European Lipoprotein Club

44th Annual Scientific Meeting

September 06-09, 2021

Evangelische Akademie

Tutzing, Germany

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Monday Sep 06

12:00 - 13:50	**Arrival, registration & lunch**
13:50 - 14:00	**Welcome** *Patrick Rensen (Leiden, The Netherlands)*
Session:	**Liver & Bile Acids**
	Chairperson: Bart van de Sluis (Groningen, The Netherlands)
14:00 - 14:30	**Invited speaker: Leanne Hodson (Oxford, UK)** The ins and outs of liver fat metabolism
14:30 - 14:45	**Bo Angelin (Stockholm, Sweden)** Understanding the feedback regulation of hepatic cholesterol breakdown to bile acids by studying the family of a patient deficient in SLC51A (OST-Alpha)
14:45 - 15:00	**Esther Verkade (Groningen, The Netherlands) *** Bile salt export pump-deficiency in Cyp2c70-knock-out mice with a humanized bile acid pool, a novel mouse model for Progressive familial intrahepatic cholestasis type 2 (PFIC2)
15:00 - 15:15	**Tarek Moustafa (Graz, Austria)** Bile acids prevent hepatic triglyceride accumulation by controlling precursors for lipid synthesis in liver and adipose tissue
15:15 - 15:30	**Dyonne Vos (Groningen, The Netherlands) *** The Parkinson's Disease protein VPS35 maintains hepatic lipid homeostasis through regulating lysosomal function and autophagy
15:30 - 15:45	**Anja Zeigerer (Munich, Germany)** Endosomal sorting complex protein Vps37a regulates hepatic glucose production via altering glucagon receptor trafficking
15:45 - 16:00	**Giannis Evangelakos (Hamburg, Germany) *** The metabolic effects of the synthetic bile acid derivative *nor*UDCA
16:00 - 16:30	**Coffee break**

** Eligible for Young Investigator Oral Award*

Monday Sep 06

Session: **Fat Signals and Cells**

Chairperson: Dagmar Kratky (Graz, Austria)

16:30 - 16:45 **Ivan Bradić (Graz, Austria) ***
Crosstalk between hepatocytes and adipocytes in LAL deficiency

16:45 - 17:00 **Paul Vesely (Graz, Austria)**
Adipose triglyceride lipase (ATGL) is needed for homeostatic control of sterol element-binding protein-1c (SREBP-1C) driven hepatic lipogenesis

17:00 - 17:15 **Gernot Grabner (Graz, Austria)**
Selective inhibition of human adipose triglyceride lipase by small molecules

17:15 - 17:30 **Carolin Muley (Munich, Germany) ***
Nfe2l1 protecs white adipocytes from cholesterol-induced inflammation

17:30 - 17:45 **Sophia Metz (Copenhagen, Denmark) ***
From variant to function: the discovery and validation of CYR61 as a regulator of body composition

17:45 - 18:00 **Fabian Finger (Copenhagen, Denmark)**
Calcium-dependent hormetic regulation in brown and white adipocytes

18:00 - 18:15 **Michelle Jaeckstein (Hamburg, Germany) ***
Vascular endothelial cells of white adipose tissue modulate de novo lipogenesis in adipocytes by the CD73-dependent generation of extracellular adenosine

18:30 - 19:30 **Dinner**

20:00 - 21:00 **Keynote Lecture**

Chairperson: Patrick Rensen (Leiden, The Netherlands)

Stephan Herzig (Munich, Germany)
Intercellular communication in energy homeostasis

21:00 – 24.00 **Bar open**

** Eligible for Young Investigator Oral Award*

Tuesday Sep 07

07:30 - 08:30	**Breakfast**
Session:	**Macrophages & Brown Fat** *Chairpersons: Alexander Bartelt (Munich, Germany) & Stefan Nilsson (Umeå, Sweden)*
08:30 - 08:45	**Alice Maestri (Stockholm, Sweden) *** The Ser251Pro SNP in perilipin 2 alters chaperone-mediated authophagy and increases macrolipophagy in human macrophages
08:45 - 09:00	**Milena Schönke (Leiden, The Netherlands) *** Time to run: late rather than early exercise decreases atherosclerosis
09:00 - 09:15	**Simon Meyer (Hamburg, Germany) *** TREM2-dependent internalization of lipoprotein particles in brown adipose tissuse macrophages in response to cold exposure
09:15 - 09:30	**Aashley Sardjoe Mishre (Leiden, The Netherlands) *** Cold-induced thermogenesis is higher in the morning compared to the evening in young lean individuals
09:30 - 09:45	**Carlotta Corban (Hamburg, Germany) *** Lipoprotein lipase expressed by vascular endothelial cells of activated thermogenic adipose tissues is dispensable for the processing of triglyceride-rich lipoproteins
09:45 - 10:00	**Sander Kooijman (Leiden, The Netherlands)** Circadian control of LPL-mediated lipolysis in brown adipose tissue
10:00 - 10:30	**Coffee break**
10:30 - 10:45	**Markus Heine (Hamburg, Germany)** Alternative energy supply mechanisms for thermogenic adipose tissues replenishment during prolonged cold exposure

** Eligible for Young Investigator Oral Award*

Tuesday Sep 07

10:45 - 11:00	**Céline Jouffe (Munich, Germany)** Genomic glucocorticoid actions in brown adipose tissue control lipid utilization
11:00 - 11:15	**Ana Bici (Munich, Germany) *** PMEPA1 is a novel regulator of brown adipocyte proteostasis and thermogenesis via TGF-beta
11:15 - 11:30	**Iuliia Karavaeva (Copenhagen, Denmark) *** The mitochondrial transporter, SLC25A34, integrates circadian and temperature cues to control adipocyte thermogenesis
11:30 - 11:45	**Christian Wolfrum (Zurich Switzerland)** Inhibition of AXL receptor tyrosine kinase enhances brown adipose tissue functionality
12:00 - 13:00	**Lunch**
13:30 - 15:30	**Networking** (**OC Meeting**)
Session:	**Fancy Lipids**
	Chairperson: Thorsten Hornemann (Zurich, Switzerland)
16:00 - 16:30	**Invited speaker: Wilfried Le Goff (Paris, France)** Importance of membrane lipid remodeling in the metabolic activation of adipose tissue macrophages during obesity
16:30 - 16:45	**Amber Meurs (Amsterdam, The Netherlands) *** Genome-wide CRISPR-Cas9 screen to identify new determinants involved in intracellular cholesterol transport
16:45 - 17:00	**Sebastian Hendrix (Amsterdam, The Netherlands) *** SPRING is a novel determinant in SREBP signalling and cholesterol metabolism
17:00 - 17:15	**Valentina Bianco (Graz, Austria) *** Consequences of intestinal LAL deficiency on whole body lipid metabolism

** Eligible for Young Investigator Oral Award*

Tuesday Sep 07

17:15 - 17:30	**Melanie Korbelius (Graz, Austria) *** The role of intestinal ATGL in intestinal and systemic cholesterol homeostasis
17:30 - 17:45	**Zachary Gerhart-Hines (Copenhagen, Denmark)** Tissue-specific roles of the mitochondrial phospholipid, cardiolipin, in the control of systemic energy homeostasis
17:45 - 18:00	**Pirkka-Pekka Laurila (Helsinki, Finland)** Spingolipids accumulate in pathological skeletal muscle and inhibition of sphingolipid *de novo* synthesis counteracts severe muscle diseases
18:00 - 19:00	**Dinner**
19:00 - 21.00	**Wine and Poster Science**
21:00 – 24.00	**Bar open**

** Eligible for Young Investigator Oral Award*

Wednesday Sep 08

07:30 - 08:30	**Breakfast**
Session:	**Lipids & Lipoproteins**
	Chairperson: Christina Christoffersen (Copenhagen, Denmark)
08:30 - 09:00	**Invited speaker Philip W. Shaul (UT Southwestern, USA)** The ins and outs of SR-B1 function in cardiometabolic health and disease
09:00 - 09:15	**Robin Verwilligen (Leiden, The Netherlands) *** *Scarb1* deficiency in zebrafish lowers female fertility in the context of unaltered plasma cholesterol levels
09:15 - 09:30	**Lei Deng (Wageningen, The Netherlands) *** Lipoprotein lipase and caveola mediate uptake of VLDL-like particles in macrophages
09:30 - 09:45	**Michael Ploug (Copenhagen, Denmark)** GPIHBP1 and ANGPTL4 utilize protein disorder to orchestrate order in plasma triglyceride metabolism
09:45 - 10:00	**Bingni Chen (Munich, Germany) *** Endothelial cannabinoid receptor CB1 deficiency decreases oxLDL uptake and attenuates vascular inflammation in atherosclerosis
10:00 - 10:15	**Matteo Pedrelli (Stockholm, Sweden)** Lipoprotein structural and functional properties prevent atherosclerosis development in brown bears (Ursus arctos)
10:15 - 10:30	**Marta Turri (Milan, Italy) *** The HDL mimetic CER-001 ameliorates lipoprotein profile in familial LCAT deficiency
10:30 - 11:00	**Coffee break**

** Eligible for Young Investigator Oral Award*

Wednesday Sep 08

Session: **Genes & Genetics**

Chairperson: Mathilde Varret (Paris, France)

11:00 - 11:15	**Grigorios Panteloglou (Zurich, Switzerland) *** The U2-spliceosome and its interactors regulate the levels and activity of the LDL receptor in humans
11:15 - 11:30	**Melina Amor (Graz, Austria)** MMP12: A potential new target for cardiometabolic diseases
11:30 - 11:45	**Ko Willems van Dijk (Leiden, The Netherlands)** Triglyceride lowering LPL alleles combined with LDL-C lowering alleles are associated with an additively improved lipoprotein profile
11:45 - 12:00	**Liv Tybjærg Nordestgaard (Copenhagen, Denmark) *** Genetic variation in ABCA1 and risk of age-related macular degeneration, dementia, and ischemic heart disease
12:00 - 12:15	**Iryna Hlushchenko (Helsinki, Finland) *** Linking cellular lipid metabolism profiles to the outcomes of cholesterol-lowering therapy in a general population cohort study
12:15 - 12:30	**Simon Pfisterer (Helsinki, Finland)** Functional analysis of LDLR variants using automated systems
12:30 - 13:30	**Lunch**
13:30 - 15.00	**Networking**

Session: **CVD & Therapy**

Chairperson: Nicola Ferri (Padova, Italy)

15:00 - 15:15	**Willemien van Zwol (Groningen, The Netherlands)** A novel gene affecting VLDL metabolism and atherosclerosis
15:15 - 15:30	**Laurent Martinez (Toulouse, France)** The Gi-coupled P2Y13 receptor signaling inhibits lipolysis and protects from metabolic syndrome and associated liver diseases

** Eligible for Young Investigator Oral Award*

Wednesday Sep 08

15:30 - 15:45	**Menno Hoekstra (Leiden, The Netherlands)** PRMT1 inhibitor TC-E 5003 reduces non-alcoholic fatty liver disease and atherosclerosis burden in western-type diet-fed LDL receptor knockout mice
15:45 - 16:00	**Danilo Norata (Milan, Italy)** PCSK9 deficiency rewires heart metabolism and drives heart failure with preserved ejection fraction
16:00 - 17:00	**General Assembly & YI Awards**
18:30 - 22.00	**Fancy dinner**
22:00 - late	**Bar open**

Thursday Sep 09

07:30 - 09:30	**Breakfast**
09:30 - 12.00	**Departure**

Oral presenters eligible for YIA

In order of appearance in the program:

Esther Verkade (Groningen, The Netherlands)
Dyonne Vos (Groningen, The Netherlands)
Giannis Evangelakos (Hamburg, Germany)
Ivan Bradić (Graz, Austria)
Carolin Muley (Munich, Germany)
Sophia Metz (Copenhagen, Denmark)
Michelle Jaeckstein (Hamburg, Germany)
Alice Maestri (Stockholm, Sweden)
Milena Schönke (Leiden, The Netherlands)
Simon Meyer (Hamburg, Germany)
Aashley Sardjoe Mishre (Leiden, The Netherlands)
Carlotta Corban (Hamburg, Germany)
Ana Bici (Munich, Germany)
Iuliia Karavaeva (Copenhagen, Denmark)
Amber Meurs (Amsterdam, The Netherlands)
Sebastian Hendrix (Amsterdam, The Netherlands)
Valentina Bianco (Graz, Austria)
Melanie Korbelius (Graz, Austria)
Robin Verwilligen (Leiden, The Netherlands)
Lei Deng (Wageningen, The Netherlands)
Bingni Chen (Munich, Germany)
Marta Turri (Milan, Italy)
Grigorios Panteloglou (Zurich, Switzerland)
Liv Tybjærg Nordestgaard (Copenhagen, Denmark)
Iryna Hlushchenko (Helsinki, Finland)

UNDERSTANDING THE FEEDBACK REGULATION OF HEPATIC CHOLESTEROL BREAKDOWN TO BILE ACIDS BY STUDYING THE FAMILY OF A PATIENT DEFICIENT IN SLC51A (OST-ALPHA)

Bo Angelin, Per Stål, Hong Jiao

Cardiometabolic Unit, Department of Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Stockholm, Sweden

Aim: Breakdown of cholesterol to bile acids (BA) in the liver is controlled through sensing their flux through the enterohepatic circulation (EHC). BA levels in ileal enterocytes are determined by the balance between luminal uptake (mainly by SLC10A2; IBAT) and venous export (mainly by the SLC51A/B; OST-alpha/beta complex). We characterized the metabolic phenotype in a patient lacking SLC51A gene function and her family.

Method: Sera obtained at 0, 90 and 180 min following a meal were analyzed for individual BA, markers of cholesterol and BA synthesis, FGF19, and lipoproteins. Whole exome sequencing (WES) was performed.

Results: The patient was the third child of unrelated healthy Swedish parents. At age one, she displayed renal tubular and hepatic dysfunction which both resolved. She had evidence of fat malabsorption and was given vitamin suppleme\ntation and medium-chain triglycerides. Levels of 7alpha-hydroxy-4-cholesten-3-one (C4) were extremely low, and at age 19 she was investigated with her family. WES in the patient revealed a missense variant at position chr3:195955754 (GRCh37) in the SLC51A gene (NM_152672: c.T596C, p.L199P). This variant is very rare, but was present in her mother and both healthy sisters. Since the father was homozygote for reference allele T, we hypothesized that there was a de novo deletion at the variant site. This was confirmed by CNV and whole genome sequencing, making the patient hemizygote for the variant. While the patient had repeatedly very low levels of C4, 0.5-0.6 nmol/mmol chol; remaining family members had normal values. Her fasting FGF19 (751 pg/mL) exceeded normal range, while serum BA were reduced and did not increase following a meal.

