worldwide, largely driven by elevated plasma cholesterol levels. The liver is the central hub for systemic cholesterol regulation, balancing synthesis, uptake, storage, and secretion. Dysregulation of these pathways directly elevates ASCVD risk. Emerging evidence indicates that males and females differ in hepatic metabolism, yet the underlying molecular mechanisms remain poorly defined. This study aimed to elucidate sex-dependent regulation of hepatic cholesterol metabolism, focusing on the lysosomal GTPase ARL8B as a potential mediator.

Method

Proteomic screening identified ARL8B as a sex-dependent candidate potentially involved in hepatic cholesterol handling. Hepatocyte-specific *Arl8b* and *Arl8a* knockout mice were generated using somatic CRISPR/Cas9 editing and studied under standard and cholesterol-rich dietary conditions in both sexes. Lysosomes were isolated using the LysoTag model via immunoprecipitation, followed by untargeted proteomic analysis of isolated lysosomes and whole-liver extracts. Primary hepatocytes were isolated to further elucidate the mechanisms by which ARL8B and its interactors impact cholesterol handling.

Results

ARL8B protein levels were elevated in females but not males in response to a cholesterol challenge. Hepatocyte-specific ARL8B deficiency in females increased plasma and hepatic cholesterol and markedly reduced LDLR levels, while males showed a substantially milder phenotype with no effect on LDLR. Lysosomal proteomics revealed that the ARL8B homolog, ARL8A, could not fully compensate for ARL8B loss but may provide partial compensation at the level of lipid droplets. Combined deletion of both ARL8 paralogs resulted in a pronounced atherosclerotic phenotype, with markedly greater severity in females, further underscoring the sex-specific dependency on lysosomal ARL8 function in hepatic cholesterol homeostasis.

Conclusions

These results highlight ARL8B as a sex-specific central regulator of lysosomal cholesterol handling. We also reveal a previously underestimated role for ARL8A, suggesting that ARL8A and ARL8B collectively safeguard hepatic cholesterol homeostasis in a non-redundant manner.

3

Integrating lysosomal proteomics and lipidomics to map hepatic molecular networks in a preclinical model of Niemann-Pick type C1 disease

Msc. Anna van Tiel[1], Dr. Cristy Verzijl[1], Msc. Marieke Smit[1], Ing. Nicolette Huijkman[1], Bsc. Niels Kloosterhuis[1], Bsc. Mariska de Graaf[1], Ing. Trijnie Bos[1], Dr. Fred Vaz[2], Dr. Karin Wolters[1], Prof. Dr. Bart van de Sluis[1]

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Aim (background & objectives)

Intracellular cholesterol trafficking depends in part on the lysosomal membrane protein Niemann-Pick type C1 (NPC1), which facilitates the transfer of free cholesterol to other organelles. Loss-of-function of NPC1 results in NPC1 disease, characterized by an accumulation of free cholesterol in lysosomes and clinically by neonatal cholestasis, hepatosplenomegaly, and progressive liver failure. Despite its clinical significance, the molecular consequences and determinants of NPC1 deficiency at the lysosomal level remain poorly understood. In this research, we aim to map the lysosomal proteome and lipidome in hepatocyte-specific NPC1 deficiency, in order to identify the molecular mechanisms underlying hepatic pathology in NPC1 disease.

Method

Hepatocyte-specific *Npc1* knockout mice were generated using hepatocyte-specific Cas9-expressing LysoTag mice. Model validity was confirmed by plasma and liver cholesterol measurements. Lysosomes were isolated from healthy and hepatocyte-specific *Npc1* knockout livers by lysosomal immunoprecipitation. Isolated lysosomes were subjected to proteomic and lipidomic profiling, and results were integrated using network-based analyses to map molecular changes associated with NPC1 deficiency.

Results

Hepatocyte-specific *Npc1* knockout mice recapitulated key clinical features typical of human NPC1 disease, including increased hepatic cholesterol levels, mainly due to free cholesterol accumulation, and elevated liver-to-bodyweight ratio. Increased lysosomal markers LAMP2A and RAB7 indicated increased lysosomal abundance. We report distinct lysosomal proteomic and lipidomic profiles of NPC1-deficient livers compared to healthy livers. Proteomic profiling revealed increased lysosomal association of mitochondrial proteins, consistent with impaired mitochondrial respiratory capacity. Lipidomic profiling revealed a relative increase in glycolipids and lyso-phospholipids alongside a decrease of fatty acids and storage lipids, reflecting broad disruption of lysosomal lipid homeostasis beyond cholesterol.

Conclusions

This study provides an integrated lysosomal proteomic and lipidomic framework that deepens our understanding of hepatic pathophysiology in NPC1 disease.

4

NPC1 trafficking via VPS41-dependent LAMP carriers regulates endosomal cholesterol homeostasis

MSc Klevis Ndoj

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Aim (background & objectives)

The Nieman Pick type 1 and 2 proteins (NPC1 and NPC2) coordinate cholesterol egress from late endosomes-lysosomes (LE/LY). Proper folding, trafficking, and localization of both NPC proteins are essential for normal LE/LY cholesterol handling. Accordingly, mutations in NPC genes cause Niemann-Pick type C (NPC) disease, a progressive neurodegenerative lysosomal cholesterol storage disorder. The routes by which NPC1 reaches the LE/LY compartment in mammalian cells is not fully elucidated.

Method

Therefore, to interrogate NPC1 trafficking we developed genome-engineered HeLa cells expressing endogenous NPC1^{mNeon} and NPC1^{mTurboID}. We demonstrate that endogenous NPC1 localizes to the LE/LY compartment and by using protein proximity-based approaches that NPC1 resides in the same membranes as Vacuolar Protein Sorting-associated protein 41 (VPS41), one of the two unique subunits of the homotypic fusion and vacuole protein sorting (HOPS) complex.

Results

Loss of VPS41 increases NPC1 and Lysosomal Associated Membrane Protein 1 (LAMP1) abundance. Paradoxically, this results in marked accumulation of lysosomal cholesterol due to a shift in their localization from LE/LY to biosynthetic vesicles called LAMP carriers, a recently described pathway that transports lysosomal-destined cargo directly from the trans-Golgi (TGN) network to LE/LY. Mechanistically, we show that ectopic mitochondrial expression of VPS41, combined with immuno-fluorescence and -electron microscopy, leads to VPS41-dependent recruitment of NPC1- and LAMP1-containing small vesicles to the mitochondria

Conclusions

This defines NPC1 as a cargo of LAMP carriers, which under physiological conditions are recruited by VPS41 to LE/LY. In conclusion, we identify NPC1 as a cargo for VPS41-dependent LAMP carriers that is important for maintaining cellular cholesterol homeostasis.

5

Secreted Lysosomal Acid Lipase Compensates for Macrophage LAL Loss and Prevents Lipid Dysregulation in Mice

Ms. Suravi Mukherjee[1], Dr. Melanie Korbelius[1], Ms. Marija Durdevic[2], Ms. Anita Pirchheim[1], Ms. Birgit Schwarz[1], Dr. Samuele Suraci[2], Ms. Silvia Rainer[1], Ms. Malena Diaz[1], Mr. Laszlo Schooltink[1], Dr. Gernot F. Grabner[1,3], Dr. Nemanja Vujić[1], Prof. Dr. Dagmar Kratky[1,3]

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Aim (background & objectives)

Lysosomal acid lipase (LAL) hydrolyzes triglycerides and cholesteryl esters at an acidic pH inside the lysosome. Mutations in the LAL-encoding *Lipa* gene cause LAL deficiency (LAL-D) in humans, a rare autosomal recessive disorder characterized by dysregulation of lipid metabolism and accelerated atherosclerosis. The pronounced infiltration of lipid- lipid-associated macrophages (LAMs) in the liver and small intestine of systemic LAL KO mice implicate macrophages in LAL-D pathology. We aim to characterize and uncover the role of infiltrating macrophages that cause ectopic lipid accumulation and lead to chronic inflammation and metabolic dysfunction in LAL-D.

Method

We applied spatial transcriptomics to small intestinal segments from WT and LAL KO mice to characterize LAMs. To elucidate the macrophage LAL contribution to LAL-D *in vivo*, we generated macrophage- and macrophage-/enterocyte-specific LAL KO mice and performed morphological, histopathological, and functional analyses under chow- and high-fat/high-cholesterol diet-fed conditions. Conditioned-medium assays and plasma analyses assessed extracellular LAL activity.

Results

Spatial transcriptomic profiling revealed LAM accumulation in the lamina propria and transcriptional reprogramming of the LAL KO intestine towards phagocytosis, lysosomal degradation, immune cell activation, and lipid uptake/storage. Adjacent enterocytes showed reduced expression of absorptive processes and epithelial cell function genes, indicating a macrophage-enterocyte crosstalk. In contrast to systemic LAL KO mice, conditional KO lines were incapable of replicating the aggravated lipid phenotype, as lipoprotein secretion, intestinal lipid absorption, and tissue lipid accumulation were largely unaffected. Despite genetic LAL-D in macrophages, LAL activity in conditioned medium experiments and LAL protein abundance in plasma supported compensation by secreted enzyme and cellular uptake.

Conclusions

LAL is secreted into the circulation and taken up by macrophages, which corrects the phenotype of conditional KO mice *in vivo*. Ongoing analyses aim to define the macrophage-enterocyte crosstalk more precisely using computational and *ex vivo* approaches to identify targeted strategies for the treatment of LAL-D.

Session B: EAS Young Fellows

6

Familial Hypercholesterolaemia in children and adolescents: Updated insights from the Familial Hypercholesterolaemia Studies Collaboration (FHSC)

Dr Kanika Dharmayat

Imperial College London, London, United Kingdom

Aim (background & objectives)

Familial hypercholesterolemia (FH) is an inherited disorder that causes elevated LDL-C levels from birth, affecting more than 25 million people worldwide. Detecting FH early in life is crucial for managing LDL-C, yet fewer than 2.5% of adults with heterozygous FH (HeFH) were identified in childhood. We assessed the presentation of HeFH in the paediatric cohort of the FHSC registry.

Method

The FHSC comprises regional/national data from multiple cohorts/registries/databases of children and adults with a clinical and/or genetic diagnosis of FH. We conducted cross-sectional analyses at registry entry of individuals (<18 years) with HeFH from 50 countries.

Results

13,000 children/adolescents were included (52.0% girls, age at diagnosis: 9.1 years [IQR: 5.4-13.0]). Approximately 53.0% are genetically diagnosed (65.0% are non-index cases; 52.0% of clinically diagnosed cases are non-index cases). Clinically diagnosed children were slightly older (9.9 vs 8.9 years). Genetic diagnosis was highest in the European region (91.3%) and revealed intra-regional variation: Western (53.7%), Southern (31.8%), Eastern (3.4%), and Northern (2.5%). Approximately 88%, 11%, and <1% had *LDLR, APOB, and PCSK9* variants, respectively. Median LDL-C was approximately 1 mmol/L higher in cases with *LDLR* variants than in *APOB/PCSK9*. No differences in lipid-lowering medication (LLM) use between the genetic (28.0%) and clinic-diagnosed (27.5%) groups. Within Europe and amongst genetic diagnoses, LLM use was highest and lowest in Western (73.0%) and Northern (2.3%) Europe.

Conclusions

Children/adolescents continue to be identified after a family member is detected first. *LDLR* variants are more frequent and associated with higher LDL-C levels than APOB/PCSK9 variants, reinforcing the clinical relevance of identifying the specific gene involved. There is intra-European variation in genetic testing and use of LLMs, pointing to opportunities for greater standardisation of care, while recognising that these differences may partly reflect variation in healthcare infrastructure, screening programs, and genetic testing policies across regions.

7

The scavenger receptor CD36 on Trem2hi macrophages drives IL-1- and IL-6-dependent inflammation and associates with plaque destabilization in humans

Dr. Mark Colin Gissler[1], Mr Timothy Mwinyella[1], Dr. Hauke Horstmann[1], Prof. Clement Cochain[2], Dr. Jessica Pauli[3], Prof. Lars Maegdefessel[3], Ms Floor van der Zalm[4], Prof. Michal Mokry[4], Prof. Florian Willecke[1], Prof. Dirk Westermann[1], Prof. Dennis Wolf[1]

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Aim (background & objectives)

In atherosclerosis, oxidized low-density lipoprotein (oxLDL) cholesterol uptake into lesion macrophages is a central mechanism of foam cell formation and lipid-associated systemic inflammation. Here, we tested whether this process requires expression of the lipid-scavenging receptor CD36 on macrophages.

Method

CD36 expression across different hematopoietic lineages was quantified by single-cell RNA-sequencing (scRNA-seq) in atherosclerotic *Ldlr*$^{-/-}$ mice and human carotid plaques. For *in vivo* studies, we generated *Ldlr*$^{-/-}$ mice with an inducible genetic deficiency of CD36 on macrophages (iMϕ-CD36). Clinical significance of CD36 in human atherosclerosis was inferred from clinical association studies.

Results

scRNAseq identified *Trem2*^{hi} macrophages as the primary cell type expressing CD36. *In vitro*, macrophage-specific CD36 deficiency reduced oxLDL uptake, foam cell formation, and ROS production. Surprisingly, plaques from hyperlipidemic iMϕ-CD36 mice had the same size and contained equal amounts of lipids as plaques from control littermates. However, plaques from iMϕ-CD36 mice had smaller necrotic cores, fewer macrophages, and contained more collagen – features of improved plaque stability in humans. CD36 induced inflammation in macrophages, as shown by reduced levels of the inflammatory cytokines IL-1b and IL-6 in iMϕ-CD36 mice and in human macrophages, and promoted the expression of modulators of the extracellular matrix and chemotaxis. Unstable human carotid plaques had substantially higher CD36-expression levels than stable lesions. Finally, lesional CD36-expression was associated with aggravated clinical symptoms and a higher rate of MACE in a cohort of 654 patients.

Conclusions

Our findings indicate that CD36 is a major driver of vascular inflammation in murine and human lesional macrophages. We validate that CD36 is a promising target to dampen lipid-associated systemic inflammation and plaque-instability in atherosclerosis.

8

Genetic testing stratifies clinically diagnosed familial hypercholesterolaemia into two cardiovascular phenotypes with different therapeutic priorities: a global analysis from the EAS-FHSC registry.

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Imperial College London, London, United Kingdom

Aim (background & objectives)

Genetic testing identifies no pathogenic variant in 40–60% of individuals with a clinical diagnosis of familial hypercholesterolaemia (FH). To date, genetic testing has served to confirm the presence of pathogenic variants and enable cascade screening; whether it carries further value in distinguishing cardiovascular phenotypes remains undetermined. We investigated whether pathogenic variant status defines distinct cardiovascular phenotypes by comparing untreated LDL-C levels, coronary artery disease (CAD) and non-LDL-C risk factor prevalence, and their contributions to CAD.

Method

Adult index cases with clinical heterozygous FH from the FH Studies Collaboration registry who underwent genetic FH testing and were untreated or had untreated LDL-C measurements were included in this cross-sectional analysis. Patients were stratified into pathogenic variant-positive and pathogenic variant-negative groups. Generalised additive models, logistic regressions, and population-attributable fractions (PAF) were used to compare groups.

Results

Among 9,367 adults included, 6,555 (70%) were pathogenic variant-positive and 2,812 (30%) variant-negative. Pathogenic variant-positive individuals had higher untreated LDL-C (median 258 vs. 224 mg/dL); variant-negative individuals exhibited greater age- and sex-adjusted prevalences of hypertension (14.4% vs. 8.4%), diabetes mellitus (3.1% vs. 1.5%), and hypertriglyceridaemia (37.3% vs. 25.3%; all $p<0.001$). Predicted CAD probability thresholds were reached up to 16 years earlier in variant-positive individuals; additional risk factors further advanced these thresholds by ~9 years in both groups. LDL-C was the dominant contributor in variant-positive individuals (20.2% of 41.9% combined PAF), whereas non-LDL-C risk factors dominated in variant-negative individuals (32.5% of 61.1%).

Conclusions

Genetic testing stratified individuals with clinically diagnosed FH into two cardiovascular phenotypes. Variant-positive individuals had earlier CAD, driven primarily by cumulative LDL-C exposure, whereas variant-negative individuals displayed a metabolic phenotype in which non-LDL-C risk factors accounted for most of the PAF for CAD. Beyond diagnostic confirmation, genetic status may inform which care-pathway is most likely to yield the greatest cardiovascular benefit.

9

The Ratio of Lipid Profile to Matrix Metalloproteinases and Serotonin Levels in Patients with Multifocal Atherosclerosis

Dr, associate professor Tetiana Motsak[1], assistant professor Denys Novyk[1], Dr, associate professor Olga Dolynna[1], Dr., associate professor Olena Kupchynska[2]

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Aim (background & objectives)

Multifocal atherosclerosis (MAS) is a severe manifestation of systemic vascular disease characterized by simultaneous involvement of several arterial beds. Matrix metalloproteinases (MMPs) and serotonin are key biomarkers of plaque destabilization, extracellular matrix remodeling, and platelet dysfunction. Assessing their relationship with lipid metabolism may improve risk stratification and secondary prevention.

Objective

To assess the relationship between lipid profile parameters and plasma levels of MMP-2, MMP-9, and serotonin in patients with MAS under the influence of treatment.

Method

The study included 72 men (67.2±6.4 years) with MAS and intermittent claudication syndrome. Group 1 (n=34) had a history of myocardial infarction, Group 2 (n=38) a history of ischemic stroke, and 18 healthy men served as controls(CG). Plasma serotonin and MMP-2,-9 levels were measured by ELISA, and lipid parameters were assessed using standard methods. Patients received adjunctive therapy with cilostazol and GABA for 16 weeks.

Results

At baseline, all patients exhibited dyslipidemia and elevated serotonin and MMP levels, without significant differences between groups. Compared with controls, serotonin levels increased 3.6–3.8-fold ($p<0.001$), LDL-C by 62.8–63.4% ($p<0.01$), MMP-2 by 57.9–58.3%, and MMP-9 by 65.1–67.8% ($p<0.01$). Strong positive correlations were observed between serotonin and LDL-C ($r=0.751$ and $r=0.762$; $p<0.05$), as well as between MMPs and LDL-C. After treatment, HDL-C increased by 13.7–14.2%, while triglycerides decreased by 13.8–14.9% ($p<0.001$). Serotonin and MMP-2 levels significantly declined in both groups, whereas MMP-9 decreased only in Group 1.

Conclusions

In MAS patients, elevated LDL-C is associated with increased serotonin and MMP-2/-9 levels, reflecting links between dyslipidemia, vascular remodeling, and atherothrombosis. Adjunctive cilostazol and GABA therapy improved lipid profiles and reduced biomarker levels, suggesting beneficial effects on disease progression.

Session C: Atherosclerosis and Vascular Biology

10

Endothelial Let-7b promotes atherosclerosis by inducing endothelial-to-macrophage-like foam cell transition through upregulating hemoglobin-alpha

Ms Yanyi Zhou[1], Ms Khadijeh Taherdangkoo[1], Mr Shilun Li[1], Ms Claudia Geissler[1], Dr Nan Li[1], Dr Lucia Natarelli[1], Dr Remco TA Megens[1,2,3], Dr Daniel Richter[4], Ms Eva Briem[4], Dr Wolfgang Enard[4], Dr Christian Weber[1,2], Dr Maliheh Nazari Jahantigh[1,2], Dr Andreas Schober[1,2]

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Aim (background & objectives)

Atherosclerosis is a chronic inflammatory vascular disease in which macrophages derived from recruited circulating monocytes take up lipid and induces plaque formation. Emerging evidence shows that endothelial cells (ECs) can also accumulate lipid droplets (LDs), and express hemoglobin subunits under inflammatory conditions. Endothelial Dicer-dependent miRNAs, like miR-103, impairs endothelial regeneration and promotes atherosclerosis. Dicer also generated the miRNA let-7b in ECs, however, its role in atherosclerosis is unclear.

Method

We generated Apoe$^{-/-}$ mice expressing mEGFP in ECs and tdTomato in other cells, with or without endothelial-specific let-7b knockout (Apoe$^{-/-}$/BMX-Cre^{ERT2}/let-7b$^{flox/flox}$/ROSA^{mTmG}) to study the effect of endothelial let-7b in atherosclerosis. Apoe$^{-/-}$/BMX-Cre^{ERT2}/let-7b$^{flox/flox}$ mice were crossed with GFP-myc-tagged Argonaute-2 (Ago2) reporter mice to study the endothelial let-7b targets *in vivo*. Mice were fed a Western diet for 4 or 12 weeks. Endothelial RISC-associated transcripts were identified through GFP-tAgo2 immunoprecipitation and prime-seq RNA sequencing. Predicted let-7b binding sites (BSs) were validated using luciferase reporter assays with wild-type or mutant 3'UTR constructs.

Results

We identified a subset of ECs on plaques that accumulated intercellular LDs and CCs, showing macrophage-like morphology. These cells resided beneath the flat endothelial monolayer, and protruded toward the lumen. They were EGFP+, confirming endothelial origin, and some expressed Mac2, suggesting transition to foam-cell-like phenotype. We term them endothelial-derived foam cells (EndoFCs).

Endothelial let-7b knockout decreased EndoFCs' proliferation, intercellular LDs and CC formation, and plaque burden. Transcriptomic profiling of endothelial RISC revealed noncanonical let-7b targets, like Hba-a1/a2. Let-7b enhances HbA expression in ECs, which was enriched in EndoFCs but low in polygonal ECs. Deletion of let-7b reduced HbA expression in ECs, suggesting a link between let-7b dependent HbA upregulation and EndoFC formation.

Conclusions

Endothelial let-7b aggravates atherosclerosis, likely by enhancing HbA expression and driving EC transformation into foam cells. This effect appears specific to endothelial let-7b rather than other let-7 family members.

11

Role of microRNAs in Foamy and Non-foamy macrophages during Atherosclerosis

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Aim (background & objectives)

Hypercholesterolemia and high-fat diets promote involvement of foamy macrophages (FMs) and inflammatory non-foamy macrophages (NFMs) in atherosclerosis. miR-147-3p is upregulated by inflammatory stimuli in macrophages and atherosclerotic lesions, suggesting a role in NFMs, but how it regulates macrophages within atherosclerotic plaques remains unknown.

Method

miR-147-3p function was examined in myeloid-specific Mir147-deficient $Apoe^{-/-}$ $Mir147^{flox/flox}LysMCre^{+}$ mice, with or without enhanced GFP expression. Using live-plaque 4D confocal imaging, we assessed lipid droplets, caspase-3 activation, apoptotic DNA, cholesterol crystal (CC) formation, mitochondrial function and macrophage behavior. We tested mitochondrial function in live-plaque tissue by Seahorse assay. GFP-tagged Argonaute 2 (tAGO2) immunoprecipitation combined with prime RNA sequencing was performed using atherosclerotic aortas from $Apoe^{-/-}$LSL-tAgo2/$Mir147^{flox/flox}LysMCre^{+}$ and control mice. The effect of the galectin-3 inhibitor GB1107 was studied using 4D live-plaque imaging.

Results

Unlike FMs, NFMs are primarily located in the plaque core and show higher miR-147-3p levels in mouse and human atherosclerosis. Myeloid Mir147 deletion increases atherosclerosis, with

enhanced CC formation and apoptotic DNA accumulation in necrotic cores. In contrast to Dicer deficiency, which primarily affected FMs, Mir147 loss specifically reduces mitochondrial activity and elevates caspase-3 activity in NFMs, and lowers spare respiratory capacity of plaque macrophages. Moreover, deleting Mir147 impairs NFM uptake of apoptotic DNA, promoting extracellular DNA accumulation and crystal formation. Additionally, Mir147 deficiency in NFMs induces endothelial caspase-3 activation and facilitates transendothelial extension of FM projections. Mechanistically, the Lgals3 transcript, encoding galectin-3, was reduced in tAGO2 immunoprecipitate after Mir147 knockout. A miR-147-3p binding site in the Lgals3 3'-UTR was functionally confirmed. GB1107 treatment reversed the Mir147 knockout effect in macrophages.

Conclusions

While Dicer primarily regulates FMs in atherosclerosis, miR-147-3p reduces atherosclerosis by suppressing harmful NFM effects on endothelial cells and enhancing apoptotic DNA clearance. Increasing miR-147-3p might thus slow necrotic core expansion and reduce atherothrombosis caused by NFM-induced endothelial damage.