Conclusions: Loss of function of the OST-alpha/beta complex leads to BA accumulation in ileal enterocytes, eliciting FXR-mediated overexpression of FGF19 that inhibits hepatic BA production and probably LDL receptor expression explaining normal LDL levels despite malabsorption.

BILE SALT EXPORT PUMP-DEFICIENCY IN CYP2C70-KNOCK-OUT MICE WITH A HUMANIZED BILE ACID POOL, A NOVEL MOUSE MODEL FOR PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS TYPE 2

Esther Verkade, Milaine Hovingh, Folkert Kuipers, Jan Freark de Boer

University Medical Center Groningen, Groningen, Netherlands

Aim: Progressive familial intrahepatic cholestasis type 2 (PFIC2) is a genetic disorder caused by mutations in the bile salt export pump (BSEP), leading to severe cholestasis and end-stage liver disease in early life. Adequate therapy is lacking and PFIC2 animal models that reflect the human disease for evaluating new treatments are not available. Bsep-KO mice do not develop liver disease, probably due to production of hydrophilic mouse-specific bile acids (BAs). We investigated whether Bsep-knock down (KD) induces PFIC2-like disease in mice without these murine BAs, *i.e.* Cyp2c70-KO.

Method: BSEP-KD was induced in Cyp2c70-KO mice and WT controls using CRISPR/Cas9-technology. Mice were sacrificed four weeks after BSEP-KD to study bile formation and liver pathology.

Results: BSEP protein expression was ~85% lower in Bsep-KD mice compared to Bsep-WT mice. Biliary BA excretion was ~45% lower and maximum biliary BA excretion capacity upon TUDCA infusion was profoundly reduced in Cyp2c70-KO Bsep-KD mice compared to Cyp2c70-KO Bsep-WT controls. As expected, the BA pool of Cyp2c70-WT mice became very hydrophilic upon Bsep-KD and hardly any liver damage was present. Surprisingly, also in Cyp2c70-KO Bsep-KD mice no cholestatic liver disease developed. Transcriptome analysis showed a strong upregulation of an alternative BA transporter, multidrug-resistance-protein 1 (Mdr1a) in Cyp2c70-KO Bsep-KD mice, suggesting a compensatory mechanism to prevent liver disease. Preliminary data indicate that Mdr1/Bsep-double KD (DKD) Cyp2c70-KO mice indeed have a reduced bile flow compared to Bsep-KD Cyp2c70-KO mice.

Conclusions: Cyp2c70-KO Bsep-KD mice present features of PFIC2 disease, namely reduced biliary BA excretion and maximum biliary BA excretion. However, upregulation of other putative BA transporters, *e.g.* Mdr1, conceivably prevents the development of cholestatic liver disease in these mice. Indeed, Mdr1/Bsep-DKD Cyp2c70-KO mice have a lower bile flow compared to Bsep-KD mice. In the near future we aim to establish whether Mdr1/Bsep-DKD Cyp2c70-KO do display PFIC2-like pathology.

Eligible for Young Investigator Oral Award

BILE ACIDS PREVENT HEPATIC TRIGLYCERIDE ACCUMULATION BY CONTROLLING PRECURSORS FOR LIPID SYNTHESIS IN LIVER AND ADIPOSE TISSUE

Alex Zaufel[1], Dagmar Silbert-Wagner[1], Judith Sommer[1], Clemens Diwoky[2], Helga Reicher[3], Jürgen Prasch[3], Martin Trötzmüller[4], Harald Köfeler[5], Dagmar Kolb[6], Nemanja Vujic[3], Ioannis Evangelakos[7], Nienke Willemsen[8], Dagmar Kratky[3], Wolfgang Sattler[3], Alexander Bartelt[8], Jörg Heeren[7], Peter Fickert[1], Tarek Moustafa[1]

[1]Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria. [2]Institute of Molecular Biosciences, University of Graz,, Graz, Austria. [3]Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Division of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria. [4]Core Facility Mass Spectrometry, Medical University of Graz,, Graz, Austria. [5]Core Facility Mass Spectrometry, Medical University of Graz, Graz, Austria. [6]Core Facility Ultrastructure Analysis, Medical University Graz, Graz, Austria. [7]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf,, Hamburg, Germany. [8]Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany

Aim: The liver represents a metabolic hub for the uptake and distribution of lipids via free fatty acids, lipoproteins and *de novo* lipid synthesis. Bile acid (BAs) regulate numerous parts of these metabolic processes by activation of receptors, including farnesoid X receptor (FXR) and Takeda G-Protein Receptor 5 (TGR5), rising targets in the treatment of hepatic and metabolic lipid disorders. Different therapeutic approaches targeting BA-signaling pathways have shown great promise for treating metabolic and chronic liver diseases. We studied the role of trans-hepatic BA-flux in the control of hepatic and systemic lipid metabolism using numerous knockout mice and explored the effect of BAs in animal models with interrupted enterohepatic circulation.

Methods: Serum/hepatic lipids, lipoprotein profile by FPLC, and lipoprotein particle disposal (Organ-specific14C-triolein / 3H-Cholesterol uptake), western-blot and qPCR of lipogenic genes and proteins from white/brown adipose and liver tissue were analyzed.

Results: We show that BAs still reduces triglyceride (TG) levels in livers of different knockout animals, including *Fxr-/-*, *Tgr5-/-*, *Ppara-/-* and *Atgl-/-*, suggesting that these genes are not essential for the BA-dependent decrease in hepatic TG levels. Gene expression analysis led us to identify lipid metabolism related genes, including *Chrebp* and *Acss2* (generates acetyl-coA from acetate) in the liver and *Fgf15* (hormone controlling BA-synthesis) in the intestine that are potently involved in lipid lowering effect in BA-treated animals. Contrary, in models with interrupted BA-flux (high liver, low intestinal BAs), high levels of TG accumulated in the liver, leading to "Steato-cholestasis", commonly described in metabolic liver diseases in childhood. Importantly, fatty acid oxidation as well as mitochondrial shape and number appeared to be essential mechanisms driving Steato-cholestasis.

Conclusion: Our findings draw a "molecular/biochemical portrait" that highlights the complex interplay between liver and intestine to allow lipid lowering effects of BAs under physiological and pathophysiological conditions.

THE PARKINSON'S DISEASE PROTEIN VPS35 MAINTAINS HEPATIC LIPID HOMEOSTASIS THROUGH REGULATING LYSOSOMAL FUNCTION AND AUTOPHAGY

Dyonne Vos[1], Andries Heida[1], Mirjam Koster[1], Joël Tissink[1], Niels Kloosterhuis[1], Marieke Smit[1], Nicolette Huijkman[1], Fulvio Reggiori[2], Muriel Mari[2], Jan Albert Kuivenhoven[1], Bart van de Sluis[1]

[1]Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands. [2]Department of Cell Biology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands

Aim: Lysosomal dysfunction and impaired autophagy have been associated with the pathogenesis of Metabolic Associated Fatty Liver Disease (MAFLD). Therefore, a better understanding of the molecular mechanism and regulation of the lysosomal system and autophagy may provide novel therapeutic targets to treat MAFLD. The Parkinson's disease *VPS35* mutation causes impaired autophagy in neurons, but the role of VPS35 in MAFLD is unknown. Here, we aimed to elucidate the role of hepatocyte VPS35 in lysosomal function and autophagy and, subsequently, in hepatic lipid homeostasis.

Method: CRISPR/Cas9 technology was used to generate VPS35-deficient (KO) Hepa1-6 cells. Lysosomal function and autophagic flux were determined in VPS35 KO and control cells. Hepatic VPS35-deficient mice (*Vps35*^{HKO}) were generated and, using electron microscopy, the architecture of the degradative compartments (DCs) in hepatocytes of our mouse model was characterized. Mice were fed either a chow or a high-fat high cholesterol (HFC) diet and, subsequently, the level of hepatic lipid accumulation was determined. The hepatic protein expression of lysosomal and autophagic markers were analyzed by immunoblotting. In addition, gene expression analyses were used to study different metabolic pathways, including lipid metabolism.

Results: Hepatic loss of VPS35 increased the number of DCs, accompanied with elevated expression of the autophagy marker LC3-II, which was confirmed in our *in vitro* model. Hepatic VPS35-deficient mice exhibited increased liver cholesterol ester concentrations, which was exacerbated by feeding the mice a HFC-diet. Interestingly, hepatic triglyceride levels were markedly decreased in *Vps35*^{HKO} mice. The increase in cholesterol esters was associated with macrovesicular steatosis and liver inflammation. In addition, hepatic VPS35 deficiency attenuated body weight gain, caused by a decreased fat mass.

Conclusions: Although additional research is required, our data suggest that VPS35 is a critical regulator of hepatic lipid homeostasis likely by regulating the lysosomal function and, subsequently, the autophagic pathway in mouse hepatocytes.

Eligible for Young Investigator Oral Award

ENDOSOMAL SORTING COMPLEX PROTEIN VPS37A REGULATES HEPATIC GLUCOSE PRODUCTION VIA ALTERING GLUCAGON RECEPTOR TRAFFICKING

Revathi Sekar[1], Karsten Motzler[1], Yun Kown[1], Bahar Najafi[1], Anna-Luisa Warnke[2], Sofiya Gancheva[3], Timo D. Müller[4], Marta Miaczynska[5], Michael Roden[3], Matthias Blüher[6], Natalie Krahmer[4], Oliver Plettenburg[2], Stephan Herzig[1], Anja Zeigerer[1]

[1]Institute for Diabetes and Cancer, Helmholtz Center Munich, Munich, Germany. [2]Institute of Organic Chemistry, Center of Biomolecular Research, Leibniz Universität Hannover, Hannover, Germany. [3]Division of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University, Düsseldorf, Düsseldorf, Germany. [4]Institute for Diabetes and Obesity, Helmholtz Center Munich, Munich, Germany. [5]International Institute of Molecular and Cell Biology, 02-109 Warsaw, Warsaw, Poland. [6]Department of Medicine, University of Leipzig, Leipzig, Germany

Aim: Hyperglucagonemia is a key driver of metabolic dysfunction in diabetes. However, the dichotomic role of glucagon receptor signaling in hepatic glucose and lipid homeostasis and its largely unexplored intracellular spatial-temporal regulation has limited its usage for anti-hyperglycemic pharmacotherapy in type-2 diabetes. Here, we identify Vps37a, a component of the endosomal sorting complexes required for transport I (ESCRT-I), to be a novel regulator of glucagon receptor trafficking.

Method: Using hepatocyte specific lipid nanoparticles and a newly developed Cy5-labeled glucagon agonist, we show that hepatocyte-specific knockdown of Vps37a causes an accumulation of glucagon receptor at early endosomes resulting in an over-activation of the cAMP/PKA/p-Creb signaling pathway, leading to increased gluconeogenic gene expression and enhanced glucose production.

Results: Strikingly, the regulatory effects of Vps37a KD on glucagon signaling were restricted to glucose metabolism, as high fat diet (HFD) treated mice displayed unaltered serum and liver lipid accumulations with no activation in fatty acid β-oxidation. Long-term reduction of Vps37a under HFD induces hyperglycemia, correlating with its reduced expression in human diabetic and obese patients.

Conclusions: Altogether, our data identify Vps37a as a key modulator of glucagon receptor trafficking and provide a molecular tool to uncouple dichotomic glucagon signaling in metabolic control, now offering the chance to harness Vps37a in tailored therapy developments.

THE METABOLIC EFFECTS OF THE SYNTHETIC BILE ACID DERIVATIVE *NOR*UDCA

Ioannis Evangelakos[1], Julia Rohde[1], Markus Heine[1], Anna Worthmann[1], Manka Fuh[1], Manju Kumari[1], Esther Verkade[2], Ludger Scheja[1], Folkert Kuipers[3], Tarek Moustafa[4], Joerg Heeren[1]

[1]University Medical Center Hamburg-Eppendorf, IBMZ, Hamburg, Germany. [2]Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, Netherlands. [3]Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, Netherlands. [4]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Medical University of Graz, Graz, Austria

Aim: Supplementation of the synthetic bile acid derivative 24-*nor*-ursodeoxycholic acid (*nor*UDCA) improves clinical outcomes in mice and humans with cholestatic or metabolic liver disease. Previous studies focused on *nor*UDCA effects on gastrointestinal and liver functions, while the potential impact on systemic lipid and energy metabolism has not been elucidated. Based on the described hypoglycemic and hyperlipidemic effects of *nor*UDCA, we here aim to investigate whether *nor*UDCA impacts lipid and energy metabolism in white and brown adipose tissue (WAT, BAT).

Method: Mice housed at different ambient temperatures were fed a chow diet supplemented ±0.5% *nor*UDCA for one week. Body and fat composition, metabolic parameters and gene expression were measured in various tissues. Lipids and bile acids as well as short-chain fatty acids (SCFAs) were determined using LC-MS/MS- and GC-MS-based approaches, respectively.

Results: Supplementation of *nor*UDCA caused a marked body weight reduction, loss of adipose tissue and secondarily lean mass. BAT lipidomic profiling revealed reduced cholesterylester and triglyceride levels after *nor*UDCA treatment, while an upregulation of thermogenic genes such as *Dio2* and *Ppargc1a* was observed in WAT. Fasting plasma glucose was reduced in *nor*UDCA-fed mice, while triglycerides and cholesterol showed an opposite trend. Additionally, *nor*UDCA supplementation strongly increased gallbladder size and changed bile acid composition in various compartments. Specifically, DCA and its respective epimers were elevated in the systemic circulation. Total SCFAs were reduced in fecal samples of *nor*UDCA treated mice, indicating that *nor*UDCA changed the composition of fiber-processing gut microbiota in the colon.

Conclusions: *Nor*UDCA reduces sizes of BAT and WAT depots regardless of housing temperature or mouse strain, while it increases their lipolytic and thermogenic activity, respectively. The *nor*UDCA-dependent effects on plasma bile acid levels may explain higher disposal of energy substrates by thermogenic adipose tissues.

Eligible for Young Investigator Oral Award

CROSSTALK BETWEEN HEPATOCYTES AND ADIPOCYTES IN LAL DEFICIENCY

Ivan Bradić[1], Katharina B. Küntzel[1], Nemanja Vujić[1], Dagmar Kratky[1,2]

[1]*Medical University of Graz, Graz, Austria.* [2]*BioTechMed-Graz, Graz, Austria*

Aim: Lysosomal acid lipase (LAL) hydrolyzes cholesteryl esters (CE) and triglycerides (TG) in lysosomes. In both humans and mice, loss of LAL activity leads to ectopic lipid accumulation, alterations in plasma lipids, and inflammation. Additionally, LAL-deficient (LAL-/-) mice have reduced body weight and lose their white adipose tissue (WAT). Although LAL-/- mice exhibit a severe phenotype on chow diet, hepatocyte-specific LAL-/- (hepLAL-/-) mice show phenotypic changes only when challenged with a high-fat/high-cholesterol diet. To determine the effects of LAL deficiency in hepatocytes on adipose tissue, we analyzed how hepatocyte-conditioned media (CM) from wild-type (WT), LAL-/- and hepLAL-/- mice influence lipogenesis and adipogenesis of 3T3-L1 fibroblasts. Moreover, we analyzed how the loss of LAL activity affects adipogenesis and lipogenesis of the stromal vascular fraction (SVF).

Method: Primary hepatocytes were isolated from LAL-/-, hepLAL-/- and WT mice by the collagenase perfusion method and seeded on collagen-coated plates in complete DMEM. Differentiation of 3T3-L1 fibroblasts into adipocytes was performed using hepatocyte-CM prepared from LAL-/-, hepLAL-/- and WT mice. SVF was isolated from WT mice and differentiated into adipocytes using differentiation media with or without the LAL inhibitor Lalistat 2. Adipogenesis and lipogenesis of 3T3-L1 adipocytes and SVF-differentiated adipocytes was assessed by qPCR, Western blot, microscopy, and lipid quantification.

Results: We observed impaired adipogenesis and lipogenesis of 3T3-L1 fibroblasts differentiated with CM from LAL-/- and hepLAL-/- mice in comparison with the ones differentiated with CM from WT mice. Furthermore, we discovered that pharmacological inhibition of LAL during SVF differentiation significantly impairs adipogenesis, lipogenesis, and lipolysis of adipocytes, indicating that LAL plays an important role not just in hepatocytes, but also in adipose tissue.

Conclusions: We conclude that secretory factors released by LAL-/- hepatocytes impair lipogenesis and adipogenesis of adipocytes. Moreover, our data reveal that loss of LAL activity in adipocytes alters adipogenesis, lipogenesis, and lipolysis.

Eligible for Young Investigator Oral Award

ADIPOSE TRIGLYCERIDE LIPASE (ATGL) IS NEEDED FOR HOMEOSTATIC CONTROL OF STEROL ELEMENT-BINDING PROTEIN-1C (SREBP-1C) DRIVEN HEPATIC LIPOGENESIS

Beatrix Irene Wieser[1], Paola Peña de la Sancha[1], Silvia Schauer[1], Helga Reicher[1], Wolfgang Sattler[1], Rolf Breinbauer[2], Martina Schweiger[3], Peter John Espenshade[4], Rudolf Zechner[3], Gerald Hoefler[1], Paul Willibald Vesely[1]

[1]Medical University of Graz, Graz, Austria. [2]Graz University of Technology, Graz, Austria. [3]University of Graz, Graz, Austria. [4]Johns Hopkins University School of Medicine, Baltimore, USA

Aim: SREBP-1c is translated as inactive precursor (P-SREBP-1c) into the ER-membrane. When unsaturated fatty acids are scarce, P-SREBP-1c is transported to the Golgi with its chaperone SCAP, where it is proteolytically activated. The emerging nuclear (N)-SREBP-1c drives fatty acid biosynthesis. During fasting, however, lipogenesis is low, and adipose-tissue lipolysis supplies the organism with fatty acids. ATGL is the rate-limiting enzyme for lipolysis, and it preferentially provides unsaturated fatty acids. Therefore, we hypothesized that ATGL-derived unsaturated fatty acids may suppress P-SREBP-1c activation.

Method: We used global, adipose-specific, or liver-specific *Atgl*-knockout mice. To liberate fatty acids from adipose-tissue, mice were fasted over-night. Alternatively, fatty acids were injected intraperitoneally. P-SREBP-1c and N-SREBP-1c were analyzed by western-blotting of microsomal- and nuclear- liver fractions. Quantitative real-time-PCR was used to measure N-SREBP-1c targets. Free fatty acids were quantified by GC/FID. GFP-tagged SCAP was used as reporter for SREBP-1c activation.

Results: P-SREBP-1c was hyper-activated in livers of global- and adipose-tissue-specific *Atgl*-knockout mice, which also showed reduced unsaturated free fatty acids in plasma and livers. Conversely, unsaturated fatty acid injection prevented P-SREBP-1c activation. Pharmacological inhibition of ATGL by Atglistatin selectively activated P-SREBP-1c in primary control-hepatocytes but not in those lacking ATGL. Mechanistically, GFP-SCAP and the Golgi-marker GM130 co-localized in ATGL-knockout hepatocytes. Conversely, addition of unsaturated fatty acids to the growth medium abrogated the co-localization.

Conclusions: ATGL lipolysis liberates unsaturated fatty acids from the adipose-tissue of mice, and in turn, suppresses lipogenic gene-expression in the liver. Mechanistically, ATGL derived unsaturated fatty acids block P-SREBP-1c/SCAP export from the ER to the Golgi-apparatus, which prevents the proteolytic activation of SREBP-1c. Our work highlights an ATGL/SREBP-1c axis that is instrumental to coordinate adipose-tissue lipolysis with lipogenesis in the liver, and whose homeostatic regulation is crucial to prevent severe diseases including diabetes, cardiomyopathy, and even cancer.

SELECTIVE INHIBITION OF HUMAN ADIPOSE TRIGLYCERIDE LIPASE BY SMALL MOLECULES

Gernot Grabner[1], Rolf Breinbauer[2], Robert Zimmermann[1]

[1]*Institute of Molecular Biosciences, University of Graz, Graz, Austria.* [2]*Institute of Organic Chemistry, Graz University of Technology, Graz, Austria*

Aim: Chronically elevated circulating fatty acids levels promote lipid accumulation in non-adipose tissues and lipotoxicity. Adipose triglyceride lipase (ATGL) critically determines the release of fatty acids from white adipose tissue and accumulating evidence suggests that inactivation of ATGL has beneficial effects on lipotoxicity-driven disorders including insulin resistance, non-alcoholic fatty liver disease, and heart failure, classifying ATGL as promising drug target. Since currently no selective inhibitors are available we aim to develop and characterize small molecule inhibitors for human ATGL.

Method: In an iterative optimization process we generated several hundred compounds and tested them for inhibition of human ATGL in *in-vitro* assays. Promising inhibitor candidates were tested upon efficacy, toxicity, stability, and off-target inhibition. Chimeric ATGL proteins and homology modeling were used to identify distinct inhibitor binding sites.

Results: Our efforts resulted in NG-497 a potent, selective inhibitor of ATGL from humans and rhesus monkeys, but not ATGL from other species or related enzymes. We demonstrate that NG-497 abolishes lipolysis in human adipocytes in a reversible manner and affects lipolysis-dependent cellular respiration. NG-497 binds ATGL within a hydrophobic cavity near the active site where we identified distinct amino acid residues determining inhibitor efficacy and species selectivity.

Conclusions: We established NG-497 as suitable tool for investigation of human ATGL function and provide important insights into enzyme – inhibitor interactions.

NFE2L1 PROTECTS WHITE ADIPOCYTES FROM CHOLESTEROL-INDUCED INFLAMMATION

Carolin Muley, Janina Caesar, Stefan Kotschi, Alexander Bartelt

Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany

Aim: Cholesterol is an important building block for lipid membranes and sterol synthesis, but accumulation of free cholesterol is linked to ER stress and inflammation. Moreover, cholesterol and inflammation are major contributors to atherosclerosis. On high-fat diet, mice accumulate tremendous amounts of free cholesterol in white adipocytes, however, how these handle excessive cholesterol is insufficiently understood. We have recently identified the ER-resident transcription factor Nfe2l1 (also Nrf1 or TCF11) as a new regulator of cholesterol homeostasis. Here we investigate the role of Nfe2l1 adipocyte function and consequences hereof in vivo and in vitro.

Method: We studied loss of adipocyte Nfe2l1 in a Cre-loxP mouse model using Adipoq-Cre for metabolic phenotyping. ER stress and inflammatory markers in WAT were assessed using RNAseq, qPCR and Western blot. Immune cell distribution in WAT was analyzed using multicolor flow-cytometry. Mechanistic studies were performed in 3T3-L1 adipocytes as well as in primary white adipocytes using RNAi. ER stress was induced with cholesterol and/or epoxomicin.

Results: Adipoq-Cre Nfe2l1 knock-out mice were more insulin-resistant on high-fat diet compared to their wild-type littermates and had severely inflamed WAT which was characterized by a marked upregulation of T cell response. In vitro, cholesterol treatment in combination with proteasomal inhibition by epoxomicin inhibited the cleavage of Nfe2l1. Consequently, loss of Nfe2l1 in white adipocytes was associated with upregulation of ER stress and inflammatory markers, which was dependent on Atf3, an ER stress transcription factor.

Conclusions: Adipocyte-Nfe2l1 is required for adaptation to high metabolic flux by maintaining proteostasis. Here, we identified a mechanism by which Nfe2l1 protects white adipocytes from cholesterol toxicity. When this mechanism is compromised, ER stress induces inflammation via Atf3 activation. Hence, our results identify a novel relay system integrating nutrient sensing and metabolic health.

Eligible for Young Investigator Oral Award

FROM VARIANT TO FUNCTION: THE DISCOVERY AND VALIDATION OF CYR61 AS A REGULATOR OF BODY COMPOSITION

Sophia Metz, Katja Thorøe Michler, Jonathan Robert Belanich, Trisha Jean Grevengoed, Tuomas Oskari Kilpeläinen, Matthew Paul Gillum

NNF Center for Basic Metabolic Research, Copenhagen, Denmark

Aim: Obesity is a major contributor to the global burden of chronic disease, and unravelling its complex metabolic interplay is key. To identify novel targets of obesity, genome-wide association studies for body composition traits are a remarkable source and variant-to-function approaches are on the rise. Here, we study the rare variant Ser316Cys in the angiogenic factor *CYR61*, previously linked to increased body-/trunk-fat percentage in the UK Biobank ($P=1.1x10^{-9}$, $P=3.8x10^{-11}$), and aim to translate its genomic associations into function and unravel the involvement of CYR61 in adipose tissue biology.

Method: To explore pleiotropic effects of CYR61-Cys316 we performed Phenome-wide association studies (PheWAS) in the UK Biobank (N~500,000). To assess functional consequences we expressed human-CYR61 in adipose tissue of mice, explored white adipocyte (WA) specific consequences of CYR61 modifications (e.g. MRI, adipocyte size and differentiation), and analysed angiogenic/binding capacities of CYR61-Cys316.

Results: PheWAS indicate an involvement of *CYR61* in the regulation of body composition; Independent of sex. Interestingly, our mouse model shows an increased body fat percentage under a high fat diet (P=0.0002), due to a switch in body composition (higher fat mass, lower lean mass), while body weight does not differ from WT mice. The mean area of WA is increased (P=0.009), reflecting adipocyte hypertrophy. *In vitro* experiments indicate that CYR61 is predominantly regulated in differentiating WA. Moreover, the variant CYR61-Cys316 decreases binding affinity to its receptor integrin-avb3 by 50% (P=0.0003), suggesting altered cell adhesion.

Conclusions: Our results extend the versatile functions of CYR61, with a critical, yet undescribed role in the regulation of body composition, mediated by angiogenesis and enhanced adipocyte growth. Ongoing *in vitro/vivo* experiments and RNAseq analysis will further define our understanding of CYR61 signalling capacities in WAT – potentially opening up a new strategy to treat obesity by lowering fat mass but retaining lean mass.

Eligible for Young Investigator Oral Award

CALCIUM-DEPENDENT HORMETIC REGULATION IN BROWN AND WHITE ADIPOCYTES

Fabian Finger, Zach Gerhart-Hines

NNF-Center for Basic Metabolic Research, Copenhagen, Denmark

Aim: Changes in adipocyte functionality and fitness, as they occur during overnutrition and obesity, are in many parts ascribed to deteriorations within the endoplasmic reticulum (ER). Such ER stress is recognized as a central component of disease progression and manifestation. Yet, in-depth knowledge about the quality and severity of this stress is limited. Thus, we set out to investigate how brown and white adipocytes change upon ER stress in a time and dose-dependent manner.

Method: To assess the progression and severity of ER stress *in vitro*, we made use of murine primary and immortalized differentiated brown and white adipocytes. Here, we analyzed the effects of thapsigargin (THG; inhibits ER calcium homeostasis) and tunicamycin (TUN; blocks ER protein folding capacity) time- and dose-dependently via qRT-PCR and globally via mRNA sequencing approaches. Translation into protein level changes were confirmed by Western blot analysis.

Results: At concentrations 1000x lower than commonly used to elicit ER stress, THG, but not TUN triggers a surprising hormetic response that includes but is not restricted to the upregulation of the key bioenergetic factor UCP1 (mRNA and protein level), both in brown and white adipocytes. This response is independent of canonical ER stress effectors (e.g. XBP1, ATF4 and ATF6), and is blunted in the presence of the calcium chelator BAPTA-AM. mRNA sequencing over several magnitudes of THG and TUN concentrations additionally reveals a complex hormetic signature which, however, does not include proadipogenic/thermogenic marker (e.g. *Pgc1a*, *Pparg*, *Prdm16*).

Conclusions: By thoroughly investigating the quality and early cellular changes upon ER stress, we uncovered a yet undescribed phenomenon in both brown and white adipocytes. This hormetic response precedes and is independent of known stress pathway activation and defines UCP1 as an early response factor for intracellular calcium alterations. Global assessment further suggests more complex adaptation processes and potential new avenues for obesity intervention.