12

Lipid droplets in age-dependent vascular dysfunction

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Aim (background & objectives)

Lipid droplets (LDs) in endothelial cells play a key role in buffering circulating lipids and maintaining vascular homeostasis. While impaired LD lipolysis is linked to cardiometabolic diseases, its role in aging remains poorly understood. This study investigates the biological significance of endothelial LDs in age-related endothelial dysfunction and examines how impaired lipolysis affects vascular function.

Method

Aged (18-month-old) and young (4-month-old) mice were used as model of aging. LD number and composition were assessed using fluorescence and Raman microscopy, respectively, while vascular function was measured by wire myography. Lipidomic profiling (GC–MS) and biochemical assays complemented the analysis. Postprandial responses in the aorta were evaluated *in vivo* following olive oil administration (10 mL/kg b.w.) and *ex vivo* by incubating aortic rings with fatty acids (oleic acid-500 μM, or palmitic acid-200 μM), with pharmacological modulation of LD abundance using atglistatin (10 μM) and metformin (200 μM).

Results

Aged mice showed increased LD accumulation in aortic endothelium and impaired vascular function compared with young controls. This was associated with unchanged plasma FFA and TAG levels, but reduced ATGL expression, and an elevated pro-inflammatory markers (elevated ICAM and arachidonic acid). In a postprandial model, TAG levels increased at 3 hours, with peak endothelial LD accumulation at 6 hours, leading to transient endothelial dysfunction. Aged mice exhibited prolonged LD accumulation (8 hours), indicating impaired lipolysis. LDs were enriched in unsaturated lipids. *Ex vivo* studies confirmed that fatty acids promote LD formation, while metformin reduced LD accumulation and restored endothelium-dependent vasodilation.

Conclusions

Aging impairs ATGL-mediated LD lipolysis in endothelium, leading to LD accumulation and vascular dysfunction. This defect is exacerbated during postprandial lipid overload. The findings highlight LD metabolism as a critical regulator of endothelial function and suggest that

enhancing LD breakdown may represent a therapeutic strategy for age-related cardiovascular diseases.

13

3D Smooth Muscle Cell Spheroids as models for Matrix Remodeling in Coronary Artery Disease

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Aim (background & objectives)

Coronary artery disease (CAD) is driven by lipoprotein accumulation within the vascular wall, inducing vascular smooth muscle cell (VSMC) phenotypic switching and extracellular matrix (ECM) remodeling. Current *in vitro* models do not adequately capture these processes due to the lack of physiological cell–matrix interactions. We aimed to establish a three-dimensional (3D) VSMC spheroid model that reflects key features of vascular remodeling. Specifically, we investigated whether 3D culture promotes disease-relevant VSMC phenotypes associated with ECM production and plasticity. In addition, we also generated endothelial spheroids for future studies on vascular cell crosstalk in atherosclerosis.

Method

Murine and human aortic VSMCs, including patient-derived cells, were cultured as scaffold-free 3D spheroids under non-adherent conditions. Cells self-assembled into multicellular aggregates, enabling tissue-like organization. Comparative analyses were performed between 3D spheroids and conventional 2D cultures. Proteomic profiling was used to assess differences in protein expression, with a focus on pathways related to ECM organization, adhesion, and cellular phenotype. Spheroid morphology and integrity were evaluated microscopically. In parallel, endothelial cells (ECs) were cultured as spheroids using the same approach to establish the basis for future co-culture systems.

Results

VSMCs formed compact spheroids. Compared to 2D cultures, 3D-grown VSMCs displayed a distinct proteomic profile consistent with a synthetic, disease-associated phenotype. This included increased expression of proteins involved in ECM production, organization, and adhesion signaling. These changes reflect key mechanisms observed in lipoprotein-driven vascular remodeling in CAD. The 3D environment enhanced VSMC plasticity, a central feature of plaque development. Additionally, endothelial spheroids were successfully generated, supporting the feasibility of modeling intercellular interactions relevant to atherogenesis.

Conclusions

This 3D VSMC spheroid model captures key pathological features of coronary artery disease by recapitulating lipoprotein-associated VSMC phenotypic switching and extracellular matrix accumulation. The model offers a new tool for dissecting pathological mechanisms in CAD and for evaluating therapeutic strategies targeting lipoprotein-driven vascular remodeling.

Session D: Lipoprotein Metabolism and Cholesterol Homeostasis

14

PGS1, a new gene involved in the regulation of plasma LDL-cholesterol levels.

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Aim (background & objectives)

Over the past few decades, many genetic loci have been associated with plasma levels of low-density lipoprotein cholesterol (LDL-c). However, most of the >500 associated variants are located in non-coding regions, making causal genes identification challenging. By integrating genetic and functional genomics datasets, we focused on the locus chr17q25. The lead LDL-c associated variants map to the first intron of PGS1 which encodes a mitochondrial enzyme catalyzing cardiolipin synthesis. We aimed to defined the role of PGS1 in cholesterol metabolism.

Method

Genetic associations were refined using LDL-c GWAS data (>1Million individuals) and GTEx expression and splicing quantitative trait loci datasets. To assess the functional role of Pgs1, we targeted the gene in the liver of Alb-Cas9-C57Bl/6J mice. Animals received AAV8 carrying Pgs1-targeting guide RNAs (n=8) or empty vectors (n=8) and were fed a chow diet.

Results

The top associated variant, rs11656568-G, induces a mis-splicing of PGS1, generating a truncated protein (p=1.2E-130). It is associated with lower total cholesterol (p=2.4E-58), LDL-c (p=5.8E-39), HDL-c (p=1.5E-42) and apolipoprotein B (p=7.5E-23). In mice, hepatic Pgs1 knockdown reduced plasma cholesterol levels by 60% (p<0.001). Hepatic cholesterol and triglyceride contents were also decreased (-75% and -35%, respectively; both p<0.01), alongside a reduced liver-to-body weight ratio (–12%; p<0.01). Fecal cholesterol content increased (+20%; p<0.01) and lipidomic profiling of feces revealed reduced intestinal cholesterol absorption, as indicated by lower sitosterol/cholesterol and campesterol/cholesterol ratios (34% and -31%, respectively; both p<0.01). The bile acid profile shifted, with decreased levels of DCA (–74%; p<0.01) and CA (–63%; p<0.05) and increased UDCA (+70%; p<0.05). Liver transcriptomic and proteomic analyses identified alterations in mitochondrial and peroxisomal pathways

Conclusions

Our human genetic and in vivo data identify PGS1 as a regulator of plasma cholesterol levels. Ongoing diet-challenge and cholesterol-flux studies will clarify the mechanisms underlying PGS1-mediated cholesterol regulation and cardiometabolic consequences.

15

TGN46 Regulates Hepatic Apolipoprotein Secretion and Is Genetically Associated with Plasma HDL Levels via the G141D Variant

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Aim (background & objectives)

Trans-Golgi network integral membrane protein 2 (TGOLN2, TGN46) is involved in protein trafficking, but its physiological role is poorly understood. Our genomic analyses (FinnGen/UK Biobank) linked the TGOLN2 locus to plasma HDL levels, identifying a missense variant (rs112065068, G141D) associated with decreased TGN46 and elevated HDL/ApoA1. We investigate how TGN46 modulates hepatic apolipoprotein trafficking and the functional impact of the G141D variant.

Method

To investigate the function of TGN46, we utilized CRISPR-Cas9 to generate TGN46 knock-out (KO) and endogenous G141D-TGN46 variant hepatic Huh7 cells. We analyzed the secretion of apolipoproteins (ApoB, ApoA1) and albumin using biochemical assays and visualized TGN morphology via immunofluorescence and electron microscopy. HDL uptake was measured by high-content imaging. Phenotypic associations were validated in FinnGen and UK Biobank datasets, focusing on non-synonymous variants and lipid profiles.

Results

TGN46 KO significantly decreased ApoB and ApoA1 but not albumin secretion, suggesting a selective role in cargo trafficking. Furthermore, TGN46 is essential for Golgi structural integrity as its absence leads to a dispersed Golgi ribbon structure. GWAS data from FinnGen and UK Biobank confirmed a strong genetic association between the TGOLN2 locus and HDL levels, highlighting the G141D variant as a key modulator. In G141D variant cell lines, we observed reduced cellular TGN46 levels and decreased HDL uptake. These cellular findings align with the clinical phenotype of altered lipid profile observed in G141D carriers, providing a potential mechanistic link between TGN46 dysfunction and elevated plasma HDL.

Conclusions

Our study identifies TGN46 as a novel regulator of hepatic apolipoprotein secretion and Golgi structural integrity. The G141D variant impairs TGN46 function and leads to an altered lipid profile, thereby providing a molecular mechanism for the genetic association between TGOLN2 and plasma HDL.

16

RIC1 as a novel regulator hepatic Apolipoprotein B secretion and systemic lipoprotein homeostasis

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Aim (background & objectives)

Apolipoprotein B (APOB) is the central structural protein of (very) low-density lipoproteins (VLDL/LDL). Although hepatic APOB synthesis, lipidation, and secretion have been extensively studied, there remain gaps in our understanding of its intracellular trafficking through the secretory pathway. Our objective is to identify novel determinants of the secretory trafficking of VLDL particles.

Method

To identify candidate regulators of this process, we performed a genome-wide CRISPR/Cas9 screen in HepG2 cells expressing endogenously mNeon-tagged APOB. Using Fluorescence-Activated Cell Sorting, this system enables efficient identification of genes whose disruption leads to intracellular APOB depletion or accumulation.

Results

In addition to established modifiers of intracellular APOB levels, our screen identified RIC1, also known as KIAA1432, as a novel regulator. We found that knockout of RIC1 in hepatocytes resulted in intracellular accumulation of APOB within LAMP1-positive endolysosomal compartments, and concomitant a marked reduction of APOB secretion into the culture medium. We demonstrate that the mechanism by which RIC1 controls APOB secretion is mediated by its role, in complex with RGP1, as a guanine exchange factor for RAB6A. Beyond APOB, RIC1 deficiency impaired the secretion of other large protein such as fibronectin. Preliminary analysis of the secretome further indicate a global reduction of protein secretion upon loss of RIC1. Ongoing studies are examining the consequences of RIC1 knockdown in primary hepatocytes and liver-specific RIC1 knockout in mice. While waiting for *in vivo* validation, human genetic data show that variations in the RIC1 locus are associated with reduced lipid plasma levels, suggesting a role for RIC1 in systemic lipoprotein homeostasis.

Conclusions

In summary, we identified RIC1 as a novel determinant of hepatic APOB and VLDL secretion and systemic lipoprotein homeostasis.

17

SMLR1: A Novel Lipidation Regulator of ApoB-Containing Lipoproteins

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Aim (background & objectives)

Small leucine-rich protein 1 (SMLR1) is an intracellular protein expressed in the liver and the small intestine and has been shown to play a role in the biogenesis of very low-density lipoprotein (VLDL). Here, we investigated whether SMLR1 is involved chylomicron metabolism.

Method

Whole-body *Smlr1* knockout (*Smlr1-/-*) mice were generated and kept on a chow diet. Analyses included body weight, food intake, plasma lipids and lipoproteins, and faecal lipid content. Mice were studied after an oral fat tolerance test and after the injection of poloxamer-407. Adeno-associated virus serotype 8 was used to re-introduce SMLR1 in the liver. The intracellular role of SMLR1 was studied using shRNA in a rat hepatocarcinoma cell line (McA-RH7777).

Results

Smlr1-/- mice are characterized by the accumulation of neutral lipids in the tips of the villi of enterocytes and reduced post-prandial plasma triglycerides. This phenotype persisted when SMRL1 was reintroduced in the liver. These observations combined with increased lipids in the faeces suggest that SMLR1 plays a role in chylomicron biogenesis. Interestingly, chow-fed *Smlr1-/-* mice exhibit reduced levels of cholesterol and triglycerides in the circulation without changes in APOB100 or APOB48 levels. Preliminary experiments in McA-RH7777 cells, knock down of *Smlr1* resulted in increased intracellular ApoB signal and increased lipid droplet number, area and colocalization.

Conclusions

This study identifies SMLR1 as a novel player in chylomicron metabolism. Our studies suggest that it plays a role in the lipidation of APOB, but the underlying mechanism is the topic of ongoing studies.

18

Loss of the E3 ubiquitin ligase MARCHF6 alters hepatic lipid metabolism and redox balance

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Aim (background & objectives)

Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease worldwide, however the molecular mechanisms driving its progression remain incompletely understood. Dysregulated hepatic lipid metabolism is a central pathogenic feature, and recent evidence implicates hepatic redox imbalances as a critical contributor to MASLD. Here, we identify the endoplasmic reticulum–associated E3 ubiquitin ligase MARCHF6 as a pivotal regulator of hepatic lipid metabolism and redox balance.

Method

To establish the role of MARCHF6 as an essential mediator of hepatic homeostasis in the context of MASLD, we generated a murine model with hepatocyte-specific *MARCHF6* deficiency. MARCHF6-dependent metabolic alterations in the livers were assessed using transcriptomics, proteomics, and lipidomics. Those findings were subsequently validated in primary human hepatocytes and independent human datasets.

Results

Global or hepatocyte-specific deletion of *Marchf6* induced spontaneous accumulation of triglycerides and cholesteryl esters under chow-fed conditions, revealing a cell-autonomous hepatic defect independent of caloric excess. Loss of MARCHF6 stabilized its substrate squalene epoxidase (SQLE), thereby enhancing sterol pathway flux while concomitantly activating the SREBP1-associated lipogenic transcriptional program and increasing lipoprotein clearance from the circulation. Consistently, lipidomic analyses demonstrated remodeling of the hepatic lipidome towards polyunsaturated, long-chain neutral lipids. In parallel, NADPH consumption was increased, accompanied by disruption of hepatic redox homeostasis, as evidenced by depletion of essential antioxidants like GSH and altered expression of antioxidant defense-associated proteins, including GPX4.

Conclusions

Collectively, these data establish MARCHF6 as a multifaceted gatekeeper that integrates sterol turnover, lipogenesis, and redox balance to maintain hepatic homeostasis.

Session E: From MASLD to Cardiometabolic Disease

19

Kupffer cell p38 signaling drives hepatic lipid accumulation through LXR and CD36

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Aim (background & objectives)

Kupffer cells (KCs), the liver-resident macrophages, are key integrators of metabolic and inflammatory signals during hepatic lipid overload. However, the signaling mechanisms linking lipotoxic stress to KC metabolic reprogramming remain poorly understood. Here, we aimed to identify the molecular pathways controlling KC lipid handling and their contribution to metabolic dysfunction–associated steatotic liver disease (MASLD).

Method

We used KC-specific genetic models to delete the p38 upstream kinases MKK3 and MKK6, as well as CD36, in mice subjected to diet-induced metabolic stress. Lipid uptake, KC phenotypes, and hepatic steatosis were assessed alongside systemic metabolic parameters. Mechanistic studies included phosphorylation analyses to determine p38α-dependent regulation of LXRα and its downstream targets. Paracrine effects on hepatocyte metabolism were evaluated through functional assays of lipid uptake and mitochondrial respiration.

Results

Deletion of MKK3/6 in KCs reduced CD36 expression, limited fatty acid uptake, and prevented the emergence of lipid-loaded pathogenic KC states. Mice lacking p38 signaling in KCs were protected from hepatic steatosis and maintained glucose tolerance and insulin sensitivity. Mechanistically, p38α directly phosphorylated LXRα, promoting CD36 expression and lipid accumulation in KCs. KC metabolic reprogramming also affected hepatocyte function through paracrine signaling, enhancing lipid uptake and impairing mitochondrial respiration. Notably, CD36 deletion in KCs phenocopied p38 inactivation, while KC-specific CD36 re-expression restored lipid accumulation and steatosis.

Conclusions

These findings identify a p38α–LXRα–CD36 signaling axis as a KC-intrinsic pathway that links lipotoxic stress to macrophage metabolic reprogramming and hepatic lipid accumulation. This work highlights a cell-type-specific metabolic checkpoint with potential therapeutic relevance for MASLD.

20

Effect of the Antidepressant Paroxetine on Hepatic Lipid Metabolism: Implications for Fatty Liver

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Aim (background & objectives)

Depression and anxiety are emerging mental health issues worldwide, leading to widespread use of antidepressants. They act by altering neurotransmitter reuptake, but their impact beyond the central nervous system remains poorly understood. Long-term use of antidepressants is accompanied by metabolic side effects such as weight gain and dyslipidemia. Given the liver's central role in drug metabolism and lipid handling, our study focuses on examining how antidepressants influence hepatic lipid homeostasis and further explores their potential in conditions like fatty liver.

Method

Confocal microscopy was employed for lipid droplets (LDs) quantification. Lipidomics and kit-based assays were used for triglyceride and cholesterol measurements. shRNA-mediated *CES2* knockdown was done. Paroxetine was administered in adult male zebrafish and male mice by oral gavage.

Results

Paroxetine treatment significantly reduced LDs in hepatic cell lines as well as in mouse primary hepatocytes. LDs remain unchanged in non-hepatic cell lines, indicating a liver-specific phenotype. Activity-based protein profiling (ABPP) revealed carboxylesterase 2 (CES2) as a notable esterase responsible for LDs' disappearance. RT-PCR analysis showed several-fold increase in *CES2* expression in Paroxetine condition relative to the control. *CES2* knockdown in a paroxetine background recovered LDs, further confirming a crucial role of CES2 in cholesterol ester hydrolysis. In a fatty liver zebrafish model, Paroxetine dramatically reduced hepatic lipid accumulation while increasing triglycerides in skeletal muscle, confirming its in vivo effect. Furthermore, Paroxetine administration in a murine model resulted in lowered hepatic triglycerides alongside elevated circulating triglycerides and higher esterase activity in the liver. Together, these data support that Paroxetine regulates CES2-driven cholesterol ester hydrolysis accompanied by hepatic lipid mobilization and export.

Conclusions

Our findings elucidate Paroxetine-tuned hepatic lipid metabolism through a novel CES2-linked pathway. Paroxetine's liver-specific lipid lowering effect presents a promising therapeutic candidate for repurposing in fatty liver management.

21

Next-generation beneficial bacteria combined with fecal microbiota transplantation attenuate diet-induced metabolic dysfunction-associated steatotic liver disease in mice

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Aim (background & objectives)

The gut microbiome is an emerging therapeutic target for metabolic dysfunction–associated steatotic liver disease (MASLD), yet few preclinical interventions demonstrate clear histological benefit, and combinatorial strategies remain unexplored. Here, we combined next-generation beneficial bacteria (NGBB) with fecal microbiota transplantation (FMT) to enhance engraftment and metabolic cross-feeding in a diet-induced MASLD mouse model.

Method

Male C57BL/6J mice were fed a Western-style high-fat, high-cholesterol, high-carbohydrate diet with 15% fructose water and treated with NGBB (Anaerobutyricum soehngenii, pasteurized Akkermansia muciniphila, and Bifidobacterium animalis subsp. lactis), FMT, or the combined regimen (FMT-NGBB).

Results

FMT-NGBB markedly boosted abundance of the administered bacteria. The combined therapy uniquely improved multiple histologic endpoints: it reduced hepatic triglycerides by a profound ~50% and improved steatosis, lobular inflammation, and sinusoidal capillarization. Hepatic transcriptomics revealed downregulation of fibrogenic pathways in the FMT-NGBB mice. Targeted lipidomics of portal vein plasma identified the anti-inflammatory prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) to be inversely correlated with hepatic triglyceride content. In vitro, 15d-PGJ_2 potently reduced lipid droplet accumulation in lipid-laden hepatocytes.

Conclusions

Together, these findings show that FMT facilitates engraftment of co-administered beneficial bacteria and that FMT-NGBB synergistically ameliorates diet-induced steatohepatitis. Moreover, we identified 15d-PGJ_2 as a key mechanistic mediator in this process. This work highlights the therapeutic promise of combinatorial microbiota interventions for MASLD.

22

Hepatic S1P drives cardiac dysfunction in MASLD through altered HDL/albumin distribution

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Aim (background & objectives)

Metabolic dysfunction-associated steatotic liver disease (MASLD) is associated with an increased risk of cardiovascular diseases, including arrhythmias and heart failure. However, the mechanisms linking liver injury to cardiac dysfunction remain poorly understood. Sphingosine-1-phosphate (S1P) is a bioactive lipid mediator, mainly synthetized by Sphingosine Kinase 1 (SphK1) and transported in plasma by high-density lipoproteins (HDL) (60-70%) and to a lesser extent by albumin (30-40%), where it signals through its receptors (S1PRs), regulating inflammation and cardiac fibrosis. As the liver is a major source of circulating S1P in steatosis, this pathway may represent a key liver-heart axis.

Method

We investigated the role of hepatic S1P in cardiac alterations associated with steatosis. Ten-week-old male control and liver-specific SphK1 knockout (SphK1-KO-Liver) mice were fed either a control or high-fat diet (HFD) for 16 weeks. S1P levels were measured in the liver, heart and plasma. Its distribution between HDL and albumin was then examined. Cardiac S1PRs expression, inflammatory and fibrotic markers (RT-qPCR), as well as cardiac function (echocardiography, ECG) were also assessed.

Results

In control mice, HFD increased plasma and cardiac S1P levels and redistributed circulating S1P from HDL toward albumin. This shift was accompanied by increased expression of cardiac S1PR2/3, elevated inflammatory cytokines, and signs of cardiac dysfunction, including atrial dilation. In contrast, SphK1-KO-Liver mice displayed reduced hepatic S1P production which resulted in lower plasma and cardiac S1P levels and a normalization of its plasma distribution. These changes were associated with reduced cardiac inflammation and fibrosis, decreased S1PR2/3 expression, and improved cardiac function. Notably, mice exhibited less atrial dilation, reduced susceptibility to atrial fibrillation, and preserved ventricular strain.

Conclusions

Overall, these findings suggest that hepatic S1P plays a key role in HFD-induced cardiac alterations in MASLD. Targeting hepatic S1P production could therefore represent a promising strategy to protect the heart by limiting inflammation, fibrosis, and functional impairment.

23

Lipoprotein-driven systemic crosstalk in Cardiometabolic HFpEF: Insights from a Porcine Model

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Aim (background & objectives)

Heart failure with preserved ejection fraction (HFpEF) is a complex systemic syndrome characterized by impaired ventricular relaxation and elevated filling pressures despite preserved systolic function, driven by obesity, systemic inflammation, and dysregulation of lipoprotein signaling and lipotoxicity. The latter are the shared cardiometabolic substrates for a whole range of cardiometabolic diseases affecting multiple organ systems beyond the heart. Recently, studies have shown that these organs do not simply become diseased independently but reinforce each other's pathological states through inter-organ crosstalk. In this work, we aim to study cardiometabolic HFpEF in a clinically relevant porcine model to uncover mechanisms underlying inter-organ tissue crosstalk.

Method

A large animal model of cardiometabolic HFpEF using minipigs was established. Female minipigs aged 14-18 months were fed a Western diet for 20 weeks. After overnight fasting, plasma and organ tissues were collected. The cardiac phenotype was assessed by echocardiography and invasive right- and left-heart catheterization. Hematologic parameters and lipid concentrations were analyzed. The plasma proteins were identified using LC-MS/MS Proteomics. Gene expression and histology are being performed on tissues.

Results

Development of cardiometabolic HFpEF was confirmed by invasive hemodynamics and clinical parameters. HFpEF animals developed elevated wedge pressures (>15 mmHg at rest, $p = 0.014$), obesity (40 vs 52 kg mean, $p = 0.003$), dyslipidemia (cholesterol 59 vs 470 mg/dl, $p < 0.001$, triglycerides 44 vs 139 mg/dl, $p = 0.008$), and hypertension (MAP 78 vs 138 mmHg $p < 0.001$). Plasma proteomics uncovered numerous differentially abundant proteins, with pathway analysis highlighting changes in cholesterol/lipid metabolism, complement and coagulation cascades, the proteasome, and PPAR signaling (top 5 DEPs: ApoC3, ApoB, ApoE, ApoC2, and ApoD).

Conclusions

This minipig model provides a robust preclinical platform to study HFpEF as a multisystem disease. Ongoing studies aim to elucidate mechanisms of organ–heart crosstalk with particular emphasis placed on released lipoproteins.

24

Studying the isolated machine perfused human liver reveals new insights into lipoprotein metabolism

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Aim (background & objectives)

In liver transplantation, normothermic machine perfusion (NMP) of human donor livers is used to perform viability assessment and prolong preservation time. Such setups allow for extending preservation to up to one week. Here, we used prolonged NMP to study ex situ lipid and lipoprotein metabolism in human donor livers.

Method

Twelve human donor livers not utilized for transplantation were perfused for seven consecutive days. Perfusate was collected daily to measure cholesterol, triglycerides, and run lipoprotein profiles (FPLC). Targeted proteomics was used to quantify proteins with established roles in lipoprotein metabolism. Three livers were treated with Lomitapide, an inhibitor of microsomal triglyceride transfer protein (MTTP).

Results

Perfusate cholesterol, triglyceride, and APOB levels gradually increased over five days and plateaued thereafter. Concentrations reached approximately 10% of human plasma levels. Cholesterol lipoprotein profiles on day five resembled normolipidemic plasma but LDL was increased. The apolipoprotein composition of isolated particles was similar to lipoproteins isolated from human plasma. Detected proteins included APOB, APOA1 and APOE, as well as APOA2/A4/A5C2/C3/C4/F/M, ANGPTL3/8, CLUS, LCAT, CETP, PCSK9, and LPA. Our studies revealed the production of primarily triglyceride-rich LDL-sized particles on the first day with a gradual appearance of VLDL and HDL-sized particles over consecutive days. Infusion of MTTP in the perfusate attenuated the production of APOB and APOAI-containing lipoproteins.

Conclusions

During prolonged NMP, isolated human livers secrete cholesterol, triglycerides, (apo)lipoproteins and numerous proteins typically found in the systemic circulation. Perfusate lipoproteins resemble circulating lipoproteins in size and apolipoprotein composition. This is remarkable considering the absence of peripheral lipolysis of triglycerides in VLDL in this setting. Thus, the data suggests that the liver secretes LDL-sized particles. Inhibition of MTTP resulted in attenuation of the appearance of triglycerides and APOB. This study introduces the isolated perfused human liver as a new tool to explore lipoprotein metabolism.

Session F: Adipose Tissue Biology

25

T-cell-mediated control of adipocyte function by TGF-beta-Pmepa1 signaling

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Aim (background & objectives)

Adipocytes are critical for maintaining systemic energy balance and metabolic homeostasis, and their dysfunction promotes inflammation and metabolic disease. In obesity, adipocyte dysfunction is associated with fibrosis, with TGF-β signaling recognized as a key driver. Yet, the adipocyte-intrinsic and extrinsic mechanisms involved in TGF-β-mediated crosstalk remain incompletely understood. Using an epigenetic screen, we identified Pmepa1 (prostate transmembrane protein, androgen-induced 1), which has been described as a TGF-β–inducible protein, as a potential novel adipocyte regulator. Here, we investigate the role of Pmepa1 in adipocyte differentiation and function.

Method

Pmepa1 gain- and loss-of-function models were generated in immortalized mouse adipocytes and mice. Adipocyte differentiation and function were quantified by BODIPY-based lipid accumulation, β-adrenergic–stimulated lipolysis, and Seahorse respirometry measurements. TGF-β signaling activity was assessed by pSMAD2/SMAD2 immunoblotting. Bulk RNA sequencing defined downstream transcriptional programs. Human visceral adipose tissue single-nuclei RNA sequencing datasets across BMI ranges were analyzed to map *PMEPA1* expression and identify cellular sources of TGF-β.

Results

Our epigenetic screen identified Pmepa1 as a novel adipocyte gene. In humans, *PMEPA1* expression correlated with adiposity and insulin resistance. Single-nuclei RNA sequencing identified T cells as the predominant source of TGF-β in human adipose tissue in obesity. Functionally, loss of Pmepa1 reduced preadipocyte viability and, in mature adipocytes, led to diminished lipid accumulation, impaired β-adrenergic lipolysis, and reduced mitochondrial respiration, consistent with compromised metabolic capacity. Mechanistically, Pmepa1 was induced by TGF-β and functioned as a negative feedback regulator of TGF-β signaling.

Conclusions

Adipocyte Pmepa1 controls a negative feedback loop that dampens T cell-derived TGF-β action in obesity. Pmepa1 is important for adipocyte differentiation, lipid accumulation, supports β-

adrenergic lipolysis, and sustains mitochondrial respiration. Therapeutic targeting of Pmepa1 may dampen pathological TGF-β activity and improve adipose function and/or development in obesity.

26

Ribosomal control of adipose tissue adaptive metabolism

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Aim (background & objectives)

Ageing drives obesity and type 2 diabetes as adipose tissue loses plasticity. While mitochondrial and inflammatory defects are documented, the role of protein translational capacity in metabolic adaptation is under-explored. We hypothesized that ageing induces ribosomal protein heterogeneity, exhausting adipocyte translational reserve and limiting adaptive programs. This study characterizes how adipose ribosomal heterogeneity impacts translational capacity and hormonal responses during ageing and obesity.

Method

We performed polysome profiling on aged male murine adipose tissue, using LC-MS/MS to identify age-altered ribosomal protein (RP) heterogeneity. Following RP-X (40S candidate) identification, we conducted in vitro assays in male mouse and male/female human adipocytes using lentiviral KD/OE. Metabolic capacity was assessed via Seahorse flux, lipolysis, and glucose uptake assays. In vivo, adipocyte-targeted AAV (KD/OE) was used in lean and obese mice, followed by metabolic phenotyping (GTT/ITT) and indirect calorimetry.

Results

LC-MS/MS identified RP-X as a key determinant of translational reserve, showing significantly reduced expression in aged adipose tissue. In primary cells, RP-X reduction decreased nascent protein synthesis and delayed oxidative responses to metabolic stimulation. Notably, proximal beta3-adrenergic signaling remained intact, suggesting impaired adaptive execution rather than signal reception. RP-X KD in human adipocytes mirrored these findings, blunting maximal respiratory capacity. Conversely, RP-X OE in aged adipocytes restored glucose uptake and lipolytic responsiveness. In vivo, adipocyte-specific RP-X KD reduced thermogenic capacity and worsened glucose tolerance. Remarkably, RP-X OE in HFD-fed mice preserved muscle mass compared to controls, despite similar total fat mass.

Conclusions

These findings identify RP-X as a critical regulator of adipocyte translational plasticity. Age-related RP-X decline creates a bottleneck in metabolic execution, driving impaired thermogenesis and sarcopenic obesity. Targeting ribosomal heterogeneity represents a novel therapeutic strategy to restore metabolic flexibility and preserve lean mass in age-associated obesity.

27

Extracellular GPX3 drives insulin receptor expression and improves visceral adipose tissue insulin sensitivity in obesity

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Aim (background & objectives)

Obesity-associated insulin resistance is closely linked to adipose tissue dysfunction, oxidative stress, and impaired insulin receptor (InsR) signaling. Glutathione peroxidase 3 (GPx3) is a secreted selenoprotein highly expressed in white adipose tissue (WAT) whose expression is suppressed in obesity. While GPx3 has been associated with metabolic health, its mechanistic role in regulating adipose insulin sensitivity through extracellular redox activity remains poorly understood. We hypothesized that GPx3 constitutes an extracellular redox-regulatory node linking adipose tissue function to InsR expression and insulin signaling.

Method

Human WAT transcriptomic data from adiposetissue.org were used to find associations between *GPX3* expression and metabolic parameters. Male C57BL/6N mice were subjected to therapeutic selenium supplementation via a selenium-rich high-fat diet (SRHFD) for 20 weeks after established obesity, followed by assessment of WAT redox state, insulin signaling, and glucose tolerance. Mechanistic studies employed recombinant GPx3 (rGPx3) in 3T3-L1, visceral WAT explants, stromal vascular fraction-derived adipocytes, and adipocyte organoids. Transcription factor binding to the *Insr* promoter was assessed by chromatin immunoprecipitation.

Results

In humans, WAT *GPX3* expression showed strong positive correlation with markers of metabolic health, mirrored *INSR* expression across metabolic parameters, and increased dynamically following weight loss. In mice, prolonged selenite supplementation reduced adipocyte hypertrophy, decreased oxidative damage, and enhanced visceral WAT InsR expression, insulin-stimulated AKT phosphorylation, improving glucose tolerance. Mechanistically, physiological rGPx3 concentrations increased InsR expression across all adipocyte models tested, driven by a shift in intracellular glutathione redox balance that promoted SP1 binding to the *Insr* promoter. rGPx3 additionally enhanced adipogenic differentiation, glucose consumption, and oxygen consumption. Conversely, siRNA-mediated GPx3 knockdown impaired adipogenic differentiation.

Conclusions

We identify a GPx3-SP1-InsR signalling axis as a novel mechanism by which extracellular redox activity regulates adipose insulin sensitivity. GPx3 emerges as a paracrine mediator of metabolic health in adipose tissue, suggesting that targeting extracellular antioxidant pathways may offer therapeutic opportunities in obesity.

28

Adipose tissue dysfunction promotes obesity cardiomyopathy.

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Aim (background & objectives)

Obesity, a condition resulting from an excess of adipose tissue, is a serious health problem worldwide and an important factor in the development and progression of cardiovascular diseases. It is well established that mitochondrial dysfunction in adipose tissue (AT) might contribute to obesity-related diseases. However, the specific contribution of mitochondria dysfunction in AT to the development of CVD remains to be explored.

Method

For human population studies, two cohorts were used (OBECAN and PESA cohorts). Cardiac phenotype in mice lacking PGC1α specifically in adipose tissue (PGC1α^{AdipoKO}), was studied by echocardiography, proteomics and metabolomics. Turbo-ID experiments were used to find AT secreted factors that can contribute to cardiac disease. Recovery experiments were conducted by BAT transplant and specific silencing or overexpression of adipokines.

Results

We found that adipose tissue of obese patients showed a reduced expression of PGC1α, an important mitochondrial regulator. Echocardiographic analysis of mice lacking PGC1α specifically in adipose tissue fed with normal diet showed that these mice develop a cardiac dysfunction like the one observed during obesity, which can be reverted by brown adipose tissue (BAT) transplantation from C57BL/6 mice, pointing to the BAT as one of the main drivers of this phenotype. Proteomics analysis of plasma from mice lacking PGC1α in adipocytes and obese participants from PESA cohort revealed several promising adipokines and lipokines. Overexpression of two of these batokines was confirmed to be sufficient to revert the cardiac dysfunction observed in PGC1α^{AdipoKO} and in C57BL/6 mice fed HFD.

Conclusions

In this project, we specifically demonstrate that mitochondrial dysfunction in the adipose tissue is enough to cause the cardiac defect observed during obesity. Reverting the adipokine dysfunction is enough to protect these mice, suggesting the therapeutical potential of adipokines in the treatment of CVD.

29

A new intracellular trafficking complex that regulates insulin receptor signaling

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Aim (background & objectives)

In obesity, adipocyte dysfunction is a major contributor to systemic insulin resistance and type 2 diabetes. While defects in insulin receptor (IR) signaling are well established, increasing evidence implicates intracellular IR trafficking and recycling in its regulation. However, the intracellular organization underlying the sorting and stability of IR remains incompletely understood. We previously identified the adaptor protein SHB as a regulator of insulin signaling and adipocyte metabolism. Here, we investigate how SHB and its newly discovered binding partners proteins define a biochemically distinct compartment that contributes to IR sorting and stability.

Method

In cultured adipocytes, we silenced SHB and assessed the effects on the transcriptome and phosphoproteome, and cellular metabolism. Using pulldown proteomics and proximity ligation we identified potential SHB interaction partners. We performed subcellular fractionation to uncover intracellular localization of SHB and interaction partners. Immunoblotting and immunofluorescence microscopy was used to study the role of the SHB complex and its components in IR trafficking. In HEK293T, GFP-tagged IR and Flag-tagged SHB and mutants were overexpressed to define relevant interaction domains.

Results

SHB depletion altered insulin signaling and profoundly disrupted adipocyte metabolism. Notably, IR stability was increased upon SHB loss. We identified a protein complex comprising SHB and trafficking regulator proteins including EPS15, PLEKHF2 and TRIM21, that orchestrates ligand-stimulated IR endocytosis and sorting. Subcellular fractionation revealed that SHB and its interaction partners co-segregate in a distinct fraction that only partially overlaps with canonical endosomal and lysosomal markers. Depletion of individual components such as TRIM21 produced insulin signaling phenotypes similar to SHB loss, indicating functional convergence within the complex.

Conclusions

Our findings identify an SHB-associated protein complex that localizes to a biochemically distinct fraction and regulates IR sorting and stability in adipocytes. This uncovers a previously unrecognized level of organization within the intracellular trafficking with implications for metabolic regulation.

Session G: Brown Adipose Tissue and Thermogenesis

30

Developmental brown fat whitening upon Nfe2l1 loss in Myf5+ progenitors

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Aim (background & objectives)

Brown adipose tissue (BAT) development involves the differentiation and maturation of Myf5-derived preadipocytes into highly oxidative, thermogenic cells. While embryonic lineage commitment of brown adipocytes is well established, the transcriptional mechanisms governing acquisition and persistence of oxidative brown adipocyte programs during development remain incompletely understood. The transcription factor Nfe2l1 plays a pivotal role in proteasomal protein recycling via the ubiquitin–proteasome system (UPS), yet its contribution to brown adipocyte maturation and functional specification is unclear.

Method

Nfe2l1 was deleted in Myf5^{+} progenitors using a Myf5-Cre/loxP system. Bulk and single-nucleus RNA sequencing of BAT from wild-type and Nfe2l1-deficient mice were performed to resolve lineage-derived adipocyte states and transcriptional changes. In parallel, siRNA-mediated knockdown of Nfe2l1 in WT1 cells was used to assess cell-autonomous effects on adipocyte differentiation *in vitro*.

Results

Male and female Nfe2l1-deficient mice were born normally but exhibited reduced BAT mass, BAT whitening, and diminished non-shivering thermogenesis. Single-nucleus RNA-seq resolved multiple Myf5-derived adipocyte states, comprising basal brown, lipogenic, white-like, and oxidative brown adipocytes. Loss of Nfe2l1 shifted adipocyte state composition toward white like adipocytes with reduced brown adipocyte states, accompanied by macrophage-dominated immune infiltration. Pseudobulk analysis of oxidative adipocytes revealed selective suppression of mitochondrial oxidative phosphorylation and respiratory gene programs. Notably, acute Nfe2l1 depletion did not impair adipocyte differentiation *in vitro*, consistent with preserved adipogenesis despite impaired oxidative maturation.

Conclusions

These findings identify Nfe2l1 as a lineage-intrinsic regulator supporting development and persistence of oxidative brown adipocyte programs. Loss of Nfe2l1 is associated with BAT whitening and tissue remodeling, highlighting proteostatic regulation as an important contributor to functional heterogeneity within BAT. As ageing is linked to brown adipocyte involution in humans, it will be intriguing to investigate the Nfe2l1-proteasome system as a protective mechanism for sustaining BAT in adult humans.

31

Co-transcription factor MafG coordinates oxidative stress and lipid metabolism in brown adipocytes with NFE2L1 and NFE2L2

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Aim (background & objectives)

During brown adipocyte differentiation, effective adipogenesis depends on coordinated metabolic remodeling, including mitochondrial biogenesis, lipogenesis, and lipid storage. These processes are coordinated by gene expression programs, mitochondrial protein synthesis and import, as well as the maintenance of proteostasis and redox balance. NFE2L1 and NFE2L2 are key CNC-bZIP transcription factors, which require small Maf proteins (e.g., MafG) as obligate dimerization partners for activity. While NFE2L1 and NFE2L2 have established roles in proteostasis and redox balance, the contribution of MafG to these processes in adipocytes remains unknown.

Method

To investigate the role of MafG during adipogenesis, we performed knockdown (KD) in WT-1 brown adipocytes and assessed transcriptional and metabolic changes using RNA-seq, qPCR, and immunoblotting. Lipid droplet formation and mitochondrial activity were evaluated by fluorescence and immunofluorescence imaging. Cellular metabolism was analyzed using Seahorse assays to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Potential protein interactions of MafG were examined by co-immunoprecipitation (Co-IP).

Results

MafG knockdown led to downregulation of gene programs associated with adipogenesis, mitochondrial maturation and function, and proteasome activity. Consistently, early MafG depletion before differentiation resulted in reduced lipid droplet size in mature brown adipocytes, accompanied by altered expression of lipid droplet-associated markers, including CIDEA and PLIN1, indicating altered lipid metabolism. Furthermore, co-immunoprecipitation confirmed interactions between MafG and NFE2L1/NFE2L2, supporting its role as a functional partner of these transcription factors.

Conclusions

Collectively, these findings identify MafG as a key regulator linking proteostasis and mitochondrial function to brown adipocyte differentiation, revealing how coordinated cellular homeostasis supports lipid metabolism in adipogenesis. MafG-dependent pathways may therefore be relevant to dyslipidemia or other lipid metabolic disorders.

32

Thermogenic function of the hyperpolarization-activated cation channel HCN2 in brown adipocytes

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Aim (background & objectives)

Despite pronounced changes in glucose and lipid metabolism, ChREBP-deficient brown adipose tissue (BAT) in mice does not exhibit overt histological or functional changes. Notably, transcriptomics of ChREBP-deficient BAT revealed upregulation of cyclic AMP signaling molecules, including HCN2, a hyperpolarization-activated cation channel previously described as a regulator of electrophysiology in heart and brain. Here, we report studies addressing the regulation and functions of HCN2 in brown adipocytes.

Method

ChREBP-deficient mice were housed at 22°C or 6°C. Gene expression was assessed by bulk and single-nuclei RNA-seq. Brown adipocytes treated with siRNA or inhibitors were used to study cellular HCN2 functions by electrophysiology, qPCR, Westernblot and respirometry.

Results

Hcn2, hardly expressed in BAT of wild-type mice at 22°C, was strongly induced by chronic housing at 6°C, an effect enhanced by ChREBP deficiency. Hcn2 was well-expressed in primary brown adipocytes and functional, as shown by whole-cell patch clamping. Accordingly, knockdown of Hcn2 using siRNA or acute inhibition with the specific HCN2 inhibitor ZD7288 strongly suppressed hyperpolarization-induced currents. Notably, prolonged treatment with the HCN inhibitors ZD7288 or ivabradine caused marked suppression of thermogenic marker genes such as Ucp1, Ppargc1a and Prdm16. Finally, norepinephrine-induced hormone-sensitive lipase phosphorylation and oxygen consumption rate were strongly attenuated by acute treatment with ZD7288, indicating a mechanistic link of HCN2 channel activity to cyclic AMP signaling.

Conclusions

Previous work showing that adrenergic activation of brown adipocytes involves plasma membrane depolarization - and suggesting that this potentiates cyclic AMP signaling - is supported by our cell culture experiments. We propose that HCN2 induction in cold-adapted and ChREBP-deficient BAT lowers the threshold for cyclic AMP signaling by tilting the resting potential of brown adipocytes toward depolarization, thereby supporting thermogenesis. Currently, AAV-based tools for overexpression and knockout of Hcn2 *in vitro* and *in vivo* are developed to further address this hypothesis.

33

UBE2H Couples Brown Adipocyte Proteostasis to Metabolic Adaptation

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Aim (background & objectives)

Brown adipose tissue (BAT) is an adaptive metabolic organ supporting energy expenditure, lipid clearance, and systemic glucose homeostasis. To sustain thermogenesis, brown adipocytes coordinate mitochondrial activity, substrate handling, and protein quality control. Although ubiquitin-proteasome system regulation is recognized as important for BAT function, the ubiquitination enzymes coupling proteostasis to metabolic remodeling remain poorly defined. We investigated UBE2H, a BAT-enriched E2 ubiquitin-conjugating enzyme, as a candidate regulator of brown adipocyte thermogenic and hormonal responsiveness.

Method

Mature brown adipocytes were subjected to siRNA-mediated Ube2h knockdown followed by transcriptomic profiling, adrenergic stimulation, respiration analysis, insulin stimulation, qPCR, immunoblotting, and phosphoproteomic analysis. UBE2H-associated protein networks were identified by immunoprecipitation-mass spectrometry of tagged UBE2H. We studied patient-derived primary human fibroblasts carrying a mutation in the UBE2H gene, c.73G>T.

Results

UBE2H protein abundance was enriched in mouse BAT and increased during brown adipocyte differentiation. Loss of Ube2h induced transcriptional remodeling of pathways associated with fatty acid metabolism, mitochondrial function, and energy homeostasis. Functionally, Ube2h knockdown markedly enhanced adrenergic induction of the thermogenic program, as reflected by increased Ucp1 expression and UCP1 protein abundance. Ube2h loss reduced insulin-induced AKT phosphorylation, indicating impaired insulin responsiveness. Phosphoproteomic profiling further supported altered insulin-linked signaling upon Ube2h loss. Interaction proteomics identified multiple components of the CTLH E3 ligase complex among UBE2H-associated proteins, with increased enrichment after insulin stimulation. This suggests that UBE2H may act within a defined ubiquitin regulatory module in response to metabolic stimulation in brown adipocytes. In patient-derived fibroblasts, UBE2H protein levels appeared reduced, consistent with variant-associated protein instability.

Conclusions

These findings nominate UBE2H as a novel brown-fat-enriched regulator linking ubiquitin-dependent protein quality control to brown adipocyte hormone responsiveness, UCP1-centered thermogenic remodeling, and metabolic adaptation. We propose that the UBE2H-CTLH axis controls the turnover of key metabolic substrates in brown adipocytes, thereby shaping thermogenesis, lipid handling, and insulin sensitivity.

34

Cold-Induced Remodeling of Cholesterol Metabolism in Brown Adipose Tissue Links Sterol Homeostasis to Thermogenic Competence

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Aim (background & objectives)

Brown adipose tissue (BAT) drives adaptive thermogenesis and improves systemic lipid handling during cold exposure. While the roles of fatty acid uptake and oxidation in BAT are well established, the contribution of cholesterol homeostasis and terminal sterol biosynthesis remains incompletely understood. We therefore aimed to define how acute cold exposure remodels cholesterol metabolism in BAT and whether these changes functionally support thermogenic activation, with a particular focus on the cold-responsive sterol biosynthetic enzyme Dhcr24.

Method

We combined transcriptional and sterol profiling of cold-exposed murine BAT with functional studies in differentiated primary murine brown adipocytes. Dhcr24 was prioritized from comparative RNA-seq of mature brown adipocytes because of its cold responsiveness and established role in cholesterol biosynthesis. Functional analyses used siRNA-mediated Dhcr24 knockdown or pharmacological inhibition of the mevalonate/cholesterol pathway with atorvastatin under control and lipoprotein-depleted conditions. Readouts included RT-qPCR, immunoblotting, confocal microscopy, NEFA release assays, Seahorse extracellular flux analysis, and high-resolution Oroboros respirometry.

Results

Acute cold induced a coordinated cholesterol biosynthesis program in BAT, with increased Hmgcr, Sqle, Cyp51, Sc4mol, Hsd17b7, and Dhcr24, whereas Sc5dl and Dhcr7 were reduced. Sterol profiling showed increased lanosterol, dehydrodesmosterol, and 7-dehydrocholesterol, while total cholesterol remained unchanged, indicating remodeling of terminal sterol metabolism. In primary brown adipocytes, Dhcr24 knockdown increased Ucp1 and Dio2, altered PLIN1- and UCP1-related protein readouts, enhanced basal and norepinephrine-stimulated NEFA release, and increased stimulated oxygen consumption, maximal respiration, spare respiratory capacity, and oxygen flux in Seahorse and Oroboros assays. Pharmacological inhibition of the mevalonate pathway suppressed the thermogenic gene program and reduced respiratory capacity, particularly under lipoprotein-depleted conditions.

Conclusions

Cholesterol metabolism is dynamically remodeled during BAT activation. Our data suggest that sterol homeostasis supports thermogenic competence by coordinating lipid storage, substrate mobilization, and mitochondrial function, and identify DHCR24 as a candidate regulator linking terminal sterol metabolism to brown adipocyte physiology.