VASCULAR ENDOTHELIAL CELLS OF WHITE ADIPOSE TISSUES MODULATE DE NOVO LIPOGENESIS IN ADIPOCYTES BY THE CD73-DEPENDENT GENERATION OF EXTRACELLULAR ADENOSINE

Michelle Y. Jaeckstein[1], Isabell Schulze[1], Michael Zajac[1], Markus Heine[1], Leon Straub[2], Ludger Scheja[1], Joerg Heeren[1]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [2]Touchstone Diabetes Center, UT Southwestern Medical Center, Dallas, USA

Aim: Endothelial and immune cells present in brown and white adipose tissue (BAT, WAT) regulate lipid and energy metabolism that is carried out mainly by the parenchymal adipocytes. In obesity, dysregulated intercellular crosstalk is closely related to metabolic abnormalities. Previously, it was shown that pharmacological activation of adenosine receptor signaling promotes beneficial metabolic effects in BAT and WAT. Adenosine can be generated by the stepwise hydrolysis of extracellular ATP, a process mediated by the two ectonucleotidases CD39 and CD73. However, the endogenous source of adenosine and its physiological relevance for intercellular communications in BAT and WAT is still unknown. Based on its endothelial cell expression, we investigated the role of endothelial CD73 for extracellular adenosine production and adipose tissue function.

Method: Magnetic-activated cell sorting (MACS) was used for cell type-specific analyses. Endothelial-specific CD73-knockout and AAV-mediated-CD73-overexpressing mice were analyzed under different conditions with respect to energy uptake, indirect calorimetry as well as gene and protein expression in WAT and BAT. Primary murine and human adipocytes were used to investigate adenosine-dependent signaling mechanisms.

Results: MACS analysis of adipose tissues showed CD73 to be mainly expressed by endothelial cells but not adipocytes. *In vivo*, cold exposed CD73-knockout mice displayed only minor differences in lipid and glucose uptake, adipose tissue histology and thermogenic function. However, impaired adenosine generation by endothelial cells resulted in elevated expression of carbohydrate-response-element-binding-protein-beta (Chrebp-beta) and *de novo* lipogenesis (DNL) marker genes in WAT. Metabolic uptake studies with radiotracers revealed enhanced uptake of glucose into WAT. On the other hand, AAV-mediated CD73 overexpression leading to higher adenosine levels in adipose tissues protected from diet-induced obesity. Mechanistic studies confirmed that adenosine regulates DNL via an AMPK-ChREBP-axis in primary adipocytes.

Conclusions: We provide evidence that endothelial cells regulate ChREBP-dependent lipid metabolism in adipocytes via CD73-mediated extracellular adenosine production.

Eligible for Young Investigator Oral Award

THE SER251PRO SNP IN PERILIPIN 2 (PLIN2) ALTERS CHAPERONE-MEDIATED AUTOPHAGY (CMA) AND INCREASES MACROLIPOPHAGY IN HUMAN MACROPHAGES

Alice Maestri[1], Peter Saliba-Gustafsson[1], Susmita Kaushik[2], Olivera Werngren[1], Matteo Pedrelli[3], Paolo Parini[3,4], Ana Maria Cuervo[2], Ewa Ehrenborg[1]

[1]Cardiovascular Medicine Unit, Department of Medicine, Center for Molecular Medicine at BioClinicum, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden. [2]Department of Developmental and Molecular Biology, Institute for Aging Studies, Albert Einstein College of Medicine, New York, USA. [3]Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet Huddinge, Karolinska Institutet, Stockholm, Sweden. [4]Metabolism Unit, Department of Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Karolinska Institutet, Stockholm, Sweden

Aim: PLIN2 is the most prominent lipid droplet (LD)-associated protein in foam cells. PLIN2 stabilizes LDs and LDs protect PLIN2 from ubiquitination. LD utilization during energy depletion occurs through macrolipophagy or cytosolic lipolysis. In both cases, PLINs need to be removed from the LD surface prior to utilization. LD-bound PLIN2 degradation is dependent on chaperone-mediated autophagy (CMA). We have recently shown that the Ser251Pro SNP in PLIN2 influences macroautophagy, cholesterol efflux, cholesterol accumulation and is associated with subclinical atherosclerosis in humans. Since a direct crosstalk between macroautophagy and CMA has been proposed, we aim to investigate whether the Ser251Pro SNP affects CMA and the degradation of PLIN2, thereby influencing macrolipophagy.

Method: WT, ATG5$^{-/-}$ and L2A$^{-/-}$ mouse embryonic fibroblasts (MEFs) transiently transfected with constructs carrying either variant of PLIN2 were loaded with oleate. LD content was measured by immunofluorescence. HEK293 cells stably transfected with constructs carrying either variant of PLIN2 were loaded with oleate. L2A protein and mRNA levels were measured by WB and qPCR, respectively. CMA activity was analyzed using a CMA reporter. In human monocyte-derived macrophages from subjects carrying either variant, loaded with oxLDL, CMA activity was analyzed by Hsc70-L2A co-localization using immunohistochemistry.

Results: Ser251Pro-dependent differences in lipid accumulation observed in WT MEF cells are abolished in ATG5$^{-/-}$ but not in L2A$^{-/-}$ MEF cells. HEK cells carrying the Pro251 allele show lower L2A expression and reduced CMA activity. Correspondingly, human macrophages carrying the Pro251 allele show decreased Hsc70-L2A co-localization compared to Ser251-carrying macrophages.

Conclusions: The allele-specific effects of Ser251Pro on lipid accumulation is dependent on macroautophagy but does not seem to be affected by CMA blockage. However, presence of the Pro251 allele reduces CMA-activity. These results suggest that the PLIN2 Ser251Pro affects macrolipophagy in a CMA-dependent manner and that this effect is cell-specific and loading-dependent.

Eligible for Young Investigator Oral Award

TIME TO RUN: LATE RATHER THAN EARLY EXERCISE DECREASES ATHEROSCLEROSIS

Milena Schönke[1,2], Zhixiong Ying[1,2], Wietse In het Panhuis[1,2], Sander Kooijman[1,2], Patrick Rensen[1,2]

[1]Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, Netherlands. [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, Netherlands

Aim: The majority of metabolic processes are under circadian regulation and its disturbance increases the susceptibility to cardiometabolic diseases. While light exposure and food intake are known circadian regulators, it is unclear whether the beneficial health effects of exercise are restricted to unique time windows. We aimed to study whether the timing of exercise training differentially modulates the development of atherosclerosis and elucidate underlying mechanisms.

Method: We endurance-trained atherosclerosis-prone female APOE*3-Leiden.CETP mice fed a Western-type diet, a well-established model for cardiometabolic disease, for one hour five times a week for four weeks either in their early or in their late active phase on a treadmill and assessed the development of atherosclerotic lesions in the aortic root.

Results: Late, but not early, exercise reduced the size of atherosclerotic lesions by as much as 40% compared to sedentary animals. Concomitantly, the greatest loss of fat mass was observed with late training (+0.43 g with early vs. -0.49 g with late training). No correlation between cholesterol exposure and lesion size was evident, indicating a modulation of vascular inflammation in early atherosclerosis with late compared to early training. Strikingly, we observed a time-of-day-dependent effect of exercise training on the composition of the gut microbiota with an increased abundance of fecal bacteria producing butyrate, a short-chain fatty acid with proposed atheroprotective properties, after four weeks of late training.

Conclusions: Together, these findings clearly indicate that timing is a critical factor to amplify the beneficial anti-atherosclerotic effects of exercise with a great potential to further optimize training recommendations for patients.

Eligible for Young Investigator Oral Award

TREM2 DEPENDENT INTERNALIZATION OF LIPOPREOTEIN PARTICLES IN BROWN ADIPOSE TISSUE MACROPHAGES IN RESPONSE TO COLD EXPOSURE

Simon P. Meyer[1], Michelle Y. Jaeckstein[1], Markus Heine[1], Joerg Heeren[1], Lidia Bosurgi[2,3], Anna Worthmann[1]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [2]I. Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [3]Protozoa Immunology, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany

Aim: Cold exposure stimulates the uptake of free fatty acids and whole lipoproteins into adipocytes and endothelial cells of brown adipose tissue (BAT), in an attempt to cover increased energy demand during non-shivering thermogenesis. The role of adipose tissue macrophages (ATM) has so far been neglected in this process. Triggering receptor of myeloid cells 2 (TREM2) binds anionic lipids and has been implicated in lipid metabolism of white adipose tissue (WAT) macrophages. Here, we aim to evaluate the impact of cold exposure on lipid internalization into BAT macrophages and the role of TREM2 for this process. Next, since phagocytosis might be the major pathway of macrophage mediated lipoprotein uptake, we aim to assess the phagocytic capacity of BAT ATM in response to cold exposure.

Method: To study lipoprotein uptake into ATM, wild type controls (WT) and *Trem2*$^{-/-}$ mice exposed to cold (24 hours at 6°C) or housed at room temperature (22-24°C) were given an oral gavage containing ^{3}H-triolein and ^{14}C-cholesterol. Subsequently CD11b+ ATM were isolated from BAT via MACS and uptake of tracers was estimated by scintillation counting. *In vitro* phagocytic capacity was assessed in MACS sorted CD11b+ ATM from BAT of warm and cold housed WT and *Trem2*$^{-/-}$ mice by measuring the uptake of apoptotic thymocytes.

Results: We found that in response to cold exposure uptake of ^{3}H-triolein and ^{14}C-cholesterol was increased in BAT ATM of WT but not of *Trem2*$^{-/-}$ mice. Similarly, BAT ATM of cold-housed mice showed higher uptake of apoptotic thymocytes compared to warm counterparts. This effect was attenuated by *Trem2* deletion.

Conclusions: Cold exposure increases phagocytic capacity of BAT ATM and lipoprotein internalization in a TREM2-dependent manner, suggesting that specific subsets adipose tissue macrophages modulate tissue homeostasis during thermogenic activation.

Eligible for Young Investigator Oral Award

COLD-INDUCED THERMOGENESIS IS HIGHER IN THE MORNING COMPARED TO THE EVENING IN YOUNG LEAN INDIVIDUALS

Aashley S.D. Sardjoe Mishre, Maaike E. Straat, Andrea Mendez-Gutierrez, Sander Kooijman, Borja Martinez-Tellez, Mariëtte R. Boon, Hermien E. Kan, Patrick C.N. Rensen

Leiden University Medical Center, Leiden, Netherlands

Aim: Brown adipose tissue (BAT) is a metabolic organ that contributes to cold-induced thermogenesis. Previously, we demonstrated that BAT activity shows a circadian rhythm in mice, with highest activity at the start of the wakeful period (Cell Rep 2018). We now aimed to investigate whether cold-induced thermogenesis displays a circadian rhythm in humans.

Method: Twenty-four lean (BMI: 22.2±2.0 kg/m^2) and young (22±3.4 years) subjects underwent a 2.5-hour personalized cooling procedure in the morning (7:30 AM) and in the evening (7:30 PM). Cold-induced thermogenesis was assessed using indirect calorimetry and using infrared thermography to measure skin temperature. Supraclavicular (*e.g.* the location of human BAT), sternal and deltoid skin temperatures were extracted from infrared thermography images using a semi-automated segmentation method. Cold-induced changes in the mean (ΔT_{mean}) and maximum (ΔT_{max}) skin temperature were determined for all three regions.

Results: Cold-induced energy expenditure continuously increased in the morning (+45±32%, p<0.001), whereas it reached a plateau after 1 hour in the evening (+30±29%, p<0.001; interaction morning *vs* evening p=0.013). This was accompanied by an increase in mean supraclavicular skin temperature after cooling in the morning (ΔT_{mean}: +0.63±0.73°C, p<0.001), but not in the evening (p>0.05), most likely due to its close vicinity to supraclavicular BAT. Sternal skin temperature also increased in the morning (ΔT_{mean}: +0.81±0.96°C and ΔT_{max}: +0.77±0.86°C, p<0.001), but not in the evening (p>0.05). In contrast, deltoid skin temperature decreased after cooling in the morning (ΔT_{mean}: -0.94±1.2°C; ΔT_{max}: -0.71±1.0°C) and evening (ΔT_{mean}: -0.98±1.2°C; ΔT_{max}: -0.70±1.2°C; all p<0.01).

Conclusions: Cold-induced thermogenesis, judged by energy expenditure and supraclavicular skin temperature, is higher in the morning than in the evening, which likely reflects a circadian rhythmicity in human BAT activity. This suggests that this rhythm should be considered when targeting BAT to improve cardiometabolic health.

Eligible for Young Investigator Oral Award

LIPOPROTEIN LIPASE EXPRESSED BY VASCULAR ENDOTHELIAL CELLS OF ACTIVATED THERMOGENIC ADIPOSE TISSUES IS DISPENSABLE FOR THE PROCESSING OF TRIGLYCERIDE-RICH LIPOPROTEINS

Carlotta Corban, Markus Heine, Ludger Scheja, Joerg Heeren

Department of Biochemistry and Molecular Cell Biology, University Medical Clinic Hamburg-Eppendorf, Hamburg, Germany

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

Method: Transgenic mice lacking LPL in endothelial cells (*Lpl*$^{fl/fl}$-*Cdh5*$^{Cre+}$), brown adipocytes (*Lpl*$^{fl/fl}$-*Ucp1*$^{Cre+}$) and respective littermate controls (*Lpl*$^{fl/fl}$-*Cdh5*$^{Cre-}$; *Lpl*$^{fl/fl}$-*Ucp1*$^{Cre-}$) were exposed to a sustained cold environment. For cell type-specific analyses, LPL and marker gene expression was determined in adipocytes versus stromal vascular fraction (SVF). Body and fat weights were determined, metabolic turnover studies using radioactive tracers as well as gene and protein expression were performed to study lipid disposal and thermogenic responses in adipose tissues.