Session H: Metabolic Adaptation Across Organs

35

Intestinal lipid uptake: the role of basolateral lipid supply

PhD Melanie Korbelius[1], MSc. Laura Liesinger[2], Univ.-Prof. Ruth Birner-Gruenberger[2], PhD Karoline Fechter[1], Univ.-Prof. Dagmar Kratky[1,3]

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Aim (background & objectives)

Systemic lipid levels are balanced by the rate of intestinal lipid absorption, lipoprotein secretion, and lipoprotein clearance from the circulation. The small intestine forms the boundary between endogenous and exogenous lipids, thus significantly contributing to whole-body lipid homeostasis. Comparable with adipocytes, cells of the intestinal epithelium (enterocytes) can store excessive lipids in form of cytosolic lipid droplets (cLDs), which can be mobilized upon need to maintain energy supply to peripheral tissues in intraprandial periods. Recent data from our lab highlighted the existence of at least two different cLD pools deriving from different sources, i.e. the intestinal lumen or the circulation.

Method

To visualize intestinal lipid uptake not only from the diet (apical uptake), but also back from the circulation (basolateral uptake) *in vivo*, wildtype (WT) mice were administered either orally or intravenously with fluorescently-labelled lipids. While oral lipid supply resulted in fluorescence detection in stomach and intestine, intravenously administered lipids were solely deposited in the small intestine. To investigate this pathway in detail, mice were restricted to endogenous lipids by using a fat-free diet (FFD).

Results

In WT mice, FFD feeding compared to an acute dietary lipid challenge completely changed intestinal gene expression profile with a clear shift towards boosted cholesterol metabolism. However, we failed to detect cLDs in enterocytes of FFD-fed WT mice, indicating a rapid turnover of basolaterally absorbed lipids. However, FFD feeding of mice lacking the major cytosolic triglyceride lipase ATGL together with its coactivator CGI-58 exclusively in enterocytes (Atgl/Cgi-58 iDKO), resulted in massive cLD accumulation in enterocytes, thereby identifying ATGL as a critical player in catabolizing cLDs of endogenous origin.

Conclusions

In summary, our data provides new insights into intestinal lipid uptake from the basolateral side, an underestimated mechanism which possibly influences circulating plasma lipid levels and represents a potential pharmaceutical target for treating hyperlipidemia in the future.

36

Myocyte Nfe2l1-proteasome function links dietary protein to energy metabolism

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Aim (background & objectives)

Skeletal muscle mass and function are critical determinants of metabolism. Adaptation to energetic challenges, such as dietary protein availability is regulated by mTORC1 signaling. As a potential downstream effector of mTORC1, Nuclear factor erythroid 2-like-1 (Nfe2l1) coordinates protein quality control with amino acid recycling by regulating proteasome function. However, its role in muscle adaptation to dietary protein fluctuations remains unclear. Here, using conditional transgenic mice, we investigate the contribution of Nfe2l1-mediated proteasomal amino acid recycling to the interplay between dietary protein, muscle function, and physical capacity.

Method

Tamoxifen-inducible Acta1-CreERT2 Nfe2l1 knockout mice (mKO) and Cre-negative littermates were analyzed under chow diet and 4-weeks leucine restriction by indirect calorimetry, treadmill, and grip-strength analysis. Body composition was assessed by EchoMRI. Tissue morphology, mitochondrial oxidative capacity, and fiber type composition were evaluated using histological, histochemical, and immunofluorescent staining. Molecular changes were examined by transcriptional and translational profiling of gastrocnemius muscle (GC). Proteasomal activity was assessed using fluorometric- and in-gel activity assays, combined with a protein ubiquitination assay. Cell-autonomous effects were analyzed in C2C12 mouse myocytes via siRNA-mediated Nfe2l1 knockdown.

Results

Loss of Nfe2l1 induced a fast-to-slow fiber-type switch and resulted in lean phenotype. mKO mice displayed aberrant mitochondrial bioenergetics in GC. Nfe2l1 deficiency reduced proteasome activity, reshaped ubiquitin landscapes, and impaired protein turnover through limited amino acid recycling *in vivo and in vitro*. The importance of Nfe2l1 in myocyte adaptive capacity is underscored by upregulation of mTORC1 signaling. Interestingly, under leucine restriction, control and Nfe2l1-deficient mice maintained body weight due to reduced energy expenditure and food intake.

Conclusions

Nfe2l1 deficiency functionally mimics dietary leucine restriction likely by limiting proteasome-dependent amino acid recycling. Our findings highlight the intrinsic link of nutritional stress with myocyte biology and physical adaptation. Thus, Nfe2l1 may offer opportunities for personalized nutritional and therapeutic strategies targeting obesity-linked metabolic diseases.

37

From injury to degeneration: delayed lipid-mediated neurotoxic mechanisms in an in-a-dish astrocyte model of repeated mild traumatic brain injury

Dr Luise Schlotterose[1], Dr Michel Struwe[2], Professor Axel Scheidig[2], Bsc. Niek Blomberg[3], Professor Martin Giera[3], Professor Kirsten Hattermann[4], Dr Mootaz Salman[1]

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Aim (background & objectives)

Traumatic brain injury (TBI) is a leading cause of long-term disability and death, often triggering progressive brain cell loss that impairs motor function and cognition. Especially repeated mild TBI (rmTBI) can leave lasting neurological effects and increase the risk of neurodegenerative diseases. Despite its prevalence, current treatments largely manage symptoms, with few options targeting the underlying cellular mechanisms. Astrocytes and neurons are key responders to brain injury: astrocytes maintain homeostasis, while neurons influence astrocytic activity. However, molecular crosstalk between them after rmTBI remains poorly understood.

Method

To address this, we have developed an in vitro model using primary human brain cells. These cells are cultured in a modular system that mimics rmTBI by applying mechanical injury to replicate trauma. This model enables the study of how astrocyte-neuron interactions influence injury progression and facilitates the discovery of therapeutic targets.

Results

We reproduced key features of TBI, including oxidative stress, and cellular swelling. In addition, we detected increased lipid droplet accumulation in injured astrocytes, along with elevated levels of apolipoprotein J (Clusterin) in the conditioned medium. These observations prompted a lipidomics analysis of the astrocyte-conditioned medium, through which we identified with Diacylglycerols (DG) a specific lipid species that was increased following injury – a known activator of protein kinase C (PKC). Given the potential neurotoxicity of astrocytes, we evaluated the effects of conditioned medium from injured astrocytes on human neurons. We observed decreased neuronal functionality and increased apoptosis which could be reversed by inhibiting PKC. These results indicate the DG-PKC-axis as a potential mechanism by which injured astrocytes induce neurotoxicity.

Conclusions

By focusing on the dynamic interplay between astrocytes and neurons, this research offers new insights into the pathophysiology of TBI. It also provides a powerful platform for identifying novel drug targets and signalling pathways, ultimately advancing strategies to improve brain health and recovery following TBI.

38

ANGPTL3 Deficiency Reveals Divergent Oxidative Remodeling in Skeletal Muscle and Heart

Dr Ottavia Terenghi, Dr Lorenzo Da Dalt, Ms Giulia Giancane, Ms Laura Gullà, Dr Annalisa Moregola, Ms Patrizia Uboldi, Prof Fabrizia Bonacina, Prof Giuseppe Danilo Norata

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Aim (background & objectives)

Angiopoietin-like 3 (ANGPTL3) inhibits capillary lipases and regulates systemic lipid trafficking. ANGPTL3 deficiency causes profound hypolipidemia by relieving inhibition of capillary lipase activity, thereby altering lipid substrate availability to peripheral oxidative tissues, including skeletal muscle and heart. However, how chronic remodeling of circulating lipid flux affects tissue-specific oxidative metabolism remains unclear. We investigated the metabolic and transcriptional consequences of ANGPTL3 deficiency in skeletal muscle and heart.

Method

Angptl3-deficient (KO) mice and WT littermates were fed a chow diet for 16 weeks. In vivo metabolic phenotyping included lipid, glucose, insulin, pyruvate, and lactate tolerance tests, together with assessment of hepatic lipoprotein production after Poloxamer injection. Skeletal muscle and heart were analyzed ex vivo by metabolomics and bulk RNA sequencing.

Results

Angptl3KO mice displayed marked baseline hypolipidemia, blunted lipid excursion after oral fat load, and reduced hepatic lipoprotein production 4 hours after Poloxamer injection, without overt hepatic steatosis or detectable ectopic lipid accumulation. In skeletal muscle, transcriptomic analysis revealed enrichment of lipid, steroid, amino acid, and α-ketoglutarate metabolism, together with increased oxidative phosphorylation. Peroxisomal protein import and FAK signaling were also upregulated, while actin-associated contractile programs were reduced, suggesting enhanced mitochondrial oxidative programming and focal adhesion signaling but attenuated cytoskeletal remodeling. In contrast, the fasted heart showed upregulation of cholesterol import and protein metabolism, accompanied by downregulation of lipid metabolic pathways and negative enrichment of oxidative phosphorylation and fatty acid oxidation.

Conclusions

ANGPTL3 deficiency elicited divergent metabolic transcriptional responses in skeletal muscle and heart. Skeletal muscle showed enhanced oxidative, lipid-related, peroxisomal, and focal adhesion programs, consistent with adaptive oxidative and mechano-metabolic remodeling. Conversely, the fasted heart displayed reduced fatty acid oxidation and oxidative phosphorylation signatures. These findings uncouple systemic hypolipidemia from ectopic lipid accumulation and suggest that ANGPTL3 loss reshapes substrate handling and energetic adaptation in a tissue-specific manner.

Poster session 1 (P1-P14)

P1. Min Mao, Munich, Germany*
Stabilization of adipocyte beta-catenin is a hallmark of proteasome dysfunction-linked lipodystrophy

P2. İlker Gümüş, Potsdam, Germany*
Investigation of molecular dynamics of caveolae in diet-induced obesity

P3. Alba Mena Gómez, Munich, Germany*
Chemerin-derived peptides regulate adipocyte inflammation and atherogenesis

P4. Angelina Markshausen, Düsseldorf, Germany
Mechanistic aspects of aldehyde dehydrogenases driving sex-specific disturbances in adipose tissue development by prenatal obesogenic exposure

P5. Asude Berber, Graz, Austria*
Reciprocal modulation of adipose tissue remodeling by cancer cachexia and cold exposure

P6. Marie Albrecht, Hamburg, Germany*
Maternal lipidome is shaped by maternal overweight and obesity during pregnancy

P7. Habiba Hayat, Prague, Czech Republic*
Role of Von Willebrand Domain-containing Protein 8 (Vwa8) in lipid metabolism and brown and beige adipocyte thermogenesis

P8. Ludger Scheja, Hamburg, Germany
Mechanisms underlying increased lipoprotein lipase activity in ChREBP-deficient brown adipose tissue

P9. Pablo Fernández, Alcorcón, Spain*
A novel role for CLDN1 in the metabolism and thermogenesis of brown adipocytes

P10. Maliheh Nazari Jahantigh, München, Germany
Myeloid let-7b Exacerbates Atherosclerosis

P11. Marta Rojas Torres, Seville, Spain*
Menopause as a turning point: sex-specific inflammatory proteomic signatures of subclinical atherosclerosis

P12. Menno Hoekstra, Leiden, the Netherlands
Cationic liposome-mediated delivery of T0901317 to macrophages fails to reduce atherosclerosis susceptibility in male apolipoprotein E knockout mice

P13. Clara Rossi, Milan, Italy*
Simvastatin modulates the pro-angiogenic secretome of senescent vascular smooth muscle cells in atherosclerosis

P14. Malena Diaz, Graz, Austria*
Consequences of lysosomal acid lipase overexpression on atherosclerosis

Poster session 2 (P15-P30)

P15. Iris Lahdeniemi, Helsinki, Finland
Effect of high intensity statin medication on circulating foamy monocytes in healthy individuals

P16. Lorenzo Da Dalt, Milan, Italy
OPA1 Drives Organ-Specific Metabolic Reprogramming in the Liver and Heart to Prevent Diet-Induced Insulin Resistance

P17. Mariia Khamdan, Munich, Germany*

Regulation of MAFLD by the nuclear ubiquitin-proteasome system

P18. Dinah Remo, Paris, France*
ABCG1-mediated protection from hepatic lipotoxicity reduces MASH and atherosclerosis

P19. Umesh Tharehalli Mathada, Groningen, the Netherlands
Hepatic loss of VPS35 aggravates diet-induced Metabolic dysfunction-associated steatohepatitis (MASH)

P20. Ilaria Micallo, Amsterdam, the Netherlands*
SPRING sets SREBP apart: specificity and evolution of a key regulatory axis

P21. Aldo Grefhorst, Amsterdam, the Netherlands
Liver sinusoidal endothelial cells protect hepatocytes against steatosis via paracrine factors

P22. Ankia Visser, Groningen, the Netherlands*
Hepatic VPS41 plays an essential role in systemic cholesterol homeostasis

P23. Jesús Roca, Seville, Spain*
Altered Metabolomic and Monocyte Transcriptional Profiles Reshapes the Menopausal Inflammatory Landscape in Healthy Women

P24. Eija Laakkonen, Jyväskylä, Finland
Systemic Metabolic Effects of Finnish Sauna Bathing Assessed by NMR-Based Metabolomics

P25. Carlos Caballero, Madrid, Spain*
High-unsaturated-fat diet prevents cardiac dysfunction and modulates the metabolic switch associated with anthracycline-induced cardiotoxicity in a porcine model

P26. Krzysztof Czamara, Kraków, Poland
Dissociation between early recovery of systemic glucose tolerance and delayed perivascular adipose tissue-linked reversal of endothelial dysfunction after HFD withdrawal

P27. Sharon Angelini, Ferrara, Italy*
Extracellular vesicles released by palmitic acid- but not TNFα-treated adipocytes induce skeletal muscle insulin resistance

P28. Andrej Gorbatenko, Amsterdam, the Netherlands
Pharmacological Modulation of Stearoyl-CoA Desaturase expression in metabolic disorders

P29. Claudia Greco, Parma, Italy*
Relationship between cholesterol accumulation and cellular senescence in an in vitro model of vascular cells

P30. Lorenzo Arnaboldi, Milano, Italy
Different size-separated subfractions of extracellular particles secreted by four tumor cell

lines reveal distinct lipid-protein signatures and latent proteomic cargo programs, suggesting specific biological effects

Poster session 3 (P31-P49)

P31. Giorgia Marodin, Padua, Italy*
Investigating the interplay between the E3 ubiquitin ligases IDOL and RNF130 in LDLR degradation

P32. Katharina Barbara Küntzel, Copenhagen, Denmark
Bridging lipid classes: N-acyl taurines at the interface of fatty acid and bile acid metabolism

P33. Ivan Bradić, Odense, Denmark*
Activation of vesicular transport pathways clears lysosomal cholesterol

P34. Jalil Daher, Balamand, Lebanon
Myeloperoxidase-oxidized LDL: a molecular bridge linking atherosclerosis, depression and psoriasis?

P35. Jennifer Härdfeldt, Stockholm, Sweden
Effects of prolonged fasting and sleep deprivation on triglyceride-rich lipoprotein remodelling in healthy adults

P36. Antoine Rimbert, Nantes, France
Can we predict cardiovascular risk in FH-patients using polygenic predispositions?

P37. Paula Cabré Fernandez, Barcelona, Spain*
HDL Proteomic Signatures of Cardiovascular Resilience in Familial Hypercholesterolemia

P38. Alina Iakubovskaia, Helsinki, Finland*
The effects of myocardial infarction recovery and anti-inflammatory drug on LDL composition and atherogenic properties in NSTEMI patients

P39. Francesca Zimetti, Parma, Italy
Apolipoprotein E4 drives pcsk9 upregulation and amplifies pcsk9-mediated impairment of neuronal cholesterol homeostasis: implications for Alzheimer's disease

P40. Zohre Pourkarami, Helsinki, Finland*
Expansion of in-depth functional characterization of LDLR gene variants and implications for cardiovascular risk assessment

P41. Mathilde Varret, Paris, France
LIPA gene variants and familial hypercholesterolemia

P42. Sif Grau Kaad, Copenhagen, Denmark*
Exploring the role of ApoM in heart faillure with preserved ejection fraction induced by chronic kidney disease in vivo

P43. Katsuyuki Nakajima, Maebashi, Japan
Remnant clearance capacity explains the clinical divergence of severe hypertriglyceridemia

P44. Giosiana Bosco, Catania, Italy*
Effect of inclisiran on lipid and mechanical vascular profiles in familial hypercholesterolemia subjects: results from a single lipid center real-world experience

P45. Lucia Sanchez Schneider, Copenhagen, Denmark*
Whole genome sequencing and variant effect predictions across diverse ancestries reveal the full functional spectrum of APOE variation

P46. Simon Pfisterer, Helsinki, Finland
Functional data for LDLR variant classification: comparative insights from high-content microscopy and flow-cytometry assays

P47. Miklas Larsen, Odense, Denmark*
Single-cell analysis of calcium signaling in macrophages reveals activation by aggregated LDL and modulation by neurosteroid

P48. Liv Rabøl Andersen, Copenhagen, Denmark*
Impact of female reproductive factors on lipids and lipoproteins

P49. Vibeke Akkerman, Odense, Denmark*
Quantitative live-cell imaging of Low-Density Lipoprotein reveals the role of neurosteroids in macrophage foam cell formation

Poster presenters eligible for YIA

Poster #	Name	City	Country
P1	Min Mao	Munich	Germany
P2	İlker Gümüş	Potsdam	Germany
P3	Alba Mena Gómez	Munich	Germany
P5	Asude Berber	Graz	Austria
P6	Marie Albrecht	Hamburg	Germany
P7	Habiba Hayat	Prague	Czech Republic
P9	Pablo Fernández	Alcorcón	Spain
P11	Marta Rojas Torres	Seville	Spain
P13	Clara Rossi	Milan	Italy
P14	Malena Diaz	Graz	Austria
P17	Mariia Khamdan	Munich	Germany
P18	Dinah Remo	Paris	France
P20	Ilaria Micallo	Amsterdam	The Netherlands
P22	Ankia Visser	Groningen	The Netherlands
P23	Jesús Roca	Seville	Spain
P25	Carlos Caballero	Madrid	Spain
P27	Sharon Angelini	Ferrara	Italy
P29	Claudia Greco	Parma	Italy
P31	Giorgia Marodin	Padua	Italy
P33	Ivan Bradić	Odense	Denmark
P37	Paula Cabré Fernandez	Barcelona	Spain
P38	Alina Iakubovskaia	Helsinki	Finland
P40	Zohre Pourkarami	Helsinki	Finland
P42	Sif Grau Kaad	Copenhagen	Denmark
P44	Giosiana Bosco	Catania	Italy
P45	Lucia Sanchez Schneider	Copenhagen	Denmark
P47	Miklas Larsen	Odense	Denmark
P48	Liv Rabøl Andersen	Copenhagen	Denmark
P49	Vibeke Akkerman	Odense	Denmark

Poster session 1 (P1-P14)

P1

Stabilization of adipocyte beta-catenin is a hallmark of proteasome dysfunction-linked lipodystrophy

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Aim (background & objectives)

Adipose tissue formation and expansion are essential for metabolic homeostasis, and dysfunction of the ubiquitin–proteasome system (UPS) impairs adipogenesis and contributes to systemic metabolic disorders. However, the mechanisms by which the UPS regulates adipocyte function remain unclear. Here, we investigate the role of the Wnt signaling effector beta-catenin in the reciprocal interplay with proteasome activity and its impact on adipogenesis and lipid metabolic homeostasis.

Method

In vivo, human RNA-seq datasets and adipocyte-specific Nfe2l1 knockout mice (both sexes) were used to assess the impact of proteasome dysfunction on canonical Wnt signaling and systemic metabolic homeostasis. In vitro, we used 3T3-L1 cells and primary preadipocytes to assess the effects of genetic and pharmacological inhibition of proteasome activity on adipogenesis and lipid metabolism. To further define the role of beta-catenin, we applied the beta-catenin antagonist iCRT3 and RNAi-mediated knockdown of Ctnnb1, which encodes beta-catenin, and examined its impact on adipogenesis and the UPS.

Results

Adipoq-Cre Nfe2l1 knockout mice showed age-dependent lipodystrophy and severe insulin resistance in vivo, with stronger effects in females and in subcutaneous versus visceral adipose tissue. Furthermore, flow cytometry revealed impaired adipogenesis, with an increased proportion of adipocyte progenitors within the stromal vascular fraction. In vitro, proteasome inhibition or RNAi-mediated knockdown of Psmb4 maintained higher active beta-catenin levels and suppressed adipogenic gene induction during differentiation. Silencing Ctnnb1 or treatment with iCRT3 effectively restored adipogenesis under proteasome-inhibited conditions, highlighting a critical role for beta-catenin in UPS-mediated regulation of adipogenesis. Moreover, reduction of beta-catenin altered ubiquitin levels and proteasome activity, supporting a functional link between beta-catenin and the UPS.

Conclusions

Dysfunction of the UPS stabilizes beta-catenin activity, thereby activating canonical Wnt signaling, and inhibiting adipogenesis. Our findings suggest that targeting proteasome-mediated beta-catenin regulation may provide a novel therapeutic approach to promote adipogenesis and

improve lipid metabolism in individuals with adipocyte dysfunction or proteasome-related lipodystrophy.

P2

Investigation of Molecular Dynamics of Caveolae in Diet-Induced Obesity

Msc Ilker Gümüs

Nutritional Science, Potsdam, Germany

Aim (background & objectives)

Caveolae are 50–100 nm plasma membrane invaginations essential for signaling and lipid homeostasis. This study investigates how caveolar movement and density are remodeled during obesity. Specifically, we aim to: (I) map the spatial distribution of caveolar proteins across tissues, and (II) identify cellular steps in caveolae-mediated lipid uptake to clarify their role in metabolic pathogenesis.

Method

Male C57BL/6J mice were fed standard chow, a high-carbohydrate diet (HCD), or a 40% high-fat diet (HFD) for 1, 4, 8, and 16 weeks. Adiposity was monitored via NMR. Quantitative biochemistry (Western Blot, qPCR) was performed on white adipose tissue (WAT), brown adipose tissue (BAT), heart, and skeletal muscle. Tissues were stabilized via cardiac perfusion for immunofluorescence (IF) and transmission electron microscopy (TEM) to visualize membrane dynamics.

Results

HFD-fed mice showed significant weight gain after one week and increased fat-to-lean mass ratios from one month onward. No significant changes occurred in HCD or chow groups, identifying dietary fat as the primary driver of obesity. Molecular remodeling was tissue-specific: BAT acted as an early responder, upregulating Cav1 and Cavin1 at one month. Conversely, WAT showed a delayed response, peaking at four months. In chronic obesity, WAT exhibited a protein-mRNA mismatch; while Cav1 and Cavin1 protein levels rose, mRNA levels remained stable. Crucially, the stabilizing proteins EHD2 and Cavin2 were significantly downregulated in WAT, suggesting a loss of structural integrity. Skeletal muscle and heart remained largely unaffected.

Conclusions

Diet-induced obesity triggers complex, lipid-driven caveolar remodeling. The late-stage downregulation of EHD2 and Cavin2 in WAT indicates a transition from functional to dysfunctional caveolae. This suggests caveolar integrity is uniquely sensitive to dietary fats, providing a mechanistic link between membrane dynamics and the pathogenesis of obesity.

P3

Chemerin-derived peptides regulate adipocyte inflammation and atherogenesis

M. Sc. Alba Mena Gómez[1,2,3], B.Sc. Glykeria Rovatsou[1,2], M. Sc. Julia Schulz[4], Prof. Dr. Yvonne Döring[3,4], Prof. Dr. Alexander Bartelt[1,2,3]

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Aim (background & objectives)

Obesity-associated inflammation is a central driver of atherosclerosis, with adipose tissue acting as a key immunometabolic regulator. Perivascular adipose tissue (PVAT) modulates vascular function through the secretion of adipokines and inflammatory mediators. The chemerin/ChemR23 axis has emerged as an important signaling pathway at the interface of metabolism and immunity, yet its adipocyte-intrinsic role in regulating inflammatory responses remains incompletely understood. This study aimed to address this gap using controlled in vitro approaches in adipocytes.