Results: Turnover studies showed that cold-induced triglyceride uptake into BAT was impaired in *Lpl*$^{fl/fl}$-*Ucp1*$^{Cre+}$. These mice showed no impaired cold tolerance, which is probably explained by an increased glucose uptake into BAT, compensating the diminished lipid uptake. Similar studies performed in *Lpl*$^{fl/fl}$-*Cdh5*$^{Cre+}$ and *Lpl*$^{fl/fl}$-*Cdh5*$^{Cre-}$indicated that LPL expressed by vascular endothelial cells has no substantial impact on lipoprotein disposal, glucose uptake and adaptive thermogenesis.

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

Eligible for Young Investigator Oral Award

CIRCADIAN CONTROL OF LPL-MEDIATED LIPOLYSIS IN BROWN ADIPOSE TISSUE

Robin van Eenige[1], Wietse In het Panhuis[1], Milena Schönke[1], Sander Kersten[2], Patrick Rensen[1], Sander Kooijman[1]

[1]Leiden University Medical Center, Leiden, Netherlands. [2]Wageningen University & Research, Wageningen, Netherlands

Aim: Brown adipose tissue (BAT) displays a high-amplitude day-night rhythm in the uptake of triglyceride-derived fatty acids (Van den Berg, Cell Rep 2018), an effect driven by glucocorticoids and the sympathetic nervous system (Kroon, Mol Metab 2021). The aim of the current study was to obtain more insight in the molecular mechanism underlying the circadian metabolic activity in BAT.

Method: BAT was collected from C57BL/6J mice at 3-hour intervals over a period of 24 hours. RNA-sequencing was performed and rhythmic genes were identified using JTK cycle. Angptl4 knockout and overexpressing mice were injected in the early light or early dark phase with glycerol tri[^{3}H]oleate containing TG-rich lipoprotein-like particles, and BAT was collected to determine the uptake of [^{3}H]oleate and perform further molecular analyses.

Results: Out of the 13,547 identified transcripts in BAT, a total of 5,442 genes were found to be oscillating. Among the oscillating genes with the highest amplitude were those encoding for the mitochondrial complexes and genes involved in the regulation of de novo lipogenesis and extracellular lipolysis. Transcription factor enrichment on the top 10% oscillating genes revealed a central role for peroxisome proliferator-activated receptor gamma (PPAR-γ) in the regulation of circadian gene expression. PPAR-γ regulates, among others, the uptake of lipids through transcriptional control of *Angptl4*, one of the top oscillating genes. Subsequent kinetic experiments revealed that circadian abundance and activity of lipoprotein lipase (LPL) and circadian uptake of [^{3}H]oleate by BAT was attenuated in *Angptl4* engineered mice, with persistent high uptake in *Angptl4* knockout mice and low uptake in *Angptl4* overexpressing mice.

Conclusions: Circadian metabolic BAT activity is characterized by high-amplitude rhythms in lipolytic processes. PPAR-γ activity, via the regulation of *Angptl4* expression, most likely links glucocorticoid and sympathetic signaling to rhythmic uptake of TG-derived fatty acids by BAT.

ALTERNATIVE ENERGY SUPPLY MECHANISMS FOR THERMOGENIC ADIPOSE TISSUES REPLENISHMENT DURING PROLONGED COLD EXPOSURE

Markus Heine, Ludger Scheja, Joerg Heeren

Department of Biochemistry and Molecular Cell Biology, University Medical Clinic Hamburg-Eppendorf, Hamburg, Germany

Aim: Under conditions of cold activation, the brown adipose tissue (BAT) supplies the body with heat while consuming large amounts of energy substrates such as lipids from the blood. CD36-deficient animals show impaired uptake of lipids into oxidative organs and are sensitive to acute cold exposure. However, in an approach that have been successfully developed for UCP1-deficient mice, we were able to acclimate CD36-deficient mice to sustained cold conditions. Therefore, we aim to investigate how CD36-deficiency leads to alternative energy supply mechanisms of thermogenic adipose tissues under prolonged cold conditions.

Method: For this purpose, we followed organ uptake of radiolabeled lipid and glucose tracers in wildtype and CD36 knockout mice which are housed at prolonged cold conditions. Transcriptional changes were analyzed in liver and thermogenic adipose tissues Energy expenditure and core body temperature was studied in mice exposed to sustained cold by indirect calorimetry.

Results: Compared to wild type controls, cold-acclimated CD36 knockout mice display similar energy expenditure rates and respiratory exchange ratio. Metabolic clearance studies using glucose and lipid tracers indicate that CD36 is important for cold-induced lipid disposal into BAT but not white adipose tissue (WAT) of cold-acclimated mice. Transcriptional studies revealed enhanced expression levels of enzymes which facilitates the production and metabolization of ketone bodies in liver and thermogenic adipose tissues.

Conclusions: These data indicate that impaired lipid uptake in CD36-deficient mice is compensated by ketone body utilization in BAT and CD36-independent lipoprotein handling in beige WAT.

GENOMIC GLUCOCORTICOID ACTIONS IN BROWN ADIPOSE TISSUE CONTROL LIPID UTILIZATION

Céline Jouffe[1], Nina Henriette Uhlenhaut[1,2], Franziska Greulich[1,2]

[1]Helmholtz Diabetes Center, Helmholtz Zentrum Muenchen, Neuherberg, Germany. [2]Metabolic Programming, TUM School of Life Sciences, Freising, Germany

Aim: Glucocorticoids (GC) are steroid hormones involved in central physiological and metabolic processes, and their actions have been linked to the pathology of metabolic syndrome. At the molecular level, GC actions are mediated by the Glucocorticoid Receptor (GR), which belongs to the nuclear hormone receptor superfamily of ligand activated transcription factors. Despite its prominent role in glucose, lipid and protein metabolism, GR's genomic actions in adipose tissue are still not fully understood. Here, we elucidate how GR binding to promoters and enhancers functionally regulates BAT physiology.

Method: We used a combination of ChIP- and RNA-sequencing, bioinformatics and in vivo experiments with GR Brown Adipose Tissue (BAT)- specific KO mice and their control littermates.

Results: Here we show that in brown adipose tissue, GR bound sites are associated with genes involved in metabolic processes. Upon acute dexamethasone treatment, GR down-regulates adaptive thermogenesis and fatty acid oxidation, while it up-regulates lipid storage, in wild type BAT. Under chronic dexamethasone treatment, GR BAT-specific KO mice exhibit a protection against lipid accumulation which is typically observed in wild type mice. RNA-seq in BAT for these mice showed an impaired expression of PPARG pathway. Taken together, our genomic data reveal a network of metabolic genes controlled by GR together with BAT-specific transcription factors, which leads to lipid accumulation and decreased thermogenesis.

Conclusions: We demonstrate that GR plays a role in the dysregulation of BAT physiology in response to chronic corticosteroid treatment.

PMEPA1 IS A NOVEL REGULATOR OF BROWN ADIPOCYTE PROTEOSTASIS AND THERMOGENESIS VIA TGF-BETA

Ana Bici[1], Sajjad Khani[1,2], Stefan Kotschi[1], Maude Giroud[1,2], Virginia Egea[1], Stephan Herzig[2], Alexander Bartelt[1,2]

[1]*Institute for Cardiovascular Prevention, Ludwig-Maximilian-University, Munich, Germany.* [2]*Institute for Diabetes and Cancer, Helmholtz Center Munich, Neuherberg, Germany*

Aim: Brown adipose tissue (BAT) mediates cold-induced non-shivering thermogenesis (NST), which depends on proper proteostasis. The transcription factor Nuclear Factor Erythroid-2, Like-1 (Nfe2l1) mediates proteasomal protein quality control during cold adaptation, however the upstream hormonal regulation remains unclear. Here we show that Prostate Transmembrane Protein, Androgen Induced-1 (Pmepa1), a component of TGF-beta signaling, is a key regulator of Nfe2l1-mediated proteostasis and NST in brown adipocytes.

Method: We performed mechanistic cell culture experiments in an immortalized brown adipocyte cell line as well as in primary brown adipocytes. Pmepa1 levels were manipulated using RNAi silencing and eukaryotic overexpression vectors. Gene expression levels and proteasomal activity were quantified by qPCR, western blot and fluorometric peptide assays. Seahorse extracellular flux analysis was used to measure bioenergetics and norepinephrine-stimulated cell respiration.

Results: Pmepa1 was highly expressed in BAT and induced during cold exposure. In brown adipocytes, silencing of Pmepa1 expression lowered Ucp1 levels, lipolysis, respiration, and NST. Interestingly, reducing Pmepa1 also led to reduced levels of Nfe2l1 and overall decreased proteasomal activity. As we found that Pmepa1 expression was regulated by TGF-beta, and in turn, TGF-beta action is dependent on Pmepa1 through Smad signaling, we focused on the role of TGF-beta in the regulation of Nfe2l1 and proteostasis. Indeed, TGF-beta dynamically regulated Nfe2l1 gene expression and proteasomal activity in a Pmepa1-dependent fashion.

Conclusions: In conclusion, the TGF-beta-Pmepa1 pathway is a novel regulator of brown adipocyte function and NST. Our results suggest that the TGF-beta-Pmepa1 pathway is a potential target for therapeutically activating BAT, as it involves a hormonal action through a transmembrane protein.

Eligible for Young Investigator Oral Award

THE MITOCHONDRIAL TRANSPORTER, SLC25A34, INTEGRATES CIRCADIAN AND TEMPERATURE CUES TO CONTROL ADIPOCYTE THERMOGENESIS

Iuliia Karavaeva[1], Samuel Trammel[1], Elahu Sustarsic[1], Tao Ma[1], Lasse Markussen[2], Lidia Argemi Muntadas[1], Yachen Shen[3], Frederike Sass[1], Sabina Chubanova[1], Olivia Johansen[1], Matthew Emmett[3], Jonas Treebak[1], Edmund Kunji[4], Mandrup[2], Matthew Gillum[1], Thomas Moritz[1], Raymond Soccio[3], Mitchell Lazar[3], Zachary Gerhart-Hines[1]

[1]NNF CBMR, Copenhagen, Denmark. [2]University of Southern Denmark, Odense, Denmark. [3]University of Pennsylvania, Philadelphia, USA. [4]University of Cambridge, Cambridge, United Kingdom

Aim: Brown adipose tissue (BAT) thermogenesis is controlled by environmental temperature and the circadian clock. Yet how these factors collectively coordinate BAT activity is still unclear. Using a comparative proteomics approach, we identified an orphan mitochondrial metabolite transporter, SLC25A34, that sits on the axes of both cold-induced, PPARa activation and circadian-regulated, REV-ERBa inhibition of BAT activity. Our aim is to investigate the role of SLC25A34 in modulating thermogenic capacity of adipocytes.

Method: Using ChIP-seq, proteomics and transcriptomics approaches, we interrogated the mechanistic underpinning of *Slc25a34* transcriptional regulation. Furthermore, we utilized genetic mouse models and primary and immortalized brown adipocyte (BA) lines to characterise the role of the transporter in BAT thermogenesis and metabolism.

Results: Expression of *Slc25a34* mRNA and protein is cold-inducible and undergoes circadian oscillation in BAT that is positively correlated with thermogenesis. Circadian expression of the transporter is controlled by the clock factor, REV-ERBa. During the sleeping period, REV-ERBa and HDAC3 suppress *Slc25a34* expression. Upon cold exposure or waking up REV-ERBa is downregulated leading to induced *Slc25a34* expression. Pathway overrepresentation analysis of proteins co-regulated with SLC25A34 in BAT during cold exposure revealed enrichment of lipid metabolic pathways. In line with this, we found that *Slc25a34* is the most induced mRNA in BAT of mice fed with ketogenic diet, suggesting its transcriptional control by PPARs, major regulators of lipid metabolism. Underscoring the role of PPARa, *Slc25a34* is the most downregulated transcript in BAT of *Ppara* knockout mice. Furthermore, the specific PPARa agonist, GW7647, dramatically induces *Slc25a34* expression in cultured BAs. Gain and loss-of-function studies in BAs showed that *Slc25a34* levels affect their norepinephrine-stimulated and maximal respiratory capacity. Moreover, *Slc25a34* knockout in BAT significantly decreases mouse energy expenditure during cold exposure suggesting important role of the transporter in BAT thermogenesis.

Conclusions: SLC25A34 is a novel effector of BAT thermogenesis whose transcriptional control by REV-ERBa and PPARa links towards circadian and temperature control of lipid metabolism.

Eligible for Young Investigator Oral Award

INHIBITION OF AXL RECEPTOR TYROSINE KINASE ENHANCES BROWN ADIPOSE TISSUE FUNCTIONALITY

Vissarion Efthymiou[1], Lianggong Ding[1], Wenfei Sun[1], Miroslav Balaz[1], Bradford Hamilton[2], Heike Neubauer[2], Leon Straub[1], Hua Dong[1], Eric Simon[2], Salvatore Modica[1], Carla Horvath[1], Caroline Moser[1], Aliki Perdikari[1], Lucia Balazova[1], Christian Wolfrum[1]

[1]ETH, Zürich, Switzerland. [2]Boehringer Ingelheim, Biberach, Germany

Aim: The current obesity epidemic and the alarmingly high prevalence of metabolic diseases render efficacious and safe treatments against these non-communicable diseases necessary. Lifestyle changes, despite being safe approaches, do not seem to exert sustainably robust effects in long-term approaches and currently only bariatric surgery constitutes an efficient treatment for obesity. Brown adipose tissue (BAT) is considered as a promising target organ with the potential to increase energy expenditure. However, no pharmacological treatments activating BAT are currently available.

Method: We used in a host of in vitro systems to study the molecular mechanism, by which AXL receptor tyrosine kinase regulates brown and brite adipocyte activity. In addition, we generated mice with brown/brite adipocyte specific ablation of AXL to determine its effect on systemic energy expenditure and lipid metabolism.

Results: We show that pharmacological and genetic inhibition of AXL receptor enhances thermogenic capacity and mitochondrial functionality of mature brown and white adipocytes. Mechanistically, we demonstrate that these effects are mediated through a fine tuning of the PI3K/AKT/PDE signaling pathway, which results in induction of nuclear FoxO1 localization under non-stimulated conditions and increased intracellular cAMP levels via PDE inhibition under isoproterenol-stimulated conditions. Lastly, we demonstrate that genetic deletion of AXL receptor in vivo enhances BAT non-shivering thermogenesis. Specifically, we show that both the congenital global AXL knockout (AXLKO) and the inducible fat-specific AXL knockout (iFAXLKO) mouse models are protected from HFD-induced obesity.

Conclusions: In conclusion, we propose that AXL receptor constitutes a promising novel target for the induction of brown adipocyte function to ameliorate obesity associated metabolic complications.

GENOME-WIDE CRISPR-CAS9 SCREEN TO IDENTIFY NEW DETERMINANTS INVOLVED IN INTRACELLULAR CHOLESTEROL TRANSPORT

Amber Meurs, Noam Zelcer

Amsterdam UMC-AMC, Amsterdam, Netherlands

Aim: Cholesterol is a fundamental component for cellular membranes and serves as a precursor for numerous biological molecules. LDL cholesterol is internalized via the LDL receptor pathway, and subsequently, cholesterol is released from lysosomes and transported to all other cellular membranes. The determinants governing intracellular cholesterol transport, and particularly transport to the ER, are not fully elucidated. Therefore, we developed a genome-wide CRISPR-Cas9-based screen to interrogate intracellular cholesterol transport.

Method: We used AMC cells that lack SQLE, a rate-limiting enzyme for cholesterol production, rendering them critically dependent on exogenous LDL-derived cholesterol. We applied a genome-scale CRISPR-Cas9 library to these cells to identify genes that essential for survival in the presence of LDL as the sole cholesterol donor.

Results: When cultured in lipoprotein-deficient serum AMC cells die within 5 days. Their survival can be fully rescued in an NPC-dependent manner by including exogenous LDL or by adding methyl-b-cyclodextrin-complexed cholesterol in the medium. Moreover, the survival can also be rescued by heterologous expression of SQLE. To interrogate cholesterol transport, we applied a genome-wide CRISPR-Cas9 sgRNA library (18,000 genes, 5x sgRNAs/gene) to identify genes that are essential for LDL-dependent growth in AMC cells. As a control, AMC-SQLE cells that stably produce SQLE were used. Our results will be presented.

Conclusions: We have developed a sensitive genome-wide live-dead CRISPR-Cas9 screen to identify determinants of intracellular transport of LDL-derived cholesterol.

Eligible for Young Investigator Oral Award

SPRING IS A NOVEL DETERMINANT IN SREBP SIGNALLING AND CHOLESTEROL METABOLISM

Sebastian Hendrix, Noam Zelcer

UMC Amsterdam, Amsterdam, Netherlands

Aim: Disturbed lipid metabolism is a key contributor to development of cardiovascular diseases. We recently identified SPRING as a new determinant of sterol regulator element- binding protein (SREBP) mediated transcriptional activation of cholesterol and fatty acid synthesis, and of low-density lipoprotein uptake. SPRING encodes a Golgi-resident, glycosylated membrane protein that is ubiquitously expressed. In this study we aim to further investigate and characterize this previously unknown regulator of lipid metabolism.

Method: To elucidate the role of SPRING *in vivo* we are studying the consequences of hepatic loss of SPRING expression in mice. To understand its mechanism of action, we investigated levels and interactions of SPRING and other SREBP-related proteins and its influence on lipid-associated pathways in SPRING KO and WT cells.

Results: SPRING KO cells fail to robustly activate SREPB target genes and therefore cholesterol biosynthesis and lipoprotein uptake in response to sterol depletion. Global ablation of SPRING is embryonically lethal. However, liver-specific deletion of SPRING in mice abrogates the SREBP-mediated hepatic feeding response. Mechanistically, we found that SPRING is required for retrograde transport of SCAP from the Golgi to the ER. Furthermore, we show that SPRING interacts with Site 1 protease (S1P), which leads to the cleavage of SPRING. Although the presence of SPRING is needed for proper SREBP signalling, our data suggest that cleavage of SPRING is not required for this process. Additionally, we demonstrate that the cleaved SPRING is secreted and further studies are warranted to elucidate the exact role of this secreted form.

Conclusions: Our data further establish the role of SPRING as a novel regulator of the SREBP pathway and thereby may help to develop mechanism-based strategies to treat dysregulated lipid metabolism.

Eligible for Young Investigator Oral Award

CONSEQUENCES OF INTESTINAL LAL DEFICIENCY ON WHOLE BODY LIPID METABOLISM

Valentina Bianco, Melanie Korbelius, Nemanja Vujic, Dagmar Kratky

Medical University of Graz, Graz, Austria

Aim: Lysosomal acid lipase (LAL) is the only enzyme responsible for the degradation of cholesteryl esters and triglycerides in the lysosome at an acidic pH. Mutations in its gene are responsible for two rare autosomal recessive diseases, depending on the residual activity of the enzyme. One of the most common symptoms of LAL deficiency is lipid malabsorption throughout the small intestine accompanied by macrophage infiltration. We aim to investigate the consequences of whole-body and intestinal LAL deficiency on lipid metabolism and absorption.

Method: We collected three parts of the small intestine (duodenum, jejunum, ileum) and livers from mice with a global (LAL KO) or intestine-specific deletion of LAL (iLAL KO) and wild-type littermates. We isolated RNA and proteins and quantified lipids. Lipoprotein secretion and cholesterol absorption were also assessed.

Results: In LAL KO mice, we observed tremendous lipid accumulation in the small intestine, especially in macrophages in the lamina propria. In addition, chylomicron secretion and cholesterol absorption were greatly reduced. Despite significantly reduced LAL activity in iLAL KO enterocytes, no significant differences were found in villus morphology, lipid parameters, and expression of lipid transporters in the small intestine of iLAL KO mice.

Conclusions: Despite the high expression of LAL in the small intestine and the pronounced lipid accumulation in the small intestine of LAL KO mice, iLAL KO mice do not recapitulate this phenotype. These findings suggest that the loss of LAL exclusively in enterocytes is not sufficient to trigger lipid accumulation in the small intestine, implicating an important role of macrophages in this process. Further investigations on the metabolism of these mice have to be performed to elucidate the role of LAL in enterocytes and macrophages in lipid absorption.

Eligible for Young Investigator Oral Award

THE ROLE OF INTESTINAL ATGL IN INTESTINAL AND SYSTEMIC CHOLESTEROL HOMEOSTASIS

Melanie Korbelius, Nemanja Vujic, Silvia Rainer, Dagmar Kratky

Medical University of Graz, Graz, Austria

Aim: Enterocytes of the small intestine (SI) play a crucial role in maintaining systemic lipid levels by regulating dietary lipid absorption and lipoprotein secretion in the postprandial state. An excessive amount of triglycerides (TGs), originating from dietary sources or lipoprotein remnants, is transiently stored in cytosolic lipid droplets (cLDs). Mice lacking adipose TG lipase (ATGL) in the SI display massive accumulation of cLDs, indicating a crucial role of this lipase in cLD breakdown. As lack of ATGL affected not only TG levels but also cholesterol absorption, we hypothesized that ATGL overexpression in the SI might have beneficial effects on lipid homeostasis in the intestine and throughout the body.

Method: To investigate the role of intestinal ATGL on intestinal and systemic lipid metabolism, we generated mice overexpressing ATGL specifically in the SI (Atgl iTg).

Results: Atgl iTg mice only displayed mildly induced enzymatic activity despite drastically elevated *Atgl* mRNA levels (up to 120-fold). Intestinal TG concentrations were significantly reduced 4 h after an acute lipid load but only slightly diminished after high fat/high cholesterol diet (HF/HCD) feeding. Interestingly, plasma cholesterol levels were significantly lower in Atgl iTg mice in the fasted state and after acute lipid challenge, consistent with delayed cholesterol absorption when mice were fed chow diet. Conversely, we observed accelerated cholesterol absorption after 5 weeks of HF/HCD feeding. Gene expression analysis revealed modulation of PPARa targets on HF/HCD, whereas the decreased plasma cholesterol in chow diet-fed mice was more likely due to changes in HDL synthesis and secretion.

Conclusions: We conclude that overexpression of the major lipase ATGL solely in the SI has beneficial effects on TG concentrations mainly in the jejunum. Unexpectedly, it acts as a critical player in regulating cholesterol homeostasis in the duodenum by increasing intestinal and circulating cholesterol concentrations when mice are fed standard chow diet.

Eligible for Young Investigator Oral Award

TISSUE-SPECIFIC ROLES OF THE MITOCHONDRIAL PHOSPHOLIPID, CARDIOLIPIN, IN THE CONTROL OF SYSTEMIC ENERGY HOMEOSTASIS

Zachary Gerhart-Hines, Mikkel Frost, Tao Ma, Fabian Finger, Lucile Dollet, Iuliia Karavaeva

University of Copenhagen, Copenhagen, Denmark

Aim: The mitochondrial inner membrane is a dynamic interface that supports bioenergetic processes, intracellular communication, and transport of metabolites, lipids, and ions. The phospholipid, cardiolipin, is one of the defining components of this membrane and is classically ascribed an indispensable role in mitochondrial structure and function. Disruption of cardiolipin biology is causally linked to numerous pathophysiologies, including diabetes, nonalcoholic fatty liver disease, Parkinson's disease, and, most notably, Barth syndrome. Yet how cardiolipin controls the metabolic functions of different tissues and how these tissues respond to loss of cardiolipin remains surprisingly unknown.

Method: We have generated inducible gain and loss-of-function genetic models of cardiolipin synthase 1 (Crls1) in skeletal muscle to investigate fiber type specific responses to CL depletion using global gene, protein, metabolite, and lipid profiling. We will further compare our new findings in muscle to our previous brown and white adipose *Crls1* KO models to determine conserved and tissue specific adaptations.

Results: We found that tissue-specific loss of cardiolipin had dramatically different outcomes on energy expenditure and whole-body glucose homeostasis. Loss of cardiolipin in skeletal muscle unexpectedly and markedly improved glycemic control in contrast to our earlier findings that cardiolipin deficiency in brown adipocytes significantly reduced organismal insulin sensitivity and glucose tolerance. Moreover, as opposed to the dogmatic belief that cardiolipin is ubiquitously required for mitochondrial function, glycolytic skeletal muscle fibers fully retained respiratory capacity in the face of cardiolipin depletion through fiber type and metabolic re-programming.

Conclusions: Our collective findings on tissue-specific roles of cardiolipin reshape the fundamental understanding of mitochondrial structure and function with potential therapeutic implications for phospholipid disorders.

SPHINGOLIPIDS ACCUMULATE IN PATHOLOGICAL SKELETAL MUSCLE, AND INHIBITION OF SPHINGOLIPID *DE NOVO* SYNTHESIS COUNTERACTS SEVERE MUSCLE DISEASES

Pirkka-Pekka Laurila[1,2], Peiling Luan[1], Nadege Zanou[3], Martin Wohlwend[1], Tanes Imamura de Lima[1], Hector Gallart-Ayala[3], Julijana Ivanisevic[3], Nicolas Place[3], Johan Auwerx[1]

[1]École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland. [2]National Institute for Health and Welfare, Helsinki, Finland. [3]University of Lausanne, Lausanne, Switzerland

Aim: We recently established the role of sphingolipids in age-related sarcopenia (Laurila et.al.). Here, we study the role of sphingolipids in severe muscle diseases.

Method & Results: Here, we discover and establish the link between sphingolipid metabolism and severe muscle diseases. Transcripts of sphingolipid *de novo* synthesis pathway, including *SPTLC1, SPTLC2, CERS2, and DEGS1*, are upregulated in a robust and global fashion in muscle biopsies of individuals with different muscle diseases, including Duchenne muscular dystrophy (DMD), Emery-Dreifuss muscular dystrophy, and Fascioscapulohumeral muscular dystrophy. We focus on DMD. Myoblasts derived from DMD patients displayed increased protein abundance of these genes, combined with accumulation of sphingolipids, including sphinganine, dihydroceramides, and ceramides. To study whether inhibition of sphingolipid biosynthesis could ameliorate muscle disease, we treated mdx mice, a mouse model of DMD, with myriocin, an inhibitor of sphingolipid biosynthesis. Myriocin treatment resulted in reduction of plasma and skeletal muscle sphingolipids, and improved cellular membrane integrity usually lost in dystrophies. Furthermore, stem cell depletion normally observed in dystrophies was prevented by reduction of sphingolipid synthesis. Augmented inflammation, normally present in dystrophic muscle was inhibited by myriocin, directing skeletal muscle macrophage phenotype from proinflammatory M1 to anti-inflammatory M2 state. Myriocin also improved the Ca^{2+} physiology of skeletal muscle. We also compared sphingolipid inhibition to glucocorticoid treatment, the best and only approved treatment for dystrophies. Relative to glucocorticoid treatment, myriocin treated mice displayed higher functional performance in tests for running and grip strength than glucocorticoid treated mice, and improved tissue morphology, as measured by inflammation and fibrosis. Combination therapy of myriocin and glucocorticoid alleviated DMD phenotype more than either compound as a monotherapy.

Conclusions: Our study identifies a novel link between sphingolipid metabolism and muscular dystrophies. As inhibition of sphingolipid synthesis was able to target many pathogenetic pathways simultaneously, it presents as a strong candidate for treatment of muscular dystrophies.

SCARB1 DEFICIENCY IN ZEBRAFISH LOWERS FEMALE FERTILITY IN THE CONTEXT OF UNALTERED PLASMA CHOLESTEROL LEVELS

Robin Verwilligen, Lindsay Mulder, Jeroen Bussmann, Menno Hoekstra, Miranda van Eck

LACDR, Leiden, Netherlands

Aim: Zebrafish are an upcoming animal model to study lipid metabolism. *Scarb1* plays an important role in high-density lipoprotein (HDL) metabolism and maintenance of the hormonal milieu. Importantly, as opposed to commonly used murine models, zebrafish do express cholesteryl ester transfer protein, an mediator of lipid transfer between HDL and apolipoprotein B-containing lipoproteins in humans. Previous studies in patients with genotypic variants of *scarb1* have suggested a role of *scarb1* in fertility. Because the reproductive regulation systems are highly similar between human and zebrafish, we examined that zebrafish may be a better animal model to study role of *scarb1* in fertility and total body cholesterol metabolism.

Method: *Scarb1* knockout (*scarb1*$^{-/-}$) zebrafish were generated using CRISPR-Cas9 technology.