Method

Mouse 3T3-L1 and primary adipocytes were used to dissect the functional role of ChemR23 signaling under inflammatory conditions. Cells were stimulated with distinct chemerin isoforms (chemerin-9 and chemerin-15) or lipopolysaccharide (LPS) to model inflammatory activation. To specifically interrogate the contribution of ChemR23, both genetic and pharmacological approaches were employed: receptor expression was silenced using siRNA targeting Cmklr1, and receptor activity was inhibited using the small-molecule antagonist α-NETA. Inflammatory responses were assessed by quantifying the expression and secretion of cytokines and chemokines at the mRNA and protein levels.

Results

Activation of adipocytes with inflammatory stimuli confirmed a robust induction of cytokine and chemokine expression. Pharmacological inhibition of ChemR23 using α-NETA attenuated inflammatory responses, particularly under LPS stimulation, supporting a role for ChemR23 signaling in adipocyte inflammation. Treatment with distinct chemerin isoforms further supported a modulatory role of this pathway, suggesting ligand-dependent effects on inflammatory signaling. Complementary genetic targeting of Cmklr1 provided additional evidence for the involvement of ChemR23 in inflammatory modulation in adipocytes.

Conclusions

These findings suggest that ChemR23 contributes to the regulation of adipocyte inflammatory responses, with its inhibition leading to a reduction in inflammation. This supports the concept that the chemerin/ChemR23 axis contributes to adipocyte-driven inflammatory signaling and

may represent a potential target to modulate adipose tissue inflammation in cardiometabolic disease.

P4

Mechanistic aspects of aldehyde dehydrogenases driving sex-specific disturbances in adipose tissue development by prenatal obesogenic exposure

M.Sc. Angelina Markshausen[1], Dr.rer.nat. Marten Schouwink[1], Dr.rer.nat. Bengt Belgardt[2,3], Dr.med. Regina Ensenauer[4]

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Aim (background & objectives)

Maternal obesity before and during pregnancy causes overweight/obesity in the offspring. Our NMRI mouse model of obesity in pregnancy indicated female-specific fetal programming effects on adipocyte development, with aggravated offspring obesity and hyperglycemia upon postnatal high-calorie feeding. Analysis of *ex vivo* differentiated preadipocytes of female mouse embryos confirmed epigenetic changes evident in genes associated with fat cell differentiation. Further, proteomics data pointed to a dysregulation in fatty acid and lipid metabolic processes. Notably, transcription of aldehyde dehydrogenase (Aldh) 1a7 was downregulated, which persisted until adulthood. In humans, the closest homologue to Aldh1a7 is Aldh1a1. We hypothesize that human Aldh1a1 is possibly functionally similar to Aldh1a7, linking prenatal obesogenic exposure to the subsequent adipose tissue disturbances in offspring later in life.

Method

To elucidate the mechanistic role of Aldh1a7 and Aldh1a1 in adipocyte development, we knocked down both candidates in the murine 3T3-L1 and the human white preadipocyte cell model SGBS, respectively. Both cell lines underwent adipocyte differentiation, and were at different stages of differentiation analyzed by qPCR, western blot, and immunostaining.

Results

Aldh1a7 knockdown in 3T3-L1 cells impaired early adipogenesis, with upregulated mRNA expression of genes that maintain the preadipocyte state, including Aldh1a1. Knockdown of Aldh1a1 in human SGBS cells strongly reduced lipid droplet formation and mRNA expression of early transcription factors within the first 24 h of differentiation, without isoform upregulation. Supplementing retinoic acid (RA), normally provided by Aldh1a1, did not fully restore adipogenesis, and antagonizing RA receptor signaling had only moderate adipogenesis-reducing effects.

Conclusions

These data show that Aldh1a1 is essential in early human adipocyte differentiation, similar to Aldh1a7 in a murine environment, probably independent of RA. Further research involving female and male human adipose derived stem cells will help clarifying the underlying

mechanistic aspects of the dysregulation of Aldh isoforms and the female-specific impaired adipocyte phenotype.

P5

Reciprocal modulation of adipose tissue remodeling by cancer cachexia and cold exposure

PhD student Asude Berber[1], Postdoctoral Researcher Isabella Pototschnig[1], Lab technician Sandra Eder[1], PhD Thomas Rauchenwald[1], PhD Student Michaela Lang[1], Professor Martina Schweiger[1,2]

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Aim (background & objectives)

Aim: Cancer-associated cachexia (CAC) is a multifactorial metabolic syndrome characterized by severe loss of muscle and fat mass. Tumor-derived factors and an altered immune cell profile contribute to systemic inflammation and catabolic reprograming of muscle and adipose tissue (AT). Adipose tissue macrophages (ATMs) are central to tissue homeostasis and regulate AT catabolic remodeling upon cold exposure in mice, a condition associated with reduced inflammation and improved metabolic homeostasis. The study aims to investigate how CAC changes ATM phenotypes and whether cold exposure, by being an anti-inflammatory trigger, prevents CAC-induced catabolic remodeling of AT.

Method

Methods: CAC was induced by injecting $1x10^6$ cancer cells (CHX207 or LLC) into the right hindlimb muscle of male C57BL6/J mice. Adipose tissue macrophages (ATMs) were isolated from subcutaneous white adipose tissue (sWAT) of pre-cachectic tumor-bearing and control (PBS-injected) mice, and analyzed by bulk RNA sequencing. To evaluate the impact of cold exposure, tumor-bearing mice were maintained under cold (16°-4°C) conditions from the onset of measurable tumor growth until the experimental endpoint.

Results

Results: Transcriptomic analyses revealed clear changes in ATM gene expression patterns indicating early immune reprogramming in tumor-bearing mice, with an enrichment of adaptive immune-related pathways and pathways associated with extracellular remodeling. Although cold exposure did not prevent AT loss in tumor bearing mice, sWAT showed a reduction in inflammatory markers' gene expression compared to tumor bearing mice housed at room temperature. Interestingly, CAC completely diminished the ability to induce Uncoupling Protein 1 (*Ucp1*) expression in sWAT of cold exposed tumor bearing mice.

Conclusions

Conclusion: My data so far show that ATMs gene expression changes during CAC. Cold exposure interferes with CAC-induced transcriptional changes and vice versa.

P6

Maternal lipidome is shaped by maternal overweight and obesity during pregnancy.

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Aim (background & objectives)

Maternal overweight and obesity (OWO) predispose to pregnancy complications and are linked to, e.g., cardiovascular disease later in the life of both mother and offspring. Dyslipidemia is highly prevalent in OWO individuals but little is known about specific, OWO-driven lipidome changes during pregnancy and childhood. This study aimed to identify differences in the serum lipidome between pregnant women with OWO or normal weight (NW), and their children from birth to ten years of age. Furthermore, we aimed to assess a ceramide-based risk score (CERT) that predicts cardiovascular events later in life.

Method

Serum lipidome analysis was performed in 98 pregnant participants (58 NW, 40 OWO) from a longitudinal birth cohort study at three timepoints during pregnancy and childhood, respectively. 717 lipid species across 16 lipid classes were consistently quantified using mass spectrometry. Differences in lipid species abundance between NW and OWO groups were assessed using FDR-corrected Wilcoxon rank-sum tests. CERT score was calculated from ceramide species based on a published reference and groups were compared using adjusted multiple linear regression. Child's sex (58 male, 40 female) was considered in adjusted models.

Results

Serum lipid class concentrations showed dynamic changes throughout pregnancy in both NW and OWO groups, with increases for most lipid classes. When considering individual lipid species, the top elevated lipids in OWO vs. NW were triglycerides, most of which carried fatty acid 20:4 or 20:5. Conversely, lipid species most attenuated in OWO vs. NW were phospholipids carrying fatty acid 18:2. Ceramide concentrations did not differ between groups at the class level, but CERT continued to rise throughout pregnancy with significantly higher values in OWO mothers. No group differences were found in children, and child's sex did not affect CERT.

Conclusions

Pregnancy is characterized by a rapidly changing lipidome which could serve as a potential marker for cardiovascular risk later in life.

P7

Role of Von Willebrand Domain-containing Protein 8 (Vwa8) in lipid metabolism and brown and beige adipocyte thermogenesis

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Aim (background & objectives)

Obesity is a worldwide health problem for whole population and promotes other metabolic problems such as diabetes, metabolic syndrome and cardiovascular disorders etc. Increasing energy expenditure via brown fat thermogenesis and fatty acid oxidation (FAO) is considered as a potential mechanisms for the management of obesity. Here we focused on poorly characterized mitochondrial and peroxisomal protein Vwa8, a putative AAA+ ATPase with a dynein conformation.

Method

To study the role of Vwa8 protein in the context of browning and mitochondrial fatty acid oxidation, we developed Vwa8 KO mice. We used male mice in our studies.

Results

The Vwa8 KO mice showed higher heat production as well as decreased respiratory exhange ratio, indicating a stronger preference for FAO at the end of the resting phase of circadian rhythm, However the overall oxygen consumption remain unchanged in brown adipose tissue.KO mice showed browning of the subcutaneous adipose tissue (sWAT) indicated by increase in mitochondria content and lipid droplet multilocularity. This was also confirmed by increase UCP1 content and increase lipolysis in beige adipocyte differentiated from KO sWAT vascular stromal fraction. However, beta adrenergic stimulation by CL316243 showed only a slight increase of energy expenditure in Vwa8 KO mice (50 % of basal), while WT mice showed almost four times increase in energy expenditure

Conclusions

In conclusion, Vwa8 affects mitochondrial substrate preference and increase browning in sWAT and may involve in canonical BAT thermogenesis and brown fat recruitment.

P8

Mechanisms underlying increased lipoprotein lipase activity in ChREBP-deficient brown adipose tissue

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Aim (background & objectives)

De novo lipogenesis enzymes are largely controlled by carbohydrate response element-binding protein (ChREBP) in murine brown adipose tissue (BAT). Accordingly, ChREBP-deficient BAT exhibits markedly diminished endogenous fatty acid (FA) synthesis, without, however, leading to altered BAT lipid content or overt defects in thermogenesis. As a potential compensating mechanism, we observed increased uptake of FA from triglyceride-rich lipoproteins (TRL). Here, we describe experiments addressing mechanisms underlying the increased TRL disposal in BAT of ChREBP-deficient mice.

Method

Mice housed at various temperatures were used for gene expression analysis, immunohistochemistry and metabolic flux studies with radioactive tracers. Protein abundance was determined by Westernblot and proteomics. AAV vectors were employed for genetic interventions in primary brown adipocytes.

Results

BAT-specific ChREBP knockout mice exhibited increased FA uptake from circulating TRL compared to control mice when housed at 22°C or 6°C. FA uptake closely correlated with lipoprotein lipase (LPL) on BAT capillaries, as assessed by immunohistochemistry. Mechanistically, the LPL induction in ChREBP-deficient BAT was linked to increased Lpl mRNA expression. Furthermore, expression of known posttranslational LPL regulators was altered: the LPL chaperone LMF1 was increased and the LPL modulator Angptl8 was decreased whereas Angptl4 was unaltered. Surprisingly, mRNA expression of the LPL inhibitor apolipoprotein C3 (ApoC3) could be detected in BAT and was downregulated in ChREBP knockouts. Apoc3 overexpression in brown adipocytes did not affect intracellular LPL but decreased heparin-releasable extracellular LPL, suggesting that brown adipocyte Apoc3 modulates LPL maturation and/or stability. Currently, AAV-based BAT-specific genetic models to functionally assess the role of Angptl8, Apoc3 and other LPL modulators are under development.

Conclusions

The reduction of endogenous FA in ChREBP-deficient BAT is compensated by increased LPL-mediated FA uptake. Several candidate LPL regulators identified in the current study will be further investigated to assess their functional relevance for the control of LPL activity in BAT.

P9

A novel role for CLDN1 in the metabolism and thermogenesis of brown adipocytes

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Aim (background & objectives)

There are no previous studies regarding the role of tight junctions' (TJ) molecules in energy metabolism, specifically in adipose tissue. Here, we aim to decipher the potential role of CLDN1, a TJ contributor, in brown adipocytes function and biology.

Method

Two different immortalized brown preadipocytes cell lines from mice (WT-1 and pBAT immortalized preadipocytes) were used to study the expression of *Cldn1*. In addition, siRNA-mediated silencing of *Cldn1* (si*Cldn1*) was performed to evaluate the effect on adipocytes' differentiation, thermogenic activity and metabolism. Immunofluorescence staining was also performed to determine the cellular location of CLDN1.

Results

Cldn1 is increased in mature adipocytes compared to preadipocytes (4-10 folds increase; $p < 0.01$). Moreover, *Cldn1* expression was also induced by thermogenic stimulation (4-5 folds increase; $p < 0.05$).

The bulk-RNAseq in pBAT adipocytes showed that the absence of *Cldn1* caused a downregulation of genes involved in ATP synthesis, mitochondrial and ER function (*Atp5a1, Coa5*). Furthermore, si*Cldn1* cells showed an impaired respiration rate upon adrenergic stimulation and metabolic signalling pathways including extracellular matrix, triglycerides metabolism or ER were altered in basal conditions and/or upon thermogenic stimulation.

The siRNA-mediated downregulation of *Cldn1* (10 folds decrease; $p < 0.01$) did not affect the differentiation of WT-1 and pBAT preadipocytes. However, in the WT-1 cells the absence of *Cldn1* slightly affected adipocytes' respiration through the differentiation process and the norepinephrine-induced respiration rate. The lack of *Cldn1* also induced a higher glycerol production in response to CL treatment. Immunofluorescence staining showed that CLDN1 is located not only in the membrane but also in the cytosolic region within the brown adipocytes.

Conclusions

Results suggest that CLDN1 may play an important role in brown adipocytes' energy homeostasis and metabolism. Further studies would be needed to determine the mechanism of action in the regulation of brown adipose tissue biology and function.

P10

Myeloid let-7b Exacerbates Atherosclerosis

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Aim (background & objectives)

Atherosclerosis is a chronic inflammatory disease initiated by the subendothelial retention and modification of apolipoprotein B–containing lipoproteins, particularly low-density lipoprotein (LDL), leading to progressive plaque formation. Macrophages are central mediators of this process, as they internalize modified lipids and differentiate into foam cells that sustain inflammation and promote plaque progression and instability. MicroRNAs, acting via Argonaute 2 (AGO2) within the RNA-induced silencing complex, are critical post-transcriptional regulators of macrophage gene expression, orchestrating lipid metabolism and inflammatory signaling. Among these, the evolutionarily conserved microRNA let-7b has been implicated in pathways relevant to cardiovascular disease; however, its myeloid-specific role and underlying molecular mechanisms in atherogenesis remain poorly defined.

Method

We generated a myeloid-specific let-7b knockout mouse model with membrane-targeted EGFP labeling of plaque macrophages (Apoe–/–; LyzMCre+mTmG/let-7bflox/flox). To delineate the underlying molecular mechanisms, we performed myeloid-specific AGO2 immunoprecipitation using mice expressing Myc-GFP–tagged AGO2 (tAGO2) in the presence or absence of let-7b. AGO2-bound transcripts from lesional macrophages were subsequently analyzed by RNA sequencing and binding site analysis was performed using targetscan.

Results

Efficient and selective deletion of let-7b in lesional macrophages was confirmed by in situ PCR in aortic root sections. Following 12 weeks of Western diet feeding, myeloid-specific loss of let-7b resulted in a marked reduction in atherosclerotic lesion size compared with control mice (Apoe–/–LyzMCre+mTmG/let-7bwt/wt). This was accompanied by decreased macrophage accumulation, reduced cholesterol crystal deposition, lower lipid content, and reduced apoptosis within plaques, indicating an overall attenuation of disease severity. We identified 23 candidate let-7b-regulated genes involved in endocytosis, inflammatory signaling, cytoskeletal organization, and mitochondrial metabolism. Let-7b binding site further revealed conserved sites in six key genes, including Frmd4b, Myocd, Alpk1, Plcb4, Utrn, and Slc25a21.

Conclusions

Let-7b promotes atherosclerosis by modulating macrophage inflammatory and lipid-handling pathways. Conserved binding sites in key targets support a direct regulatory role and highlight its therapeutic potential.

P11

Menopause as a turning point: sex-specific inflammatory proteomic signatures of subclinical atherosclerosis

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Aim (background & objectives)

Cardiovascular disease risk rises sharply after menopause, yet the mechanisms linking endocrine ageing to early atherosclerosis remain poorly defined. Identifying molecular signatures of subclinical plaque could enable earlier risk stratification, especially in women, where current clinical tools may miss female-specific pathways.

Method

We profiled 161 inflammatory and cardiovascular proteins (Olink) in 163 women and 66 men from the HARMONi cohort, with menopausal staging, plaque assessment across five vascular territories, and SCORE2 risk classification. Sex- and menopause-stratified analyses were adjusted for age and BMI.

Results

The menopausal transition produced a coherent proteomic remodelling independent of chronological ageing, anchored by lipid and adipogenic mediators. This signature evolved across menopausal stages, establishing menopause as a discrete biological transition. This female-specific remodelling emerged on top of pre-existing sexual dimorphism and ultimately shaped plaque-associated biology in a sex-divergent manner. Ten male- and eight female-specific inflammatory proteins were associated with subclinical plaque, with no shared molecules between sexes. The female signature pointed to matrix remodelling, Th17/IL-17 inflammation and LDL-receptor biology, while the male signature involved Wnt/Notch signalling and chemokine activation. Strikingly, two thirds of postmenopausal women with plaque were classified as low-risk by SCORE2, and in this clinically silent group, the female-specific signature was able to identify women with subclinical atherosclerosis.

Conclusions

These findings position menopause as a turning point that reshapes the female cardiovascular proteome. The pathways driving subclinical atherosclerosis in women converge on lipoprotein biology and chronic inflammation. Crucially, current tools fail in this population. A female-specific inflammatory signature could complement current risk calculators, supporting molecular profiling in the next generation of female-specific cardiovascular prevention.

P12

Cationic liposome-mediated delivery of T0901317 to macrophages fails to reduce atherosclerosis susceptibility in male apolipoprotein E knockout mice

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Aim (background & objectives)

Activation of the nuclear transcription factor liver X receptor (LXR) in macrophages is considered beneficial in the combat against atherosclerotic cardiovascular disease. In the current study we evaluated the therapeutic potential of cationic liposome-mediated targeted delivery of LXR agonist T0901317 to macrophages.

Method

Egg-phosphatidylcholine (EPC), cholesterol, and N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N-trimethylammonium methyl-sulfate (DOTAP) were used to generate cationic liposomes with or without T0901317 of 150-160 nm in size and a zeta-potential of 30-31 mV. Ex vivo and in vivo experiments were performed using male C57BL/6 wild-type and apolipoprotein E (apoE) knockout mice.

Results

Exposure of thioglycollate-elicited macrophages from C57BL/6 mice to T0901317-containing cationic liposomes stimulated ABCA1 and ABCG1 gene expression levels and increased cholesterol efflux to apolipoprotein A1 and high-density lipoprotein. Thus, T0901317 maintains its therapeutic efficacy upon encapsulation into cationic liposomes. Liposome-mediated intraperitoneal delivery of T0901317 (5 mg/kg) prevented the development of fatty liver or hyperlipidemia, adverse effects commonly associated with oral T091317 administration, in chow diet-fed male apoE knockout mice. In line with an anti-inflammatory role for LXR, peritoneal macrophages isolated from T0901317 liposome-treated apoE knockout mice exhibited a 23% reduction in interleukin-6 secretion. Interestingly, an unexpected 5.8-fold higher peritoneal foam cell extent was observed after 8 weeks of T0901317 liposome treatment. This increase could be related to 128% higher macrophage transcript levels of the PXR target gene CD36, a scavenger receptor that mediates lipoprotein uptake. Atherosclerotic lesion sizes were not different in the aortic root of T0901317 liposome-treated and control apoE knockout mice. However, the lesional collagen / macrophage ratio, a surrogate measure for atherosclerotic plaque stability, increased >3-fold in response to liposomal T091317 delivery.

Conclusions

We have shown that cationic liposome-mediated intraperitoneal delivery of the LXR agonist T0901317 increases macrophage foam cell formation without altering atherosclerosis susceptibility in chow diet-fed male apoE knockout mice.

P13

Simvastatin modulates the pro-angiogenic secretome of senescent vascular smooth muscle cells in atherosclerosis

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Aim (background & objectives)

Atherosclerosis, the leading cause of cardiovascular disease, progresses from fatty streaks to unstable plaques that may rupture, triggering thrombotic events. A hallmark of plaque instability is intraplaque angiogenesis (IPA), the formation of fragile, leaky neovessels that promote, inflammation, plaque growth and hemorrhage. While hypoxia is a known trigger, additional pro-angiogenic mechanisms remain unclear. As an age-related disease, atherosclerosis is characterized by the accumulation of senescent vascular smooth muscle cells (VSMCs), which, despite being non-proliferative, are metabolically active and adopt a senescence-associated secretory phenotype (SASP) that promotes chronic inflammation. Although the detrimental roles of senescent VSMCs in atherosclerosis are increasingly recognized, it remains unexplored whether SASP secreted by senescent VSMCs could stimulate IPA. We aim to investigate the interplay between VSMC senescence, SASP and IPA and to assess the effect of simvastatin.

Method

By integrating transcriptomic, histological and functional analyses, we evaluate the impact of senescent VSMCs.

Results

scRNA-seq and immunohistochemistry revealed increased senescent VSMCs in unstable versus stable human carotid plaques. To further study VSMC senescence, we developed two *in vitro* models, replicative and doxorubicin-induced senescence, which showed increased production of pro-angiogenic factors, including VEGF. Conditioned media from senescent cells promoted angiogenesis response in endothelial cells (ECs). We found that simvastatin treatment effectively attenuated these pro-angiogenic effects *in vitro*. However, given that IPA often persists *in vivo* even in statin-treated patients, we investigated the molecular regulation of the SASP. Notably, blocking VEGFR2 in ECs abolished the SASP-induced pro-angiogenic effects, confirming the VEGF/VEGFR2 axis as a key mediator.

Conclusions

VSMC senescence actively contributes to plaque instability through a strong pro-angiogenic-SASP. While simvastatin modulates these pathways, the persistence of IPA in clinical settings highlights the need for novel additional therapeutic strategies. Targeting VEGF/VEGFR2 axis may provide an effective approach to address vascular senescence offering benefits that go beyond traditional lipid-lowering therapies.

P14

Consequences of lysosomal acid lipase overexpression on atherosclerosis

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Aim (background & objectives)

Lysosomal acid lipase (LAL) is the sole lysosomal enzyme capable of catabolizing triacylglycerols and cholesteryl esters (CE). In patients with LAL deficiency (LAL-D), these lipids accumulate in lysosomes of various cell types, particularly in macrophages. Among other manifestations, LAL-D patients present increased atherosclerosis susceptibility. Interestingly, genome wide association studies have identified risk variants for coronary artery disease within the *LIPA* gene encoding for LAL. Paradoxically, these variants were associated with increased *LIPA* expression in monocytes/macrophages. In our study, we aim to decipher the consequences of increased *LIPA* expression on atherogenesis.

In the early stages of atherosclerosis, an accumulation of free cholesterol (FC) in the lysosomes of macrophages can cause a disruption of lysosomal acidification, leading to impaired lysosomal hydrolysis. We hypothesize that increased *LIPA* expression promotes lysosomal FC accumulation, resulting in loss of LAL activity and elevated CE levels within the lysosomes, ultimately leading to a LAL-D-like phenotype in macrophages.

Method

To study increased *Lipa* expression in atherogenesis, we generated *Ldlr*-deficient mice with an overexpression of *Lipa* in hematopoietic cells. To this end, we isolated stem cells from bone marrow of wild-type mice, transduced them with lentivirus containing GFP or the *Lipa* gene for control and overexpressing mice, respectively, and transplanted the cells into female *Ldlr*-deficient mice. After 10 weeks on Western-type diet, we evaluated atherogenesis using histological and biochemical assays.

Results

Flow cytometry and DNA analysis confirmed the overexpression of *Lipa* after transplantation. Body weight gain, tissue weights, plasma lipid parameters, and blood cell counts were comparable between the groups. Aortic root staining, however, revealed a decrease in plaque area in the aortic sinus of *Lipa* overexpressing mice.

Conclusions

We concluded that LAL may have an anti-atherogenic role. We are currently characterizing *ApoE*-deficient mice with myeloid cell-specific *Lipa* overexpression to confirm our results and decipher the contribution of LAL to atherosclerosis.

Poster session 2 (P15-P30)

P15

Effect of high intensity statin medication on circulating foamy monocytes in healthy individuals

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Aim (background & objectives)

Circulating foamy monocytes (CFMs) are pro-inflammatory, display increased trans-endothelial migration, and are important drivers of atherosclerotic plaque progression. Elevated CFMs have mainly been demonstrated in familial hypercholesterolemia and hypertriglyceridemia. We aimed to determine if CFMs can be reliably quantified in healthy individuals and reduced by high-intensity statin treatment.

Method

We performed an interventional clinical study with 11 healthy volunteers receiving 40 mg atorvastatin daily for 4 weeks. Plasma and white blood cell samples were collected weekly, including baseline and one-week washout. CFMs were quantified by measuring monocyte lipid droplet (LD) number, size, area, percent LD-positive cells, and low-density lipoprotein (LDL) uptake with a previously established semi-automated high-content based analysis platform.

Results

After one week of treatment, LDL-C, total cholesterol, and apolipoprotein B (ApoB) decreased by 52%, 30%, and 38%, respectively. LDL-C further decreased during treatment, reaching a 63% reduction from baseline by week four. One week after treatment, LDL-C, total cholesterol, and ApoB increased by 74%, 23%, and 37%, respectively, but remained below pre-treatment levels. At baseline, 15.6% of monocytes were LD-positive and 0.29 LDs were detected per monocyte. The number of LDs decreased in a stepwise manner with treatment and reached the lowest level (0.13, $p < 0.01$, Wilcoxon signed-rank test) at washout. Similar reductions were observed for the percent of LD-positive monocytes (8.7% at wash out, $p < 0.01$). Interestingly, reduced monocyte lipid storage coincided with increased cellular LDL uptake, suggesting enhanced monocyte LDL uptake when lipid storage is lowered.

Conclusions

Our results highlight a novel aspect on how atorvastatin can influence inflammation and atherosclerotic plaque progression through reducing circulating foamy monocyte abundance.

P16

OPA1 Drives Organ-Specific Metabolic Reprogramming in the Liver and Heart to Prevent Diet-Induced Insulin Resistance

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Aim (background & objectives)

Mitochondrial dynamics are increasingly recognised as central regulators of whole-body metabolic homeostasis. Optic atrophy 1 (OPA1) controls inner mitochondrial membrane fusion, cristae architecture, and oxidative phosphorylation efficiency; however, how OPA1-driven mitochondrial remodelling coordinates systemic insulin sensitivity across organs remains incompletely defined. Here, we tested whether OPA1 overexpression protects against diet-induced insulin resistance through organ-specific metabolic rewiring with distinct consequences in the liver and the heart.

Method

We compared transgenic mice overexpressing OPA1 with wild-type controls under chow and high-fat diet (HFD) conditions. Using metabolic cages, insulin-resistance phenotyping, Seahorse analyses in primary hepatocytes, biochemical profiling, and histological assessment of liver and heart, we examined mitochondrial function, lipid handling, and tissue remodelling.

Results

OPA1 overexpression protected mice from HFD-induced insulin resistance but elicited divergent metabolic programmes across organs. In the heart, OPA1 increased oxidative/energy metabolism, as shown by a higher ratio of (C16 + C18)/C0, an index of CPT1 activity (0.1271 vs 0.8391; p = 0.0418), and a higher ratio of (C16 + C18:1)/C2, an index of CPT2 activity (0.0216 vs 0.1142; p = 0.0237). Cardiomyocyte cross-sectional area was reduced (3085 vs 2456 μm^2; p = 0.0456), fibrosis was increased (0.1505 vs 0.2287; p = 0.0393), and lipid deposition during HFD was higher (0.0834 vs 0.1473 µg triglycerides/µg protein; p = 0.0414). In contrast, in the liver, OPA1 promoted an anabolic metabolic state characterised by reduced hepatic lipid accumulation together with increased lipoprotein biosynthesis (higher lipoprotein production after 4h form the injection of poloxamer 407 (1305 vs 1520 mg/dl Triglycerides; p=0.3494), consistent with a rewiring of hepatic lipid partitioning rather than a simple reduction of lipid flux.

Conclusions

OPA1 is a systemic determinant of metabolic resilience that buffers diet-induced insulin resistance via organ-specific mitochondrial remodelling: a catabolic, energy-producing programme in the heart and an anabolic, lipoprotein-producing programme in the liver.

P17

Regulation of MAFLD by the nuclear ubiquitin-proteasome system

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Aim (background & objectives)

The ubiquitin - proteasome system (UPS) maintains cellular homeostasis and metabolism by regulating gene expression, cell cycle and stress responses via targeted protein modification and degradation. While proteasomes are found in both cytoplasm and nucleus, the role of nuclear proteasomes for the turnover and stability of transcription factors remains unclear. Given the profound influence of diet on hepatic gene expression and metabolic function, we hypothesized that nuclear UPS contributes to the transition from normal hepatocyte physiology to Metabolic Associated Fatty Liver Disease (MAFLD) in diet-induced obesity (DIO) by regulating the stability and function of key transcription factors.

Method

To explore compartment - specific proteasome function, nuclear and cytoplasmic fractions were isolated from liver tissue of male C57BL/6J mice subjected to either a high-fat diet (HFD)-induced obesity (DIO) or standard control diet for 10 weeks. Fraction purity was validated via immunoblotting. Proteasomal activity was assessed using fluorogenic substrates, native PAGE, and proteomic profiling of ubiquitinated proteins (ubiquitomics). To mimic MAFLD in vitro, AML12 cells were loaded with fatty acids and TGF-β1 and used for mechanistic experiments.

Results

Total hepatic proteasomal activity remained unchanged between diets; however, nuclear extracts from DIO mice showed reduced proteasome function compared to controls. Ubiquitomics combined with gene ontology analysis was started to explore protein signatures potentially linked to hepatic transcriptional regulation by the UPS. Consistent with in vivo findings, MAFLD-like AML12 cells recapitulated nuclear proteasome activity pattern, with trypsin-like activity showing the most pronounced change upon fatty acid and TGF-β1 treatment.

Conclusions

Metabolic stress is associated with a decline in nuclear proteasomal activity, suggesting compartment-specific regulation of UPS function. The underlying mechanisms remain unclear but may involve reduced proteasomal subunit abundance or impaired transport. Elucidating the role of nuclear proteasomes in liver physiology under obesogenic conditions could provide new insights into transcriptional regulation in MAFLD and identify potential therapeutic targets.

P18

ABCG1-mediated protection from hepatic lipotoxicity reduces MASH and atherosclerosis

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Aim (background & objectives)

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common chronic liver disease and an independent risk factor for atherosclerotic cardiovascular diseases (ASCVD). Metabolic dysfunction-associated steatohepatitis (MASH), the intermediate reversible step of MASLD, is characterized by lipid accumulation and inflammation in the liver. An excess of ceramides in hepatocytes promotes lipotoxicity and MASH, implying that hepatic ceramides export could protect from these deleterious outcomes. Our preliminary data uncovered a role of the membrane ATP-Binding Cassette G1 (ABCG1) transporter in the net export of cellular ceramides to high-density lipoproteins (HDL) which is accompanied by an increase of their atheroprotective cholesterol efflux capacity.

Therefor, our objective is to determine whether ABCG1 may reduce hepatic lipotoxicity and ASCVD during MASH development.

Method

Our project combines in vitro and in vivo methods.

Results

Immunohistochemical analysis of human liver biopsies reveals that ABCG1 is highly expressed in hepatocytes. However, its expression is reduced by fatty acids in primary human hepatocytes (PHH) as well as in liver biopsies from patients with MASLD. Transcriptomic and lipidomic analyses in control and ABCG1 deficient PHH (from 5 donors) highlight the importance of ABCG1 in lipotoxic pathways contributing to MASH. On the contrary, expression of ABCG1 in HepG2 cells, an ABCG1-deficient hepatocellular carcinoma cell line, reduces lipotoxicity. In accordance with a protective role of the ABCG1 / HDL axis in the liver, ceramide content of HDL is associated with their capacity to promote cholesterol efflux and is inversely correlated with MASH score and coronary artery calcification in a cohort of 222 patients with type 2 diabetes. These results were validated in a mouse model expressing human ABCG1 in hepatocytes and susceptible for both MASLD and atherosclerosis.

Conclusions

Our results identify a non-canonical protective function of ABCG1 / HDL axis in the export of hepatic ceramides, which could protect from hepatic lipotoxicity, MASH development and ASCVD.

P19

Hepatic loss of VPS35 aggravates diet-induced Metabolic dysfunction-associated steatohepatitis (MASH)

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Aim (background & objectives)

Metabolic dysfunction-associated fatty liver disease (MAFLD) is the most common chronic liver disease, driven by the obesity pandemic and metabolic diseases such as type 2 diabetes and hyperlipidaemia. It encompasses a spectrum of diseases ranging from simple fatty liver to more complex conditions like metabolic dysfunction-associated steatohepatitis (MASH), which may progress to cirrhosis and liver cancer. Recently, we demonstrated that the endosomal sorting complex plays an important role in regulating hepatic and systemic cholesterol homeostasis, and its disruption leads to liver malignancies. However, the precise contribution of this complex to MASLD development and progression remains largely elusive.

Method

To investigate retromer function in MASLD, we created liver-specific VPS35 ablation using the Cre-Lox system. Control and VPS35-deficient (Vps35-KO) mice were fed a high-fat high-cholesterol (HFC) diet for 12 weeks. We assessed liver histology for steatosis, fibrosis, and inflammation using immunostaining. Additionally, hepatic and plasma lipid and liver enzyme levels were measured to reflect MASLD progression in our mouse model.

Results

Liver-specific Vps35 ablation resulted in loss of VPS26A and VPS29, leading to destabilisation of the retromer complex. Further, Vps35-KO mice showed markedly increased plasma and hepatic cholesterol content. Plasma triglycerides were unaffected; however, liver triglycerides were significantly reduced after 12 weeks of HFC feeding. The increase in plasma cholesterol was ascribed to elevated low-density lipoprotein cholesterol. Elevated hepatic cholesterol levels were accompanied by infiltration of macrophages and T-cells, along with increased liver fibrosis. Plasma levels of the liver enzymes alanine aminotransferase and aspartate aminotransferase were also elevated, indicative of liver damage. Furthermore, Ki67 and BrdU staining revealed that hepatic Vps35 deficiency markedly increased hepatocellular proliferation, a hallmark of hepatocellular carcinoma.

Conclusions

This study demonstrates that VPS35 plays a key role in the progression of diet-induced MAFLD towards fibrosis and potentially hepatocellular carcinoma.

P20

SPRING sets SREBP apart: specificity and evolution of a key regulatory axis

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Aim (background & objectives)

Lipid metabolism is a strictly regulated process that has a pivotal role in the survival of cells. SREBP transcription factors are master regulators of de novo lipogenesis (DNL). After their synthesis as precursors in the ER, they require translocation to the Golgi and subsequent initial cleavage by Site-1 Protease (S1P). As a novel regulator of lipid metabolism, SPRING plays a crucial role in the S1P-mediated maturation of SREBPs, by enhancing maturation of S1P iself. S1P has several cleavage substrates, including ATF6 and GNPTAB, and is therefore implicated in a variety of cellular processes. Our initial observations suggest SPRING may be required only for the correct processing of SREBPs. This study aims to evaluate the requirement of SPRING for S1P-mediated cleavage of its substrates, and to assess whether SPRING's function is evolutionary conserved.

Method

To assess whether SPRING controls the S1P-dendent processing of its substates we used an overexpression strategy. SPRING^{KO} cell lines were co-transfected with constructs encoding substrates of S1P with or without the co-overexpression of SPRING. Furthermore, we assessed whether SPRING function is evolutionary conserved by comparing human, mouse, Drosophila and Arabidopsis thaliana SPRING. HEK293 cells were transfected with these SPRING constructs and their ability to support S1P proteolytic maturation and SREPB signaling was evaluated.

Results

Preliminary data from immunoblot analyses show that another S1P substrate, ATF6, can be processed even in absence of SPRING. Ongoing structural/functional experiments will reveal whether this is the case for other S1P targets. Preliminary findings suggest that dmSPRING can functionally complement the mammalian isoform.

Conclusions

SPRING seems to be an SREBP-specific partner for S1P, but further analyses are required to clarify the underlying mechanism and explain why other S1P substrates escape the need for SPRING. Additionally, SPRING is conserved among animal species and exerts the same effect on hS1P. Its evolutionary origin is being investigated.

P21

Liver sinusoidal endothelial cells protect hepatocytes against steatosis via paracrine factors

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Presented by Dr Aldo Grefhorst

Aim (background & objectives)

Emerging evidence highlights the crucial role of liver sinusoidal endothelial cells (LSECs) in early metabolic dysfunction-associated steatotic liver disease (MASLD), yet the direct interaction of steatotic hepatocytes with LSECs is understudied. This study aimed to elucidate how LSECs regulate hepatocyte lipid metabolism under steatotic conditions.

Method

HepG2 cells were co-cultured with human-derived LSECs (hLECs) or with aortic endothelial cells (HAECs) as controls, with or without oleic/palmitic acid (OA/PA) loading. Lipid droplets and triglyceride (TG) content were assessed. Secretomic analysis was performed for hLECs on medium of characterized paracrine factors. The measurements of hLEC/HepG2 were replicated in primary murine hepatocytes/LSECs.

Results

Co-culture with hLECs, but not HAECs, significantly reduced lipid droplet and triglyceride accumulation in hepatocytes under OA/PA exposure. This was accompanied by decreased expression of lipogenesis gene *SCD1*, and the lipid transporter *CD36*, with an increased level of *PLIN2* in hepatocytes. More lipid droplets were accumulated in hLECs in co-culture with HepG2, whereas less in HAECs. Inhibiting CD36 in hLECs impaired their lipid uptake[OH1] , leading to failure to reduce OA/PA-induced lipid overloading in HepG2. Secretome analysis revealed LSEC-specific upregulation of paracrine factors relevant to inflammatory response and extracellular matrix (ECM) remodeling.

Conclusions

This study demonstrates that LSECs protect hepatocytes from steatosis by actively taking up fatty acids via CD36, thereby reducing lipid accumulation and altering key metabolic genes. Moreover, LSECs secrete a distinct set of paracrine factors, suggesting their role extends to modulating the inflammatory and fibrotic microenvironment in early MASLD.

P22

Hepatic VPS41 plays an essential role in systemic cholesterol homeostasis

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Aim (background & objectives)

We recently identified ARL8B, a lysosomal GTPase, as a novel hepatic cholesterol regulator. ARL8B recruits the HOPS complex, which is essential for lysosomal fusion. Previously, we showed that hepatic ablation of ARL8B disrupts cholesterol metabolism and reduces the lysosomal abundance of VPS41, a core HOPS subunit. Whether the loss of VPS41 itself is sufficient to disrupt cholesterol homeostasis and thereby contribute to the ARL8B phenotype remains unknown. This study, therefore, investigates the consequences of targeting hepatic VPS41 on cholesterol metabolism.

Method

Using somatic genetic editing, we targeted hepatic VPS41 in mice (*Vps41*^{HepKO}) and studied the effects of VPS41 deficiency on cholesterol homeostasis on standard chow conditions and after a cholesterol challenge. Plasma lipids were measured using commercial enzymatic assay kits. Plasma lipoprotein profiles were determined with FPLC. Liver lipid content was quantified after lipid extraction using the Bligh and Dyer method. Quantitative real-time PCR was used to determine the expression of cholesterol synthesis genes. Western blotting was used to assess protein expression of lysosomal cholesterol-handling proteins, and lipoprotein and scavenger receptors.

Results

Targeting of VPS41 (*Vps41*^{HepKO}) in female mice resulted in ±30% increase in plasma cholesterol levels compared to control (*EV*^{Hep}) mice on a control diet and after a cholesterol challenge of 1 week. While hepatic cholesterol was not different between *Vps41*^{HepKO} and *EV*^{Hep} mice on a control diet, cholesterol content in the liver of *Vps41*^{HepKO} mice increased ±35% compared to *EV*^{Hep} mice after the cholesterol diet, mainly due to cholesterol esters. Western blotting revealed a significant decrease in LDLR and SR-B1 but not LRP1. In addition, there was a significant increase in NPC1 and ARL8B protein levels in *Vps41*^{HepKO} mice.

Conclusions

These findings indicate that VPS41 plays an important role in systemic cholesterol homeostasis, and thus, its reduction in ARL8B-deficient livers may contribute to disturbed cholesterol handling.

P23

Altered Metabolomic and Monocyte Transcriptional Profiles Reshapes the Menopausal Inflammatory Landscape in Healthy Women

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Aim (background & objectives)

The menopausal transition induces profound physiological remodelling, including hormonal fluctuations, detrimental lipid profile changes, and metabolic shifts that alter susceptibility to immune-modulated conditions. While monocytes are known major contributors to systemic inflammation, how menopause affects their activation and transcriptional landscapes to promote inflammation-related diseases remains unclear.

Method

We analysed monocyte distributions, HLA-DR levels, and transcriptomes in 127 healthy women (67 premenopausal [preM]; 59 postmenopausal [postM]). Moreover, we analysed serum metabolomics to dissect metabolite changes. To decouple menopausal inflammatory effects from aging and adiposity, we estimated an age- and body mass index (BMI)-adjusted inflammatory index based on GlycA. This Inflammatory Burden Index (IBI) was used to further stratify postM women and identify inflammatory phenotypes.

Results

Menopausal status did not significantly alter the proportions of monocyte subpopulations. Instead, it impacted their activation state via altered HLA-DR levels, alongside profound transcriptomic differences. PostM women showed marked enrichment in immunosenescence and stress pathways, including TNFa signalling, hypoxia, and interferon responses. Crucially, stratifying this cohort based on the IBI score identified a distinct postM subgroup with higher inflammation than would be expected for their age and BMI. This subgroup exhibited significant modulation of multiple pathways, including chemokine signaling, mTOR, lipid and energetic metabolism. Importantly, this high-inflammatory postM group was linked to alterations in serum metabolites and circulating lipid profiles.

Conclusions

Menopause is associated with monocyte activation and a marked remodelling of their global transcriptome. Integrating these transcriptional changes with those observed in serum metabolites provides a more comprehensive understanding of the impact of menopause on innate immunity and inflammatory signalling. Our adjusted index identified a distinct pro-inflammatory monocyte signature in a healthy postM subgroup with elevated GlycA, providing critical mechanistic insights into how menopause modulates susceptibility to diseases with a strong inflammatory component.

P24

Systemic Metabolic Effects of Finnish Sauna Bathing Assessed by NMR-Based Metabolomics

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Aim (background & objectives)

Frequent heat exposure, such as sauna bathing, is associated with reduced risk of adverse cardiovascular outcomes. However, biochemical pathways through which sauna may exert its vascular health effects remain uncertain. This study aimed to investigate the effects of Finnish sauna bathing on blood-based cardiometabolic biomarkers. Specifically, we examined two exposures: the acute effects of a single 30-minute Finnish sauna session and the effects of an 8-week exercise-plus-sauna intervention on circulating metabolomic biomarkers related to lipid and amino acid metabolism

Method

The study included two complementary populations: (1) an acute sauna exposure study including 63 habitual sauna users (46% females) with at least one cardiovascular risk factor (Acute Sauna Study), and (2) an 8-week randomized controlled trial (Sauna RCT) including 47 sedentary non-regular sauna users (87% females) randomized to exercise, exercise plus sauna, and control arms. The exercise intervention consisted of concurrent training three times per week, with the sauna group additionally completing 15 min of post-exercise sauna bathing. Circulating metabolites were quantified using high-throughput 1H NMR spectroscopy at pre, post and post 30 min time points in the acute sauna study and at baseline and post-intervention in the Sauna RCT.

Results

Acute sauna exposure produced consistent metabolic shifts, including significant reductions in concentrations of plasma triglycerides and VLDL particles, decreases in branched-chain and aromatic amino acids, and increases in ketone bodies, indicating enhanced lipid oxidation and transient metabolic stress. In the Sauna RCT, sauna exposure resulted in additional improvements beyond exercise alone, including reductions in LDL particle number and

concentration, greater decreases in VLDL subclasses, and increases in HDL particle size, suggesting synergistic metabolic effects when heat exposure is combined with exercise training.

Conclusions

Finnish sauna bathing induces favourable acute alterations in lipid and amino acid metabolism, and when combined with regular exercise, augments longer-term improvements in lipoprotein profiles.

P25

High-unsaturated-fat diet prevents cardiac dysfunction and modulates the metabolic switch associated with anthracycline-induced cardiotoxicity in a porcine model.

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Aim (background & objectives)

Anthracycline-induced cardiotoxicity is linked to a metabolic switch marked by impaired myocardial fatty-acid metabolism and increased oxidative stress. We aimed to restore myocardial fatty-acid metabolism and ATP production through a high–unsaturated-fat diet.

Method

Large White pigs (male and female) were randomized to receive a chow diet or a HUFD (80% feed, 10% extra-virgin olive oil, 10% nuts). Half of each dietary group received six intravenous doses of doxorubicin (25 mg/m^2). Pigs were allocated to 4 groups: Control, HUFD, Doxo and Doxo+HUFD. Serial advanced multimodal cardiac imaging and invasive pressure–volume loop analysis were performed to evaluate ventricular function and contractility. At study completion, myocardial tissue underwent mitochondrial structural analysis, high-resolution respirometry, and proteomic and metabolomic profiling.

Results

The prevalence of anthracycline cardiotoxicity was markedly reduced in the Doxo+HUFD group compared with Doxo alone (14.3% vs. 50%). HUFD significantly attenuated the doxorubicin-induced decline in left ventricular ejection fraction (−12.9% ±11.5 in Doxo vs. +0.4% ±5.3 in Doxo+HUFD, $P<0.05$). Markers of myocardial fibrosis and edema were also reduced with HUFD, assessed by extracellular volume fraction (ECV) and T2 mapping, respectively: (ECV: 32.8%±3.31 vs. 27.37±4.53, *P-diet* =0.004; T2: 50.09ms±3.47 vs. 45.82±1.23, *P-diet* =0.002). Cardiac contractility reveals an improvement with HUFD, as reflected by the ESPVR slope (0.67±0.4 vs. 1.03±0.33; *P-diet* =7.58e-4). Omics analyses revealed that HUFD increased myocardial unsaturated fatty-acid content and upregulation of pathways related to fatty-acid oxidation, cardiac contraction, and antioxidant defense. Mitochondrial structural and functional analyses revealed that HUFD prevented the classical doxorubicin-induced mitochondrial fission and increased F-pathway–linked respiration and maximal electron transport chain capacity.

Conclusions

The high-unsaturated-fat diet ameliorates significantly the effects of doxorubicin on left ventricle ejection fraction and there is a tendency to improve other cardiac parameters such as fibrosis, edema and cardiac contractility.

P26

Dissociation between early recovery of systemic glucose tolerance and delayed perivascular adipose tissue-linked reversal of endothelial dysfunction after HFD withdrawal

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Aim (background & objectives)

High-fat diet (HFD) feeding leads to insulin resistance, impairment of endothelial function, and perivascular adipose tissue (PVAT) lipid remodeling and dysfunction. Some reversal effects occur only after HFD withdrawal and return to a normal diet, but recovery process and PVAT's role in vascular regulation remain unexplored. Thus, we investigated how rapidly systemic glucose tolerance, PVAT lipid profile, and endothelial function are normalized following HFD withdrawal.

Method

A multimodal characterization of thoracic versus abdominal aorta (TA and AA, respectively) and adjacent PVAT was involved to evaluate the early and late effects of diet change after eight weeks of HFD feeding (60 kcal% of fat with 1% cholesterol). C57BL/6J male mice were examined by magnetic resonance imaging to characterize endothelial function in vivo, Raman spectroscopy to assess PVAT lipid unsaturation, and by RT-qPCR for mRNA expression. Plasma lipid profile and assessment of insulin resistance based on the glucose tolerance test supplemented findings.

Results

After one week of HFD withdrawal, reversal of insulin resistance was observed, followed by partially recovered endothelial function in TA and normalization of lipid unsaturation of AA PVAT correlating with *Scd1* expression. However, full reversal of endothelial dysfunction was delayed and was associated with transcriptomic alterations in PVAT, but not in the vascular wall, manifested by altered gene expression of *Insr*, *Irs1/2*, *Edn1* and *Gucy1b1,* and adipokines (*Lep, Nampt,* and *Adipoq*).