Results: *Scarb1*$^{-/-}$ zebrafish lack yellow pigment, confirming the absence of functional scarb1 since the development of yellow xanthophores depends on *scarb1*. Introduction of a mutated *scarb1* allele did not affect embryonic development. However, when *scarb1*$^{-/-}$ zebrafish were intercrossed, the total number of eggs laid was significantly lower than in wild-type (WT) breedings (-45%, $p<0.05$). The genotype-associated infertility could be traced back to female animals as males lacking *scarb1* produced normal offspring. Strikingly, we observed that both male and female adult *scarb1-/-* zebrafish exhibited normal plasma cholesterol levels (WT 4.92±0.2 vs *scarb1*$^{-/-}$ 4.39±0.3 µg/µL; $p>0.05$). Furthermore, stress-induced cortisol secretion did not differ between WT and *scarb1*$^{-/-}$ zebrafish larvae (1.65±0.1 vs 1.79±0.2 ng/mL; $p>0.05$). In addition, HDL lipid clearance and total body expression of genes involved in cholesterol metabolism were not different between WT and *scarb1*$^{-/-}$ zebrafish larvae.

Conclusions: We have shown that *scarb1* deficiency in zebrafish is linked to reduced female fertility in the context of unaltered plasma cholesterol levels. Our studies (1) argue against a major role for *scarb1* in zebrafish cholesterol metabolism and (2) provide new proof for a primary role of *scarb1* in fertility.

Eligible for Young Investigator Oral Award

LIPOPROTEIN LIPASE AND CAVEOLA MEDIATE UPTAKE OF VLDL-LIKE PARTICLES IN MACROPHAGES

Lei Deng[1], Anouk L. Feitsma[2], Rinke Stienstra[1], Sander Kersten[1]

[1]Wageningen University and Research, Wageningen, Netherlands. [2]Friesland Campina, Amersfoort, Netherlands

Aim: The lipolysis function of Lipoprotein lipase (LPL) is considered mandatory for cells to take up exogenous triglycerides (TAGs). However, how macrophages take up excess lipids and what are the processes that internalized lipids going through are still not clearly uncovered.

Method: In our study, we created a very low-density lipoproteins (VLDL)- like emulsion with a droplet size of 60-80 nm. The roles of LPL and caveola during VLDL uptake by RAW 264.7 and human primary GM-CSF macrophages had been investigated using specific inhibitors and siRNA interference. The localization of the fluorescent labelled lipid droplets (LDs) and cellular organelles were determined using confocal microscopy and quantified by flow cytometer. Whole transcriptome data and qPCR data were used to indicate the uptake of lipids by macrophages on mRNA level.

Results: The results show that the block of binding terminal or removal of whole molecule of LPL, instead of just block its catalytic domain, could significantly decrease or even eliminate the uptake of VLDL particles by macrophages. Interestingly, we observed that the inhibition of caveola protein or knock down of its encoding genes could block the VLDL uptake process as well. We also found VLDL-derived lipolysis products were exported from lysosome to cytoplasm via NPC1 protein and parallelly these lipids can be translocated to endoplasmic reticulum directly by Stard3.

Conclusions: To conclude, the caveola-mediated endocytosis has been approved as a new mechanism for macrophages beside LPL to uptake extracellular TAGs. In this process, the VLDL-derived lipolysis products can be intracellularly transported from lysosome to cytoplasm and endoplasmic reticulum accordingly by NPC1 and Stard3.

Eligible for Young Investigator Oral Award

GPIHBP1 AND ANGPTL4 UTILIZE PROTEIN DISORDER TO ORCHESTRATE ORDER IN PLASMA TRIGLYCERIDE METABOLISM

Katrine Zinck Leth-Espensen[1,2], Kristian Kølby Kristensen[1,2], Anni Kumari[1,2], Anne-Marie Lund Winther[1,2], Stephen G Young[3], Michael Ploug[1,2]

[1]Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark. [2]Biotech Research Innovation Centre, Copenhagen, Denmark. [3]Department of Medicine, UCLA, Los Angeles, USA

Aim: The complex between lipoprotein lipase (LPL) and its endothelial receptor (GPIHBP1) is responsible for the lipolytic processing of triglyceride-rich lipoproteins (TRLs) along the capillary lumen. LPL activity is regulated in a tissue-specific manner by endogenous inhibitors (angiopoietin-like [ANGPTL] proteins 3, 4, and 8), but the molecular mechanisms are incompletely understood.

Aim: To delineate the molecular mechanisms of LPL regulation within the intravascular unit.

Methods: Biophysical methods such as HDX-MS, DSF, SPR, and SAXS to study molecular interactions in highly purified proteins.

Results: ANGPTL4 catalyzes the inactivation of LPL monomers by triggering the irreversible unfolding of LPL's α/β-hydrolase domain. Here, we define the binding site for ANGPTL4 on LPL and map the unfolding trajectory that culminates in the collapse of LPL's catalytic site. This unprecedented regulatory mechanism is context dependent and is driven by the inherent instability of LPL's α/β-hydrolase domain (Tm of 34.8 °C), which is dramatically increased by GPIHBP1 binding (Tm of 57.6°C), while ANGPTL4 lowers the onset of LPL unfolding by ~20°C. Of note, Kersten Sander showed that ANGPTL4 sensitizes LPL to PSCK3 cleavage in *trans*-Golgi of adipocytes and we now show that this is cleavage is promoted by ANGPTL4-catalyzed LPL unfolding.

Conclusion: ANGPTL4-catalyzed unfolding of LPL's α/β-hydrolase domain provides an unprecedented molecular mechanism for the irreversible inhibition of LPL activity and increased cleavage by PCSK3 in adipocytes. It will be important to establish whether the ANGPTL3/ANGPTL8 utilizes a similar mechanism for LPL inhibition in oxidative tissues and measure the impact of ApoA5 on this mechanism.

ENDOTHELIAL CANNABINOID RECEPTOR CB1 DEFICIENCY DECREASES OXLDL UPTAKE AND ATTENUATES VASCULAR INFLAMMATION IN ATHEROSCLEROSIS

Bingni Chen[1], Raquel Guillamat Prats[1], Yvonne Jansen[1], Remco Megens[1], Beat Lutz[2,3], Susanna Hofmann[4], Stephan Herzig[5,6], Sabine Steffens[1,7]

[1]Institute for Cardiovascular Prevention, Ludwig-Maximilians-University, Munich, Germany. [2]Institute of Physiological Chemistry, University Medical Center, Mainz, Germany. [3]Leibniz Institute for Resilience Research, Mainz, Germany. [4]Institute for Diabetes and Regeneration, Helmholtz Diabetes Center, Helmholtz Zentrum, Munich, Neuherberg, Germany. [5]Institute for Diabetes and Cancer, Helmholtz Zentrum Munich, Munich, Neuherberg, Germany. [6]Joint Heidelberg-IDC Translational Diabetes Program, Heidelberg University Hospital, Heidelberg, Germany. [7]German Center for Cardiovasular Research, partner site Munich Heart Alliance, Munich, Germany

Aim: The endocannabinoid system has emerged as an important lipid signaling system in various pathophysiological conditions, including metabolic disorders and atherosclerosis. Since endothelial dysfunction plays a critical role in the early stages of atherosclerosis, we here aim to study the effect of endothelial cell-specific CB1 deficiency on atherosclerotic plaque development and composition.

Method: We generated endothelial cell-specific CB1 knockout mice by breeding BmxCreERT2 with CB1-flox mice. The transgenic mice were under ApoE-/- background and received 4 or 16 weeks Western Diet (WD; 0.15% cholesterol). Immune status and atherosclerotic plaque progression were assessed by flow cytometry, qPCR, Western Blot, and histology.

Results: Endothelial CB1 deficiency (EC-CB1-KO) in female mice attenuated plaque development in the aortic roots (EC-CB1-WT 752478 ± 27719, n=9; EC-CB1-KO 631143 ± 29851, n=11; $P<0.01$) and abdominal aortas, accompanied by more stable plaque phenotype with increased collagen at advanced stage (16 weeks WD). Interestingly, the effect of endothelial CB1 deficiency on lesion development was not observed in male mice, suggesting that endothelial CB1 might play a sex-specific role in atherosclerosis. Besides, female EC-CB1-KO mice exhibited a significant decrease in aortic adhesion molecule ICAM-1 and VCAM-1 expression. Moreover, ex vivo imaging of carotid arteries via 2-photon microscopy revealed less endothelial oxLDL uptake (WT $5.75 \times 10^{-3} \pm 4.98 \times 10^{-4}$, n=5; KO $3.345 \times 10^{-3} \pm 7.69 \times 10^{-4}$, n=6; $P<0.05$; particles per cell length), which might explain the increased plasma cholesterol levels in EC-CB1-KO mice. Furthermore, a significant reduction of aortic endothelial caveolin-1 (CAV-1) protein expression was found in female EC-CB1-KO mice.

Conclusions: Our results indicate that endothelial CB1 contributes to atherosclerosis by modulating endothelial oxLDL uptake and vascular inflammation, possibly involving CAV-1, which deserves further investigation.

Eligible for Young Investigator Oral Award

LIPOPROTEIN STRUCTURAL AND FUNCTIONAL PROPERTIES PREVENT ATHEROSCLEROSIS DEVELOPMENT IN BROWN BEARS (URSUS ARCTOS)

Matteo Pedrelli[1], Paolo Parini[1], Jonas Kindberg[2], Jon M. Arnemo[3], Ingemar Bjorkhem[1], Ulrika Aasa[4], Maria Westerståhl[1], Anna Walentinsson[5], Chiara Pavanello[6], Marta Turri[6], Laura Calabresi[6], Katariina Öörni[7], Germán Camejo[1], Ole Fröbert[8], Eva Hurt-Camejo[5]

[1]Karolinska Institutet, Huddinge, Sweden. [2]5. Norwegian Institute for Nature Research, Trondheim, Norway. [3]6. Swedish University of Agricultural Sciences, Umeå, Sweden. [4]Umeå University, Umeå, Sweden. [5]AstraZeneca, Gothenburg, Sweden. [6]Universtitá di Miliano, Milano, Italy. [7]Wihuri Research Institute, Helsinki, Finland. [8]Örebro University, Örebro, Sweden

Aim: Despite having high levels of plasma cholesterol (TC) and triglycerides (TG) free-ranging brown bears seem resistant to atherosclerosis. This apparent paradox might be explained by specific lipoprotein structure and functions during hibernation (winter) and active state (summer).

Method: Plasma from the same wild free-ranging Swedish brown bears (n=10) were sampled in winter and summer. Lipoproteins were separated by size exclusion chromatography, ultracentrifugation and gel-electrophoresis. Lipoprotein composition and function, as LDL binding to arterial proteoglycans (PGs) and cholesterol efflux capacity (CEC) were studied. Samples from 14 human subjects were studied for comparative purpose. Data are presented as median (10^{th} - 90^{th} percentile).

Results: During hibernation bear LDL carried 4.6 (2.3-5.9) mmol/L cholesterol esters (CE), 1.5 (1.1-2.4) mmol/L unesterified (UC), 3.7 (2.1-4.9) mmol/L TG and 2.5 (1.8-3.4) mmol/L phospholipid (PL). Those levels were higher than in summer and when compared to human. Yet, LDL percentage lipid composition did not change in bears from winter to summer, but it differed between species. Human LDL were smaller than bear LDL, which were proportionally richer in TG (winter 38 (32-41) %, summer 37 (28-47)% vs human 12 (10-20)); $p<0.001$) and poorer in CE (winter 33 (23-41)%, summer 23 (18-34)% vs human 48 (46-52)%; $p<0.01$)). Bear LDL showed pre-beta electrophoretic mobility and had 5-10 times lower binding to arterial proteoglycans than human LDL. Plasma CEC was higher in bears than in human, especially the HDL CEC mediated by the ATP-binding cassette transporter AI.

Conclusions: Despite high TC and TG levels, bear lipoprotein profile was less atherogenic than the human one. This was due to low LDL affinity for PGs, secondary to increased TG and PL, and to low positive surface charge. High plasma CEC may play a role. We provided further mechanistic insights for the atherosclerosis development, which is driven by the circulating lipoprotein composition and functions more than plasma absolute lipid levels.

THE HDL MIMETIC CER-001 AMELIORATES LIPOPROTEIN PROFILE IN FAMILIAL LCAT DEFICIENCY

Marta Turri[1], Chiara Pavanello[1], Arianna Strazzella[1], Patrizia Tulissi[2], Stefano Pizzolitto[3], Giovanna De Maglio[3], Riccardo Nappi[2], Laura Calabresi[4], Giuliano Boscutti[2]

[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Milano, Italy. [2]Nephrology, Dialysis and Renal Transplantation Unit, S. Maria della Misericordia Hospital, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy. [3]Pathology Unit, S. Maria della Misericordia Hospital, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Udine, Italy. [4]Nephrology, Dialysis and Renal Transplantation Unit, S. Maria della Misericordia Hospital, Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), Milano, Italy

Aim: Familial LCAT deficiency (FLD) is a rare recessive disorder of lipid metabolism with no cure. The major cause of morbidity and mortality in FLD is renal failure. Lipoprotein X, an abnormal lipoprotein, is the main accountant for nephrotoxicity. In a mouse model of LCAT deficiency CER-001 reduced renal impairment. CER-001 has thus been tested in a FLD carrier with extremely fast recurrence of renal damage to slow the progression of renal decline.

Method: CER-001 was infused at the dose of 10 mg/kg 3 times per week for 3 weeks, followed by 2 times per week for 3 weeks, and then once per week for 6 weeks. Plasma samples were collected at all visits, before CER-001 administration, and at the end of treatment. A complete lipid-lipoprotein profile was determined using a Roche Integra c311 analyzer. The 1.020–1.063 g/mL lipoprotein fraction was separated at each timepoint by ultracentrifugation and analyzed by fast performance liquid chromatography. HDL subclass distribution was characterized by 2D-electrophoresis. ApoA-I in urine was analyzed by western blotting.

Results: CER-001 did not substantially alter lipid levels; nevertheless after 3 weeks of treatment triglycerides transiently decreased and unesterified cholesterol transiently raised. Interestingly, the HDL mimetic produced a remodeling of lipoprotein profile, reducing circulating lipoprotein X in favor of normally sized LDL particles. This remodeling occurred slowly, suggesting a progressive disassembling of LpX, which likely acts as shuttle/sink of phospholipids and unesterified cholesterol. HDL subclass distribution analysis showed no significant changes. Accordingly to its short half-life, CER-001 was not detected in plasma, but it was found in patient's urine.