Conclusions

HFD reversal quickly restored systemic insulin sensitivity and PVAT lipid unsaturation, but endothelial function response was location-dependent, with the TA showing quicker restoration, associated with brown-like PVAT. Delayed response of AA to HFD withdrawal can be attributed to alterations in AA PVAT evoked by impaired guanylate cyclase signaling pathway and disruption in adipokine secretion.

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P27

Extracellular vesicles released by palmitic acid- but not TNFα-treated adipocytes induce skeletal muscle insulin resistance

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Aim (background & objectives)

Lipotoxicity and inflammation are hallmarks of obesity and drive both adipose tissue disfunction and systemic insulin resistance. However, the individual contribution of inflammation and lipid overload to adipocyte metabolic impairment remains incompletely understood. Similarly, it is unknown whether cytokines and saturated fatty acids differently modulate adiposomes to shape skeletal muscle insulin sensitivity. Therefore, this study investigated the individual and synergistic effects of Palmitic Acid (PA) and Tumor Necrosis Factor-alpha (TNFα) on adipocyte metabolic health and impact of adipocyte-derived extracellular vesicles (EVs) on myotube insulin resistance.

Method

Human Simpson-Golabi-Behmel (SGBS) adipocytes were treated with PA, TNFα, or both for 24h after which adipocyte insulin signalling was assessed. The phosphorylation of hormone-sensitive lipase (p-HSL), the abundance of perilipin-1 (PLIN1) and the expression of peroxisome-proliferator-activated-receptor-γ (*PPARγ*) were assessed as proxies of lipid-handling capacity. Adipocyte mitochondrial membrane potential was assessed using JC-10 and the expression of pro-inflammatory mediators (*IL-6, IL-1β, MCP-1, IL-18*) by qPCR. Adipocyte-derived EVs were isolated from treatment-deprived conditioned medium and administered to immortalised human myotubes followed by the assessment of insulin-mediated phosphorylation of Akt.

Results

TNFα upregulated *IL-6*, *IL1β*, *IL-18* and *MCP*-1, while downregulating *PPARγ*, with these effects being exacerbated by the co-administration of PA. Moreover, TNFα impaired insulin-induced phosphorylation of Akt (Thr308) without affecting insulin-mediated inhibition of HSL. However, all treatments tended to reduce basal phosphorylation of HSL. Moreover, PA alone, and in synergy with TNFα, reduced the abundance of PLIN1, suggesting an instability of the lipid droplets. Finally, TNFα induced a hyperpolarisation of the mitochondria membrane. Remarkably, myotubes exposed to EVs derived from PA-laden adipocytes displayed reduced insulin-mediated phosphorylation of Akt (Ser473) along with an upregulation of *PLIN2*, a lipid-accumulation marker.

Conclusions

While inflammation, but not saturated fatty acid, directly hampers adipocytes metabolic health, lipid-laden adipocytes release EVs that promote markers of lipid accumulation and insulin resistance in myotubes.

P28

Pharmacological Modulation of Stearoyl-CoA Desaturase expression in metabolic disorders

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Aim (background & objectives)

X-linked adrenoleukodystrophy (X-ALD) is a rare metabolic disorder for which treatment options remain limited and are largely supportive. X-ALD is caused by mutations in ABCD1 gene, leading to impaired metabolism of very long chain fatty acids (VLCFAs) and toxic accumulation of them in cytoplasm. Saturated VLCFAs are particularly toxic. SCD is an essential mammalian fatty acid (FA) desaturase and increased expression of SCD has been shown to improve toxic parameters in x-ALD. The aim of this study is to identify genes whose modulation by clinically approved compounds increases SCD expression.

Method

To track SCD expression in live cells, we labeled endogenous SCD with mNeon by CRISPR/Cas9-mediated mediated homology directed repair. Whole genome CRISPR-Cas9 knockout library was used to identify genetic traits regulating SCD expression. Expression of genes were verified using FACS, Western Blot and qPCR.

Results

CRISPR-Cas9 functional genetic screen for regulators of SCD expression identified multiple candidate hits. A membrane-bound GTPase (GTX1A) was identified as a negative regulator of SCD expression. C-7, a non-prescription compound shown to inhibit GTX1A, increased SCD mRNA and protein levels across all tested human cell models, including X-ALD patient-derived fibroblasts, with protein increases up to fourfold. C-7 treatment did not induce SREBP1 pathway activation, with only a minor increase in downstream target gene expression.

Conclusions

Our study identifies genes modulating SCD expression, offers new potential mechanisms of FA saturation sensing in mammalian cells and pinpoints a new potential drug repurposing candidate for X-ALD, with potential for a disease-modifying strategy targeting the metabolic cause.

P29

Relationship Between Cholesterol Accumulation and Cellular Senescence in an In Vitro Model of Vascular Cells

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Aim (background & objectives)

Aging is a major risk factor for atherosclerosis and is associated with the accumulation of senescent vascular cells releasing pro-inflammatory mediators. A close relationship between cholesterol homeostasis and cellular senescence has been proposed, yet their reciprocal interaction remains only partially understood. This study investigated the interplay between cellular senescence and cholesterol homeostasis in THP-1 human derived macrophages and vascular smooth muscle cells (VSMCs)

Method

Senescence was induced by doxorubicin (200nM, 24h), cholesterol loading by acetylated LDL (acLDL, 50µg/mL, 24h). HDL (12.5µg/mL, 6h), normolipidemic human serum (SN2%, 6 h) or hydroxypropyl-β-cyclodextrin (HPβCD, 2.5-10mM, 18h) were used as cholesterol acceptors. Intracellular cholesterol content was measured by fluorometric assay; SA-β-Gal and p65 were assessed by Western blot, SIRT1 and IL-6 mRNA by RT-qPCR, and IL-6/IL-8 secretion by ELISA.

Results

In THP-1 macrophages, doxorubicin increased SA-β-Gal (+78%-$p<0.0001$) without affecting intracellular cholesterol, acLDL increased cellular cholesterol (+45%-$p<0.01$) and SA-β-Gal (+52%-$p<0.0001$), reduced SIRT1 (−30%-$p<0.01$) upregulated p65 (+24%-$p<0.0001$), markedly increasing IL-6 and IL-8 secretion (+6600%-$p<0.0001$; +111%-$p<0.001$). Cholesterol depletion with HDL, SN or HPβCD 2.5mM and 10mM, significantly reduced intracellular cholesterol (respectively -12,2%, -24%, -26%, -56%, vs acLDL-$p<0.0001$) and IL-6 expression (-84%, -95%, -78%, -78% vs acLDL-$p<0.001$).

In VSMCs, doxorubicin increased SA-β-Gal (+63%-$p<0.0001$) and modestly elevated intracellular cholesterol (+23%-$p<0.05$). acLDL induced cholesterol accumulation (+24%-$p<0.05$), markedly increased SA-β-Gal (+205%-$p<0.05$) and enhanced IL-6 and IL-8 secretion (+64%-$p<0.001$; +83%-$p=0.06$). Exposure to HDL, SN, HPβCD 2.5 mM and 10 mM reduced intracellular cholesterol levels (-26%-$p=0.0023$, -35%-$p=0.0006$, -20%-$p=0.0139$, -72%-$p<0.0001$ vs acLDL) while, HDL and SN decreased IL-6 expression, restoring values similar to basal (-32%-$p=0.003$ vs acLDL). HPβCD effects on IL-6 expression is under evaluation.

Conclusions

These findings reveal a tight link between cholesterol accumulation, cellular senescence and inflammation in vascular cells. Modulation of cholesterol homeostasis and its downstream inflammatory pathways may represent a promising pharmacological strategy to counteract vascular aging possibly delaying progression of age-related diseases.

P30

Different size-separated subfractions of extracellular particles secreted by four tumor cell lines reveal distinct lipid-protein signatures and latent proteomic cargo programs, suggesting specific biological effects

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Aim (background & objectives)

Extracellular particles (EPs) released by cancer cells are highly heterogeneous, but whether different subpopulations carry distinct lipid–protein signatures and biological activities remain poorly understood. To overcome this problem, we characterized EP subfractions secreted by melanoma- (LM-16), triple-negative breast cancer- (MDA-MB-231) and prostate cancer (PC-3) cell lines.

Method

EPs secreted by aforementioned cells were size-separated by differential ultracentrifugation into five subfractions (range <50/>200nm). Size was assessed by AFM, SEM and Zetasizer; lipid composition was investigated by MS/GLC and proteomics by MS, followed by unsupervised non-negative matrix factorization (NMF) of the subfractions. The optimal rank was selected through repeated-random initializations, reconstruction error and consensus-based stability metrics. Functional effects were tested in murine macrophages and human fibroblasts after 48-hour exposure.

Results

We recovered five EP subfractions corresponing in size to what theoretically calculated (among them the <50 nm exomere-enriched one). Lipidomics indicates fraction-specific lipid-class signatures, with larger particles showing a more phospholipid-rich profile and the smallest displaying enrichment in saturated fatty acids, increased rigidity and distinct compositions undergoing orthogonal validation. Proteomics identified shared and fraction-specific proteins, with MMP9 being selectively detected in exomere-enriched fractions across all the tested lines. Consensus NMF resolved the five subfractions into three functional cargo programs peaking in: smallest/exomeres (<50nm), intermediate (50-200nm) and largest (>200nm) fractions. Reactome enrichment linked: -the exomere-like program to extracellular-matrix organization, collagen formation and IGF/RTK-related signaling; -the intermediate one to membrane trafficking and immune/inflammatory signaling; -the largest-vesicle program to vesicle-mediated transport, trans-Golgi budding and ER–Golgi cargo processing. Exomere-like program was consistent with functional assays showing that exomere-enriched particles promoted inflammation/metabolic changes in macrophages and fibroblasts, suggesting a role in tumor–stroma communication.

Conclusions

Size-separated cancer EPs show lipid/protein heterogeneity. Proteomic complexity converged into three latent cargo programs, supporting an involvement of exomere-enriched particles in matrix remodeling, inflammation/metabolic reprogramming.

Poster session 3 (P31-P49)

P31

Investigating the interplay between the E3 ubiquitin ligases IDOL and RNF130 in LDLR degradation

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Aim (background & objectives)

Dyslipidemia is the leading cause of cardiovascular disease (CVD) and is characterized by high circulating LDL (Low-density lipoprotein)-cholesterol levels. Hepatic LDL receptor (LDLR) mediates LDL clearance from circulation and is a major therapeutic lipid-lowering target. The E3-ubiquitin ligases IDOL and RNF130 are key novel post-transcriptional regulators of LDLR. Despite different regulation, structures and cellular localization, acting as E3 ligases both promote ubiquitination and subsequent degradation of LDLR. This study aims to elucidate the possible interplay between IDOL and RNF130 in LDLR degradation and therefore their role in CVD.

Method

A431-RNF130^{KO} cells were cultured with FCS or sterol-depletion medium (LSM). GW3965 was used to activate LXR pathway and IDOL expression. A431-IDOL^{KO} cells were transduced with lentiviral particles encoding WT or RING-mutated hRNF130 constructs. Immunoblotting, FACS of surface LDLR and qPCR were used to evaluate intracellular, membrane proteins and transcripts levels, respectively.

Results

Surface LDLR in A431-RNF130^{KO} cells increased upon treatment with LSM, while GW3965 treatment counteracted this, reducing surface expression by ~50%. LDLR modulation was comparable in A431-RNF130^{KO} and control cells. This result was also confirmed by immunoblotting and qPCR: LDLR expression after GW3965 was unaffected by RNF130 KO, suggesting that degradation of LDLR by IDOL is independent of RNF130. Co-transfection experiments in HEK293 cells demonstrated that WT, but not RING-mutated RNF130 was able to promote degradation of LDLR. Preliminary results in A431-IDOL^{KO} cells overexpressing either WT or RING-mutated hRNF130 suggest that in absence of IDOL, over-expression of RNF130 does not decrease LDLR abundance. Ongoing experiments are exploring this observation and expanding our studies on the interplay between IDOL and RNF130.

Conclusions

This study highlights a possible functional connection between IDOL and RNF130 in degrading LDLR. We demonstrated that IDOL degrades LDLR independently of RNF130, while RNF130 may require IDOL to functionally target LDLR. Further analyses are ongoing to elucidate the underlying mechanism.

P32

Bridging lipid classes: N-acyl taurines at the interface of fatty acid and bile acid metabolism

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Aim (background & objectives)

Bile is a complex mixture containing not only the well-characterized bile acids, phospholipids, and cholesterol, all of which act as detergents and signaling molecules, but also another less-known lipid family: the N-acyl taurines (NATs). NATs are comprised of a fatty acyl chain amidated to taurine, and their amphipathic structure mirrors that of taurine-conjugated bile acids, increasing fatty acid solubility. NATs are endogenous, bioactive lipids that regulate carbohydrate and lipid metabolism and can reduce hepatic steatosis. However, in human metabolic dysfunction-associated steatotic liver disease (MASLD), NATs are elevated due to an unknown mechanism. Therefore, we studied hepatic NAT synthesis and uptake pathways and specifically scrutinized similarities between bile acids and NATs to identify the enzymes and transporters involved.

Method

We used mouse models and a human study to establish NAT absorption, uptake, and synthesis in vivo, and cell culture models to confirm NAT uptake and transport in vitro.

Results

NATs are synthesized in hepatocytes, using overlapping pathways with bile acids, namely fatty acid transport protein 5 -mediated acyl-CoA synthesis before bile acid-CoA:amino acid N-acyltransferase-mediated conjugation with taurine.

After secretion into the intestine via bile, NATs are absorbed intact in humans and rodents, and preclinical models revealed that this requires the apical sodium-dependent bile acid transporter. NATs are then taken up by hepatocytes via sodium-taurocholate co-transporting polypeptide (NTCP), as demonstrated in in vitro and in vivo models. The requirement for NTCP

as a hepatic transporter of NATs was confirmed in humans treated with an NTCP inhibitor, which increased NATs and bile acids.

As previously observed in humans, circulating NATs also increased in a mouse model of MASLD, suggesting reduced hepatic uptake via downregulation of transport proteins.

Conclusions

NATs share the hepatic synthesis and hepatic-intestinal transport machinery with bile acids, functionally connecting two classes of amphiphilic molecules and positioning them as important regulators of metabolism.

P33

Activation of vesicular transport pathways clears lysosomal cholesterol

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Aim (background & objectives)

Intracellular cholesterol transport is subject to strict regulation that involves both vesicular and non-vesicular mechanisms. Recent evidence indicates that oxysterol-binding proteins are the primary mediators of intracellular cholesterol transport. However, vesicular pathways remain essential for the trafficking of cholesterol and other lipids. Lysosomes play a central role in processing and distributing cholesterol derived from low-density lipoproteins. In Niemann-Pick disease type C1 (NPC1), lysosomal dysfunction caused by the absence or deficiency of the NPC1 transporter results in severe accumulation of free cholesterol within the lysosomal lumen

Method

This study examined vesicular mechanisms responsible for mobilizing intracellular cholesterol from lysosomes in both wild-type and NPC1-deficient cells. We employed *Drosophila* steroidogenic tissue as an in vivo model system, leveraging the fact that these organisms are cholesterol auxotrophs whose systemic cholesterol levels can be precisely manipulated. Key findings were subsequently corroborated in mammalian cell culture models.

Results

In *Drosophila*, the modulation of particular signaling pathways homologous to mammalian liver X receptor resulted in an alteration of the cholesterol content within lysosomes. Our data demonstrated that Rab8 acted downstream of these pathways, and its overexpression completely rescued lysosomal cholesterol accumulation in *Drosophila* steroidogenic tissue deficient in Npc1. Similarly, but to a lesser extent, our data and previous studies have shown that RAB8A reduces intracellular cholesterol. Thus, with the aim of further characterizing other important players in post-lysosomal cholesterol trafficking, we performed proximity labeling proteomics and stimulated emission depletion microscopy. These approaches successfully identified several potentially relevant RAB8A binding partners.

Conclusions

By elucidating how these newly identified partners integrate into the broader lipid transport machinery, we can better understand the regulation of post-lysosomal cholesterol clearance. Exploiting these regulatory nodes could ultimately yield new strategies to bypass lysosomal blockades in lipid storage disorders like NPC1.

P34

Myeloperoxidase-oxidized LDL: a molecular bridge linking atherosclerosis, depression and psoriasis?

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Aim (background & objectives)

Myeloperoxidase-oxidized low-density lipoproteins (Mox-LDL) are a pathophysiologically relevant form of oxidized LDL implicated in atherosclerosis and increasingly associated with inflammatory and neuropsychiatric disorders. Emerging evidence suggests that psoriasis and major depressive disorder (MDD) share common inflammatory pathways—particularly involving IL-17, IL-23, and TNF-α—and are both linked to increased cardiovascular risk. We hypothesize that Mox-LDL, through combined inflammatory and neuro-fibrinolytic mechanisms, may represent a molecular bridge connecting atherosclerosis, psoriasis, and depression.

Method

We developed an integrated in vitro approach using Normal Human Epidermal Keratinocytes (NHEKs) and Human Aortic Endothelial Cells (HAECs) to assess the effects of Mox-LDL on inflammatory, fibrinolytic, and neurotrophic pathways. Transcriptomic profiling (DNA microarrays), quantitative real-time PCR, and immunoassays were employed to evaluate the expression of key cytokines (IL-6, IL-8, IL-17, IL-23, TNF-α), psoriasis-related targets (CXCL3, CXCL8, S100A7, β-defensin 2), and regulators of fibrinolysis and neuroplasticity (neuroserpin, PAI-1, tPA, plasminogen, and BDNF).

Results

Preliminary data demonstrate that Mox-LDL induces a robust pro-inflammatory response in keratinocytes. Specifically, exposure to physiological concentrations of Mox-LDL significantly upregulated mRNA expression of CXCL3, CXCL8, IL-23, TNF-α, and β-defensin 2 in NHEKs, indicating activation of psoriasis-relevant inflammatory pathways. These findings support a direct role for Mox-LDL in epidermal immune activation. Ongoing analyses aim to determine whether Mox-LDL also disrupts fibrinolytic balance and BDNF-related signaling in endothelial and skin cell models.

Conclusions

Our results provide initial mechanistic evidence that Mox-LDL may contribute to the convergence of inflammatory, vascular, and psoriasis pathways. By linking Mox-LDL exposure to coordinated inflammatory responses, this study initially supports the concept of a shared molecular axis underlying atherosclerosis and psoriasis. These findings lay the groundwork for ongoing analyses that aim to determine whether Mox-LDL may also play a role in disrupting fibrinolytic signaling pathways in endothelial and skin cell models.

P35

Effects of prolonged fasting and sleep deprivation on triglyceride-rich lipoprotein remodelling in healthy adults

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Aim (background & objectives)

Prolonged fasting and sleep deprivation are major metabolic stressors that induce profound metabolic adaptation. We investigated how multi-day fasting and sleep deprivation alter circulating lipoprotein composition in relation to markers of metabolic stress.

Method

In a randomized crossover study conducted in a structured survival training setting, 12 healthy adults (5 women, 7 men) completed two interventions in opposite sequence: 66 h of fasting followed by 24 h of partial refeeding, and 55 h of sleep deprivation. Blood was sampled daily at 10:00. Serum was analysed for markers of metabolic stress and systemic adaptation, including indices of glycemic and ketotic response, lipolysis, protein catabolism, haematological responses, and organ stress using routine methods. Lipoprotein cholesterol and TGs were analyzed by FPLC, and TG:chol ratios were calculated for each fraction.

Results

Prolonged fasting was characterized by marked ketosis and depletion of triglyceride-rich lipoproteins, with lower TG, VLDL-TG, and TG:chol ratios across all lipoprotein fractions, most prominently in VLDL. Higher ketones during fasting were associated with lower VLDL-TG, consistent with a catabolic shift and a state of starvation. Partial refeeding reversed the phenotype, with attenuated ketosis and intermediate triglyceride-rich lipoprotein levels. In contrast, sleep deprivation was characterized by higher glucose and a relatively TG-enriched VLDL phenotype, despite lower absolute VLDL cholesterol. During sleep deprivation, increases in glucose were accompanied by within-subject increases in TG and VLDL-TG, linking metabolic stress to triglyceride-rich lipoprotein remodelling. Sequence order modified the magnitude of some responses, but not their direction.

Conclusions

Multi-day fasting and sleep deprivation produced distinct metabolic and lipoprotein phenotypes. Fasting depleted triglyceride-rich lipoproteins in parallel with ketosis, whereas sleep deprivation was associated with altered remodeling of triglyceride-rich lipoproteins and a more TG-enriched VLDL phenotype, suggesting impaired processing of triglyceride-rich lipoproteins.

P36

Can we predict cardiovascular risk in FH-patients using polygenic predispositions?

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Aim (background & objectives)

Patients with familial hypercholesterolemia (FH) have a substantially higher risk of developing atherosclerotic cardiovascular disease (ASCVD) compared with the general population, yet a small subset remains protected. Genetic factors are known to modify ASCVD risk, and polygenic risk scores (PRSs) can quantify such predisposition. This study evaluated genetic susceptibility to ASCVD in a genetically confirmed FH cohort and explored contributing pathways such as endothelial dysfunction.

Method

The prospective multicenter SAFIR (NCT03234127) study enrolled 511 French patients with genetically diagnosed FH (men > 40 years, women > 50 years). Among them, 340 had established ASCVD (“non-protected”) and 171 showed no atherosclerotic lesions (coronary artery calcium score = 0; “protected”). All participants underwent whole-genome sequencing. Based on a large independent ASCVD genome-wide association dataset (>185,000 individuals), we computed an ASCVD-PRS including over six million common variants weighted by effect size. A dedicated PRS for endothelial dysfunction was also calculated. Mean PRSs were compared between groups using Student’s t-tests.

Results

"Protected" FH patients displayed a significantly lower ASCVD-PRS than "non-protected" patients (18.03 ± 0.09 *vs*. 18.10 ± 0.08; p = 1.7e-14). They also exhibited lower genetic susceptibility to endothelial dysfunction (p = 2.7E-4). Further analyses are ongoing to assess additional polygenic risk factors, including hypertension, inflammation, lipoprotein(a) plasma levels.

Conclusions

Protection against ASCVD in a subset of FH patients appears to result from a polygenic origin, partly involving endothelial function. This study has two main implications. First, the use of PRSs can help refine key additional risk factors contributing to ASCVD in FH patients and improve understanding of the disease's etiology in this particularly high-risk population. Second, by incorporating PRS-based ASCVD risk assessment, we aim to provide clinicians with valuable insights to better predict patient trajectories and advance toward more personalized care for individuals with FH.

P37

HDL Proteomic Signatures of Cardiovascular Resilience in Familial Hypercholesterolemia

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Aim (background & objectives)

Background: Familial hypercholesterolemia (FH) is an autosomal dominant condition characterized by persistently elevated LDL cholesterol (LDL-C) levels from birth, with marked variability in cardiovascular event (CVE) presentation. A subset of individuals, termed "resilient", remain free of CVE beyond age 70 despite lifelong exposure to severe hypercholesterolemia. Emerging evidence suggests that HDL composition and functionality may contribute to this protective phenotype, beyond LDL-C levels. In this study, we investigated the HDL proteomic profile in FH patients to identify molecular signatures associated with resilience to CVE.

Method

Methods: HDL proteomic analysis was conducted in statin-treated FH individuals (n=76, 50% women), including patients with early CVE (n=32) and those with a resilient phenotype (n=44), as well as non-FH relatives (n=20, 50% women) from the Spanish SAFEHEART cohort. HDL particles were isolated by ultracentrifugation and analyzed using LC-MS/MS. Differentially expressed proteins were identified using bioinformatic approaches, and selected targets were validated by ELISA.

Results

Results: Proteomic analysis identified 66 proteins in HDL from FH patients, one-third involved in HDL remodeling and cholesterol metabolism, including PLTP, LCAT, and CETP. HDL levels of PLTP and CETP differed significantly between FH and non-FH subjects. Within FH, resilient subjects showed significantly higher LCAT levels. By ELISA, HDL-associated PLTP was higher in FH than in non-FH subjects (p=0.004), with increased levels in FH patients with early CVE compared to resilient individuals (p=0.009). CETP levels were lower in FH than in non-FH subjects (p=0.0174), with no differences between FH subgroups. HDL-associated LCAT levels were also lower in FH compared with non-FH relatives (p=0.007), pattern found in FH patients with early CVE (p=0.0021), while no differences were observed between resilient individuals and non-FH subjects.

Conclusions

Conclusions: Distinct HDL proteomic signatures are associated with cardiovascular resilience in FH, underscoring the relevance of HDL functionality beyond LDL-C and highlighting potential targets for improved risk stratification.

P38

The effects of myocardial infarction recovery and anti-inflammatory drug on LDL composition and atherogenic properties in NSTEMI patients

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Aim (background & objectives)

The effects of post-myocardial infarction recovery on LDL composition and properties have not been previously investigated, and the impact of anti-inflammatory therapy on these characteristics remains poorly understood. Here, we assessed the time-associated changes (baseline, 3-7 days, and 6 months) in LDL proteomic and lipidomic profiles, LDL aggregation susceptibility and clinical outcomes in patients with non-ST-segment elevation myocardial infarction (NSTEMI). In parallel, we evaluated effects of a single-dose tocilizumab (IL-6 receptor antagonist) on these parameters.

Method

The LDL was isolated from plasma samples from NSTEMI cohort (details: DOI:10.1093/eurheartj/ehw171). Proteomic and lipidomic studies were carried out with LC-MS. LDL aggregation was measured with dynamic light scattering.

Results

The results revealed significant time-associated changes in LDL profiles that were linked to post-infarction recovery rather than anti-inflammatory therapy. Notably, LDL aggregation-susceptibility decreased significantly during follow-up accompanied by a three-fold decrease in serum amyloid A2, linked to inflammation and more atherogenic LDL phenotype.

Time-associated analysis revealed a two-fold increase in apoliprotein (apo) A4, a known inhibitor of LDL oxidation. ApoF showed the most elevation during the follow-up with up to four-fold change. Given that LDL-associated apoF inhibits lipid transfer, its marked increase may contribute to remodeling of LDL lipidome. Indeed, lipidomic analysis demonstrated enrichment of LDL in triglycerides (TG) compared to baseline. Alongside these changes, lysophosphatidylcholine (LPC) (16:0), known for its atherogenic properties, was significantly decreased during the follow-up.

Conclusions

The observed reduction in LDL aggregation susceptibility is consistent with previous studies linking this property to cardiovascular risk. The results showed a post-myocardial infarction shift in lipoprotein profile with the most significant changes occurring in proteins related to lipid transport activity, suggesting an overall effect on liver and lipid metabolism. Further integration of these findings with clinical parameters will provide a more comprehensive understanding of the underlying processes.

P39

Apolipoprotein e4 drives pcsk9 upregulation and amplifies pcsk9-mediated impairment of neuronal cholesterol homeostasis: implications for alzheimer's disease

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Aim (background & objectives)

PCSK9, widely known for its role in lipid metabolism, is increasingly recognized as a key player in Alzheimer's disease (AD). We previously demonstrated that PCSK9 disrupts neuronal cholesterol homeostasis by downregulating apoE-interacting receptors and exacerbates amyloid-β (Aβ)-induced neurotoxicity and inflammation. Consistently, PCSK9 genetic ablation in AD mice models improves cognition and reduces Aβ burden and neuroinflammation. Notably, elevated PCSK9 levels in the cerebrospinal fluid of AD patients were reported – with the highest values in Apolipoprotein (Apo) E4 carriers. This evidence suggests an interplay between apoE4 and cerebral PCSK9 that remains yet unexplored. This study aims to elucidate the mechanistic relationship between ApoE4 and PCSK9 in AD.

Method

Human neuroblastoma IMR-32 cells were incubated with recombinant apoE3 (physiological isoform) or apoE4 (AD-associated isoform). PCSK9 expression and secretion were assessed by WB and ELISA assays, respectively, while SREBF expression was measured through RTqPCR. Neuronal cholesterol uptake was evaluated measuring the internalization of radiolabeled cholesterol carried by synthetic apoE-HDL.

Results

ApoE4 [2.5 - 50μg/ml] significantly increased PCSK9 expression and secretion, reaching a plateau at [10μg/ml] (+31.5%, $p<0.0001$ and +42.6%, $p<0.0001$, respectively, vs untreated cells). ApoE4 [10μg/ml] significantly induced SREBF2 gene expression (+47%, $p<0.05$ vs untreated cells). No significant effects were observed with ApoE3 [10μg/ml], suggesting an isoform-specific response. In addition, cholesterol uptake, essential for proper neuronal physiology, was significantly reduced in neurons exposed to apoE4-containing HDL (-28%, $p<0.01$ vs HDL-ApoE3) and further decreased upon PCSK9 co-incubation (-15%, $p<0.01$ vs HDL-ApoE4).

Conclusions

ApoE4 specifically induces PCSK9 expression in neuronal cells, at least in part via SREBF-mediated transcriptional regulation, providing a novel mechanism underlighting the association between ApoE4 and AD. Also, the two proteins additively contribute to impaired cholesterol uptake, The results point to PCSK9-targeted therapies as a novel pharmacological approach in AD, particularly for ApoE4 carriers, at increased risk of adverse effects from the anti-Aβ treatments.

P40

Expansion of in-depth functional characterization of LDLR gene variants and implications for cardiovascular risk assessment

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Aim (background & objectives)

Familial hypercholesterolemia (FH) is a common inherited disorder that remains substantially underdiagnosed despite its strong contribution to premature cardiovascular disease. A central barrier to improving FH diagnostics is the lack of functional evidence for a majority of LDLR variants. A recent sequence–function atlas of LDLR missense variation (Tabet et al. 2025) provided information on pathogenecity for a large set of LDLR variants. However, a more precise determination of residual LDLR activity and deeper insight into the functional consequences of individual variants on the cellular level may improve cardiovascular risk assessment.

Method

We utilized a previously established semi-automated analysis pipeline based on multiplexed high-content imaging (Islam et al. 2025) to expand in-depth functional characterization of LDLR gene variants. The resulting functional data was compared to the new sequence function map of LDLR and will be integrated with whole exome sequencing and clinical data from the UK Biobank.

Results

We expanded the in-depth functional characterization to more than 600 LDLR gene variants. Providing in-depth functional characterization regarding LDL uptake, LDLR expression, and localization. LDLR functional groups were formed based on residual LDLR activity, representing loss-of-function (0-10% residual activity), defective (10-30%), mildly defective (30-70%), and non-defective (>90%) groups. At EAS we will present the integration of the functional data with UK Biobank data to demonstrate the effects of the functional groups on the utilization of lipid-lowering therapy, circulating lipoproteins, and cardiovascular disease risk. Moreover, we will report a comparison of the two largest functional studies on LDLR activity.

Conclusions

We expect that different functional activity groups will display varying cardiovascular risk profiles, which will be highly relevant for risk assessment in familial hypercholesterolemia. This will enhance the utility of genetic testing by providing more data for personalized treatment approaches in familial hypercholesterolemia.

P41

LIPA gene variants and familial hypercholesterolemia

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Aim (background & objectives)

Familial hypercholesterolemia (FH) is characterized by increased LDL-C, resulting from impaired clearance of LDL, and risk of premature cardiovascular disease. A substantial proportion of FH cases lack pathogenic variants in canonical FH genes. While polygenic inheritance explains part of this mutation-negative group (FH/M–), additional rare variants and alternative cellular mechanisms may contribute. Lysosomal acid lipase (LAL), encoded by *LIPA*, is essential for post-endocytic lipid processing. Biallelic *LIPA* loss-of-function causes LAL deficiency, and defective lysosomal processing of LDL-derived lipids which give rise to lipid FH phenocopy. The contribution of rare heterozygous *LIPA* variants to FH/M– remains unclear.

Method

We sequenced *LIPA* in 514 unrelated FH/M– probands and assessed the burden of rare predicted damaging variants relative to population reference datasets. Functional consequences were investigated in cells from carriers of *LIPA*–p.(Pro370Gln), including LDL uptake, LDL receptor abundance, LAL enzymatic activity, transcriptomic and proteomic profiling.

Results

Six rare predicted damaging *LIPA* variants were identified and were enriched in the FH/M– cohort as compared to general population (OR = 2.34 [0.93–4.86], $p = 0.034$). One variant, p.(Pro370Gln), segregates with hypercholesterolemia in one FH/M– family. Cells from p.(Pro370Gln) carriers exhibited reduced LDL receptor ($p = 0.0002$) and impaired LDL uptake ($p < 0.0001$) consistent with an FH-like cellular phenotype. However, LAL enzymatic activity was not markedly reduced on the contrary of heterozygous *LIPA* loss-of-function variant carriers. Transcriptomic and proteomic analyses revealed alterations in endocytosis, vesicle-mediated transport, proteolysis, and intracellular trafficking pathways, which were distinct from those observed in *LDLR*-related FH.

Conclusions

Together, these findings indicate that *LIPA*–p.(Pro370Gln) carriers display an FH-like cellular phenotype that is mechanistically distinct from both *LDLR*-related FH and classical LAL deficiency. The *LIPA*–p.(Pro370Gln) molecular signatures highlight alternative intracellular pathways that may contribute to hypercholesterolemia and warrant further genetic investigation.

P42

Exploring the role of ApoM in heart faillure with preserved ejection fraction induced by chronic kidney disease in vivo

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Aim (background & objectives)

Patients with chronic kidney disease (CKD) are at high risk of developing heart failure with preserved ejection fraction (HFpEF). Reduced levels of circulating apolipoprotein M (apoM) are associated with advanced CKD and increased cardiovascular death. Furthermore, apoM is mainly associated with HDL and function as a carrier of sphingosine-1-phosphate (S1P). Together, the apoM-S1P complex is involved in many downstream signaling pathways, including endothelial barrier function, lipid turnover and inflammation. This study aims to investigate the potential protective role of apoM in CKD-induced HFpEF *in vivo*.

Method

CKD-HFpEF was induced in Wt, apoM-KO and apoM transgenic mice using a two-step surgical approach. Ischemia reperfusion injury was performed in one kidney combined with a delayed contralateral complete nephrectomy. Hypertension was induced by administration of L-NAME in the drinking water. Renal and cardiac function were monitored throughout the study using glomerular filtration rate (GFR) and echocardiography, respectively.

Results

Over the 17-week study period, Wt mice maintained a ~50 % reduction in GFR. Preserved cardiac systolic values combined with impaired diastolic values such as impaired left ventricular (LV) relaxation, increased LV filling pressures and hypertrophy confirmed the development of CKD-HFpEF phenotype. This phenotype was further exacerbated after the administration of L-NAME. ApoM transgenic mice showed a similar disease progression. In contrast, apoM-KO mice displayed a milder HFpEF phenotype and lower mortality rate in response to L-NAME treatment.

Conclusions

Contrary to our hypothesis, preliminary findings indicate that apoM deficiency protects against HFpEF development in CKD. Further characterization of structural changes, metabolic pathways and vascular response is ongoing to clarify the exact role of apoM in CKD-induced HFpEF pathogenesis.

P43

Remnant clearance capacity explains the clinical divergence of severe hypertriglyceridemia

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Aim (background & objectives)

Background:
Severe hypertriglyceridemia associated with multifactorial chylomicronemia syndrome (MCS) exhibits striking clinical heterogeneity. While some patients predominantly develop acute pancreatitis, others present mainly with atherosclerotic cardiovascular disease (ASCVD). The metabolic basis underlying this divergence remains poorly understood.

Method

We developed a dual-epitope ELISA that selectively quantifies structurally intact lipoprotein lipase (intact LPL), representing circulating full-length LPL retaining functional domains. Using fasting plasma samples from patients with severe hypertriglyceridemia, we evaluated the ratio of triglyceride(TG)-to-intact LPL as a surrogate marker of remnant clearance capacity (RCC), reflecting the balance between triglyceride-rich lipoprotein (TRL) flux and lipolytic processing capacity

Results

Marked variability in the TG-to-intact LPL ratio was observed among patients with similar TG levels. Patients with extremely elevated ratios showed metabolic features consistent with impaired TRL processing, suggesting a state in which TRL flux exceeds lipolytic capacity. In contrast, patients with moderately elevated ratios appeared to retain partial processing capacity, potentially favoring remnant persistence rather than massive chylomicron accumulation

Conclusions

These findings support a conceptual framework in which clinical manifestations of severe hypertriglyceridemia reflect the balance between TRL flux and lipolytic processing capacity. RCC, estimated by the TG-to-intact LPL ratio, may provide a clinically accessible marker of TRL processing capacity and help explain the metabolic divergence between pancreatitis-dominant and ASCVD-dominant phenotypes of MCS.

P44

Effect of inclisiran on lipid and mechanical vascular profiles in familial hypercholesterolemia subjects: results from a single lipid center real-world experience

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Aim (background & objectives)

Familial hypercholesterolemia (FH) is characterized by elevated LDL-C and an increased risk of premature cardiovascular events. Inclisiran is a small interfering RNA that inhibits hepatic PCSK9 synthesis and promotes LDL-C clearance by enhancing LDLR expression on hepatocytes. This study aimed to evaluate the efficacy of six-months add-on inclisiran on lipid profile and PWV in FH; furthermore, we investigated the association between LDL-C reduction and PWV variation.

Method

This prospective observational study involved 78 genetically confirmed FH subjects (51.3% male) with an LDL-C off target despite high-intensity statins plus ezetimibe. All subjects obtained biochemical analysis and PWV evaluation at baseline and after six months add-on inclisiran.

Results

After six months add-on inclisiran, 41 % of subjects achieved LDL-C targets. Significant reductions of LDL-C (- 41.5 %, $p < 0.001$), ApoB (- 33.7 %, $p < 0.01$), Non-HDL-C (- 35.9 %, $p < 0.001$), and Lp(a) (- 18 %, $p < 0.01$) were observed, while PWV improved by 14.4 % ($p < 0.001$). In a secondary analysis, the study population was divided in two groups according to the cardiovascular history: FH subjects without ASCVD (Primary prevention group) and FH subjects with ASCVD (Secondary prevention group). The Primary prevention group showed a higher prevalence of subjects on LDL-C target than the Secondary prevention group (59 % vs 23.1 %, $p < 0.001$). Both groups exhibited significant improvements of lipid profile and PWV (Δ - 14.1 %, $p < 0.01$ and Δ - 14.6 %, $p < 0.001$, respectively). Linear regression showed a significant association between ΔPWV and ΔLDL-C in the whole study population as well as in the Primary and Secondary prevention groups (p for all < 0.001). Moreover, multiple linear regression analysis demonstrated that in the whole population ΔPWV remained significantly associated with ΔLDL-C after adjusting for traditional cardiovascular risk factors.

Conclusions

Inclisiran significantly improved lipid profile and PWV in a cohort of FH subjects. ΔPWV was significantly associated with ΔLDL-C.

P45

Whole genome sequencing and variant effect predictions across diverse ancestries reveal the full functional spectrum of *APOE* variation

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Aim (background & objectives)

The strongest genetic risk factor for dementia and a major player in peripheral and cerebral lipid metabolism – the *APOE* gene – is a well-known modulator of atherosclerotic cardiovascular disease (ASCVD), and other common lipid-related traits and diseases. Recent evidence from clinical trials and genome-wide association studies (GWAS) highlights the importance of taking variation in this central gene into consideration when designing randomized clinical dementia trials and when performing drug discovery genomic analyses. Numerous studies have been conducted with the common ε2/ε3/ε4 *APOE* polymorphism – the evidence is however sparse concerning the totality of genetic variation in *APOE* obtained from whole-genome sequencing (WGS). We hypothesize that WGS data will reveal novel functional variation in the *APOE* gene with impact on common lipid-related traits and diseases.

Method

WGS data from the UK Biobank (UKB) and All of US was analyzed to evaluate variation in *APOE*. Variants underwent quality control procedures before functional annotation with the Ensembl Variant Effect Predictor (VEP). Splice site and truncating Loss-of-Function (LoF) variants were identified using LOFTEE, and missense variants were further classified using state-of-the-art VEPs (AlphaMissense and REVEL). Given the low frequency of most functional variants, gene-level burden testing was employed.

Results

AlphaMissense and REVEL identified functional variants that, through burden tests, were significantly associated with higher apoE (p-values<0.001) and triglyceride concentrations (p-values<0.05). These analyses were adjusted for ε2/ε4, highlighting an independent ε2-like LDL-receptor ligand defect. LOFTEE identified LoF variants associated with low apoE concentrations (p-values<0.001), which also remained unaffected by ε2/ε4 adjustment (Figure 1). Effects were largely similar in All of US, and additionally revealed ancestry specific functional variants responsible for the findings.

Conclusions

WGS data revealed novel functional impact of the *APOE* gene on common lipid-related traits, highlighting that both common and rare functional genetic information should be addressed in hypothesis generating association studies and in genomics-driven drug discovery.

P46

Functional data for LDLR variant classification: comparative insights from high-content microscopy and flow-cytometry assays

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Aim (background & objectives)

Current FH VCEP specifications of ACMG/AMP guidelines assign a higher evidence level to functional data obtained with flow-cytometry than microscopy assays for familial hypercholesterolemia (FH) gene variant interpretation. This limits the use of microscopy-derived functional data for variant classification. We compared *LDLR* variant functional data from microscopy and flow cytometry assays to evaluate whether the evidence level could be raised for microscopy assays.

Method

Fifty *LDLR* variants with available flow cytometry and high-content microscopy data were compared for LDL uptake activity, including 21 newly characterized variants by microscopy in this study. Variants were grouped by FH VCEP functional thresholds (pathogenic: <70% activity; benign: >90% activity) and results were integrated with UK Biobank data to assess associations with lipid traits.

Results

First, we validated our scalable microscopy assay with FH VCEP-classified control variants. Then we compared functional activity measured by microscopy and flow cytometry assays for 50 variants, which showed strong correlation ($r = 0.66$, $p<0.0001$) and a close average agreement (Bland–Altman bias = –0.06). Applying FH VCEP functional classification thresholds yielded broadly consistent classification in both methods, with minor shifts among categories. Integration with UK Biobank showed that individuals with variants with <70% and <50% activity exhibited higher LDL-C, total cholesterol and apolipoprotein-B levels across both methods, with stronger effects being observed for the 50% cutoff; for example LDL-C (microscopy: 4.30(<70%)/4.57(<50%) vs 3.81 mmol/L (>90%); flow cytometry: 4.0(<70%)/4.18(<50%) vs 3.83 mmol/L(>90%)).

Conclusions

Microscopy provides robust LDLR activity measurements, aligning closely with flow cytometry and lipid phenotypes. These results support reconsideration of the current evidence level of validated microscopy assays into the FH VCEP classification frameworks as a level 1 criterion

P47

Single-cell analysis of calcium signaling in macrophages reveals activation by aggregated LDL and modulation by neurosteroid

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Aim (background & objectives)

Atherosclerosis is a leading cause of mortality in developed countries and is characterized by chronic inflammation caused by accumulation of cholesterol-rich lipoproteins in the subendothelial space. This promotes macrophage activation, uptake and processing of LDL, and eventually leads to foam cell formation. Calcium signaling regulates macrophage activation and intracellular pathways linked to lipid handling. Toll-like receptor 4 (TLR4) has been implicated in recognition and processing of aggregated LDL. This study aimed to investigate how aggregated LDL modulates calcium signaling in macrophages and whether this response can be regulated by endogenous neurosteroids such as allopregnanolone.

Method

Murine J774 macrophages were exposed to aggregated LDL and subjected to live-cell calcium imaging using Cal-520 AM. Time-lapse recordings were segmented at the single-cell level, and intracellular calcium activity was quantified by spike frequency, amplitude, and duration. Allopregnanolone and its structurally related analogue epi-allopregnanolone were applied to assess inhibitory effects. Ongoing experiments will validate findings using lower-affinity calcium indicators expected to improve separation of stimulus-induced spikes from basal activity. Machine-learning based trajectory clustering will be employed to infer macrophage subpopulations, as we have shown recently for sterol-induced calcium oscillations in astrocytes.

Results

Single-cell analysis revealed heterogeneous calcium signaling across macrophage populations. Aggregated LDL increased calcium spiking compared with control conditions. Allopregnanolone reduced basal calcium activity and decreased the aggregated LDL-induced increase, although activity was not fully restored to control levels. Epi-allopregnanolone showed a similar inhibitory effect.

Conclusions

This study presents a single-cell imaging approach for resolving calcium dynamics in macrophages. Aggregated LDL promotes elevated calcium signaling, while allopregnanolone dampens, but does not abolish, this response. These findings suggest that neurosteroid modulation of TLR4-associated pathways may influence calcium dependent macrophage responses during lipoprotein processing in atherosclerosis.

P48

Impact of female reproductive factors on lipids and lipoproteins

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Aim (background & objectives)

Female reproductive factors are increasingly linked to cardiovascular disease (CVD), but the extent to which lipid status facilitates these associations remains unclear. While hypertensive disorders of pregnancy have been associated with increased CVD risk, the role of lipids across different reproductive exposures has not been thoroughly investigated. We aimed to explore whether reproductive traits and pregnancy-related complications influence lipid traits and CVD.

Method

We performed a two-sample Mendelian randomization study using genetic instruments for age at menarche, age at natural menopause, gestational diabetes, preeclampsia, and gestational hypertension. Summary statistics for lipid traits (LDL cholesterol, HDL cholesterol, triglycerides, total cholesterol, and non-HDL cholesterol) and cardiovascular outcomes, including coronary artery disease (CAD) and stroke subtypes, were obtained from large genome-wide association studies. The inverse-variance weighted method was used as the primary analysis, with sensitivity analyses; MR-Egger and weighted median.

Results

Later age at menarche was associated with a favorable lipid profile, including higher HDL cholesterol (OR 1.04 [1.10-1.07]) and lower triglycerides (OR 0.96 [0.94-0.97]), and with reduced risk of CAD and several stroke subtypes. In contrast, hypertensive disorders of pregnancy showed strong associations with increased risk of CAD and stroke (preeclampsia: OR 1.23 [1.11-1.36] and gestational hypertension: OR 1.26 [1.16-1.36] for CAD), but no association with lipid traits. Age at natural menopause showed largely null associations across lipid and CVD outcomes, while gestational diabetes demonstrated inconsistent effects.

Conclusions

Genetically proxied later age at menarche appears to decrease cardiovascular risk through lipid-related pathways, whereas hypertensive disorders of pregnancy confer substantial cardiovascular risk independent of lipid traits. This highlight heterogeneous biological mechanisms linking female reproductive health to cardiometabolic disease.

P49

Quantitative live-cell imaging of Low-Density Lipoprotein reveals the role of neurosteroids in macrophage foam cell formation

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Aim (background & objectives)

Atherosclerosis is driven by macrophage interactions with aggregated low-density lipoproteins (agLDL) within the arterial wall. This interaction leads to the formation of an extracellular, acidic, hydrolytic compartment enriched in secreted lysosomal enzymes, a process termed *digestive exophagy*, which promotes foam cell formation. Activation of Toll-like receptor 4 (TLR4) is a key step in this pathway. Although low-density lipoprotein (LDL) delivers cholesterol to mammalian cells as cholesteryl esters (CEs), the intracellular trafficking and hydrolysis of CEs remain poorly understood due to limited methodological tools. Furthermore, the role of endogenous steroid hormones in atherogenesis is not well defined.

Method

We study the dynamics of LDL-derived CEs in cells using fatty acyl chain conjugates of the intrinsically fluorescent cholestatrienol (CTL). By reconstituting CTL esters into LDL, we are able to determine intracellular trafficking routes and metabolic processing of LDL-derived cholesterol. Using quantitative fluorescence microscopy, we examined the effects of naturally occurring steroid hormone allopregnanolone (AlloP) on murine macrophage activity in extracellular digestion of lipoprotein aggregates.

Results

We found that treating murine macrophages with agLDL in combination with AlloP reduces the secretion of lysosomal enzymes, thereby inhibiting the digestion of agLDL. We are currently studying how AlloP affects the generation of free cholesterol at sites of contact between agLDL and macrophages. Preliminary findings suggest that AlloP reduces the formation of lipid droplets in macrophages.

Conclusions

These findings demonstrate that AlloP suppresses digestive exophagy in macrophages and reduces lipid accumulation. This suggests a potential role for AlloP in modulating foam cell formation and slowing the progression of atherosclerosis.

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