Conclusions: The present results demonstrate that CER-001 induces lipoprotein normalization in FLD by reducing the nephrotoxic LpX, thus leading to reduced accumulation of cholesterol in renal cells. In conclusion, CER-001 may represent a possible therapy for FLD, which has no cure.

Eligible for Young Investigator Oral Award

THE U2-SPLICEOSOME AND ITS INTERACTORS REGULATE THE LEVELS AND ACTIVITY OF THE LDL RECEPTOR IN HUMANS

Grigorios Panteloglou[1,2], Paolo Zanoni[1,2], Alaa Otman[3], Joel T. Haas[4], Roger Meier[5], Marta Futema[6], Freerk van Dijk[7], Melinde Wijers[8], Justina C. Wolters[8], Michele Visentin[9], Sven Francque[10,11], Luc Van Gaal[11,12], Andreas Geier[13], Lucia Rohrer[1,2], Steve E. Humphries[14], Bart Staels[4], Bart van de Sluis[8], Jan Albert Kuivenhoven[8], Arnold von Eckardstein[1,2]

[1]Institute of Clinical Chemistry, University and University Hospital of Zurich, Switzerland. [2]Center for Integrative Human Physiology, Univ. Zurich, Switzerland. [3]Institute of Molecular Systems Biology, ETH Zurich, Switzerland. [4]Univ. Lille, Inserm, CHU Lille, Institut Pasteur de Lille, Lille, France. [5]Scientific center for optical and electron microscopy (ScopeM), ETH Zurich, Switzerland. [6]Centre for Heart Muscle Disease, Institute of Cardiovascular Science, University College London, United Kingdom. [7]Univ. Groningen, University Medical Center Groningen, Department of Genetics, Groningen, Netherlands. [8]Department of Pediatrics, Section Molecular Genetics, Univ. Groningen, University Medical Center Groningen, Groningen, Netherlands. [9]Department of Clinical Pharmacology and Toxicology, Univ. Hospital Zurich, Switzerland. [10]Department of Gastroenterology and Hepatology, Antwerp University Hospital, Belgium. [11]Laboratory of Experimental Medicine and Paediatrics, Faculty of Medicine, University of Antwerp, Belgium. [12]Department of Endocrinology, Diabetology and Metabolism, Antwerp University Hospital, Belgium. [13]Division of Hepatology, Department of Medicine II, University Hospital Würzburg, Germany. [14]Cardiovascular Genetics, Institute of Cardiovascular Science, University College London, United Kingdom

Aim: The low-density lipoprotein receptor (LDLR) in the liver is the major determinant of LDL-cholesterol levels in humans. The discovery of novel genes that regulate the activity of LDLR could lead to the identification of pathomechanisms of hypercholesterolemia and novel therapeutic targets against atherosclerotic cardiovascular disease.

Method: We performed a genome-wide RNAi screen for fluorescent LDL uptake in Huh-7 hepatocarcinoma cells and validated our top hit genes in vitro, ex vivo as well as in population genetics datasets.

Results: In our genome-wide RNAi screen, the knock-down of 54 genes led to a significant inhibition of LDL uptake. Fifteen of these genes encode for proteins involved in splicing, especially components or interactors of the U2-spliceosome. Eleven were validated by individually targeted knock-down experiments, which confirmed their limiting role on LDL uptake into Huh-7 cells. RNA sequencing revealed that the loss of each spliceosome gene results in the selective retention of intron 3 of LDLR. The transcript is translated into an LDLR fragment, which lacks 88% of the full length LDLR and is detectable in cells and their medium upon overexpression, but neither in non-transfected cells nor in human plasma. The intron 3 retention transcript is expressed in considerable amounts in human liver and in blood cells. Its expression correlates with plasma LDL-cholesterol and age and increases after bariatric surgery. Single nucleotide polymorphisms and rare variants of one spliceosome gene, RBM25, are associated with LDL-cholesterol in the population and familial hypercholesterolemia, respectively.

Conclusions: We identified a novel mechanism of post-transcriptional regulation of LDLR activity in humans.

Eligible for Young Investigator Oral Award

MMP12: A POTENTIAL NEW TARGET FOR CARDIOMETABOLIC DISEASES

Melina Amor, Anja Dobrijević, Martin Buerger, Ivan Bradić, Katharina Kuentzel, Akhmetshina Alena, Dagmar Kratky

Gottfried Schatz Research Center, Medical University of Graz, Graz, Austria

Aim: We have previously identified the macrophage-secreted protein matrix metalloproteinase 12 (MMP12) as a massively upregulated pro-inflammatory factor in metabolic and vascular tissues in mice suffering from cardiometabolic diseases (CMDs). However, the exact effects of MMP12 in CMDs together with the mechanisms underlying its detrimental role remain elusive.

Method: To investigate the metabolic consequences of MMP12 deficiency, we used either $Mmp12^{-/-}$ and wild type (WT) mice fed with chow diet (CD) or $Ldlr^{-/-}$ and LdlrMmp12 double knockout (DKO) mice fed with high-fat sucrose-enriched diet (HFSCD) as a cardiometabolic mouse model that mimics human disease by developing insulin resistance, adipose tissue inflammation, and atherosclerosis. We also performed *in vitro* studies using stromal vascular cells (SVFs) and thioglycolate-elicited macrophages from $Mmp12^{-/-}$ and WT mice.

Results: $Mmp12^{-/-}$ mice fed with CD showed reduced plasma glucose concentrations in fed and fasted states as well as improved glucose tolerance compared to the WT controls. SVF differentiation into mature adipocytes resulted in reduced lipid accumulation and an increased expression of browning markers in $Mmp12^{-/-}$ cells. In thioglycolate-elicited macrophages from $Mmp12^{-/-}$ mice, the expression of proinflammatory genes was markedly reduced. Additionally, we isolated aortic rings from $Mmp12^{-/-}$ and WT mice and performed myography to investigate aortic contractility, which revealed an improved relaxation pattern and endothelial function in $Mmp12^{-/-}$ mice. First results in LdlrMmp12 DKO mice showed reduced body weight and body weight gain as well as plasma glucose concentrations in both fed and fasted states compared to $Ldlr^{-/-}$ mice. Interestingly, we also detected increased concentrations of MMP12 in plasma samples of 66 patients suffering from metabolic syndrome compared to 62 healthy volunteers.

Conclusions: We conclude that MMP12 may play a detrimental role in various cells and tissues and has the potential to be an interesting molecular target for the treatment of CMDs.

TRIGLYCERIDE LOWERING LPL ALLELES COMBINED WITH LDL-C LOWERING ALLELES ARE ASSOCIATED WITH AN ADDITIVELY IMPROVED LIPOPROTEIN PROFILE

Dorina Ibi[1,2], Lisanne Blauw[1], Raymond Noordam[1], Martijn Dolle[2], Wouter Jukema[1], Frits Rosendaal[1], Constantinos Christodoulides[3], Matt Neville[3], Robert Koivula[3], Patrick C.N. Rensen[1], Fredrik Karpe[3], Ko Willems van Dijk[1]

[1]Leiden University Medical Center, Leiden, Netherlands. [2]National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands. [3]University of Oxford, Oxford, United Kingdom

Aim: Mendelian randomization studies have shown that triglyceride (TG) lowering *lipoprotein lipase (LPL)* alleles and low-density lipoprotein-cholesterol (LDL-C) lowering alleles have independent beneficial associations on cardiovascular disease (CVD) risk. We aimed to provide further insight in this observation by applying Mendelian randomization analyses of genetically influenced TG and LDL-C levels on plasma metabolomic profiles.

Method: We quantified over 100 metabolomic measures in the Netherlands Epidemiology of Obesity (NEO) study (N=4,838) and Oxford Biobank (OBB) (N=6,999) by nuclear magnetic resonance (NMR) spectroscopy. Weighted genetic scores for TG via five *LPL* alleles and LDL-C via 19 alleles were calculated and dichotomized by the median, resulting in four genotype combinations of high/low TG and high/low LDL-C. We performed linear regression analyses using a two × two design with the group with genetically influenced high TG and LDL-C as a reference.

Results: Compared to the individual groups with genetically influenced lower TG or lower LDL-C only, the group with combined genetically influenced lower TG and LDL-C showed an overall independent and additive pattern of changes in metabolomic measures. Over 100 measures were different ($p<1.35x10^{-3}$) compared to the reference, with effect sizes and directionality being similar in NEO and OBB. Most notably, levels of all very-low density lipoprotein (VLDL) and LDL sub-particles were lower.

Conclusions: Our findings provide evidence that TG lowering on top of LDL-C lowering has additive beneficial effects on the lipoprotein profile compared to TG lowering or LDL-C lowering only, which is in accordance with reported additive genetic effects on CVD risk reduction.

GENETIC VARIATION IN *ABCA1* AND RISK OF AGE-RELATED MACULAR DEGENERATION, DEMENTIA, AND ISCHEMIC HEART DISEASE

Liv Tybjærg Nordestgaard[1], Ruth Frikke-Schmidt[1], Mette Christoffersen[1], Anne Tybjærg-Hansen[1], Shoaib Afzal[2], Børge Nordestgaard[2]

[1]*Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.* [2]*Herlev-Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark*

Aim: The adenosine triphosphate-binding cassette transporter A1(ABCA1) is a major cholesterol transporter highly expressed in liver, brain and the eye. ABCA1 mediates cholesterol and phospholipid efflux to lipid-poor apolipoproteins and is essential for the biogenesis of high-density lipoprotein(HDL) cholesterol in the circulation and HDL-like particles in the brain. Genome-wide association studies of age-related macular degeneration(AMD) and dementia found the *ABCA1* gene to be significantly associated with these diseases. Whether genetic variation in *ABCA1* is associated with AMD and dementia in prospective cohorts of the general population remains unknown. We tested the hypothesis that genetic variation in *ABCA1* is associated with risk of all-cause AMD, dry AMD, wet AMD, all-cause dementia, non-Alzheimer's dementia, Alzheimer's disease, and ischemic heart disease.

Method: In a prospective cohort study of the Danish general population (n=90,344), we tested the association between an *ABCA1* allele score, weighted on higher HDL cholesterol levels on a continuous scale and stratified into 3 groups (group 3=highest levels), and risk of all-cause AMD, dry AMD, wet AMD, all-cause dementia, non-Alzheimer's dementia, Alzheimer's disease, and ischemic heart disease.

Results: On a continuous scale, higher levels of genetically determined HDL cholesterol were associated with higher risk of all-cause AMD, dry AMD, and wet AMD, all-cause dementia, non-Alzheimer's dementia, but not with Alzheimer's disease or ischemic heart disease. The *ABCA1* allele score group 3 versus group 2 was associated with hazard ratios (95% confidence intervals) of 1.15(1.02-1.30) for age-related macular degeneration, 1.11(0.95-1.30) for dry AMD, 1.21(1.05-1.40) for wet AMD, 1.13(1.03-1.23) for all-cause dementia, 1.20(1.07-1.34) for non-Alzheimer's dementia, and 1.04(0.92-1.18) for Alzheimer's disease.

Conclusions: Genetic variation in *ABCA1* was associated with higher risk of age-related macular degeneration, all-cause dementia, non-Alzheimer's dementia, but not with Alzheimer's disease or ischemic heart disease.

Eligible for Young Investigator Oral Award

LINKING CELLULAR LIPID METABOLISM PROFILES TO THE OUTCOMES OF CHOLESTEROL-LOWERING THERAPY IN A GENERAL POPULATION COHORT STUDY

Iryna Hlushchenko, Mohammad M. Islam, Simon G. Pfisterer

Faculty of Medicine, University of Helsinki, Helsinki, Finland

Aim: Currently, there are no good tools to identify patients who do not respond well to statin therapy and are in need of more effective drugs such as PCSK9 inhibitors. Our goal is to define how alterations in cellular lipid metabolism, such as LDL uptake and lipid storage, influence the response to cholesterol-lowering medication.

Method: Previously, we established a multiparametric analysis platform to quantify LDL uptake and lipid storage in leukocytes from human subjects and showed that quantification of cellular lipid metabolism allows to identify poor statin responders among heterozygous familial hypercholesterolemia patients. (Pfisterer et al., bioRxiv, doi:10.1101/2021.04.19.440471). Here we use this pipeline to analyze 400 subject samples from the FINRISK 2012 cohort study, including 200 recipients of cholesterol-lowering medication. For each subject we have access to drug reimbursement, NMR metabolomics and clinical follow-up data.

Results: So far we have analyzed more than 300 samples and found that LDL uptake and lipid mobilization, the velocity with which cells deplete their lipid reservoirs, varies up to 8-fold between individuals. Interestingly, for subjects on cholesterol-lowering medication, reduced LDL uptake correlates with increased cholesterol and cholesteryl esters in small and medium LDL and VLDL particles. This correlation profile is changing for control subjects, highlighting that alterations in cellular lipid metabolism can influence the effect of cholesterol-lowering drugs on a patients lipoprotein profile.

Conclusions: This is the first real-world study attempting to link alterations in cellular lipid metabolism with differential outcomes of cholesterol-lowering therapy. In the following months we will finalize the sample analysis and perform subgroup analysis for different cholesterol-lowering medications to establish novel patient stratification tools for hypercholesterolemia.

Eligible for Young Investigator Oral Award

FUNCTIONAL ANALYSIS OF LDLR VARIANTS USING AUTOMATED SYSTEMS

Mohammad M. Islam[1], Iryna Hlushchenko[1], Attila Beleon[2], Peter Horvath[2,3], Simon Pfisterer[1]

[1]*Faculty of Medicine, University of Helsinki, Helsinki, Finland.* [2]*Biological Research Center, Szeged, Hungary.* [3]*Institute for Molecular Medicine Finland, Helsinki, Finland*

Aim: Familial hypercholesterolemia (FH) is mostly caused by mutations in the low-density lipoprotein (LDL) receptor (*LDLR*) gene. However, only a small fraction of *LDLR* variants is functionally characterized, limiting the use of genetic tools for early diagnosis of FH and complicating the characterization of FH patients.

Method: We established automated systems for large-scale *LDLR* variant characterization, regarding cellular LDL uptake, LDLR localization and lipid storage. For this purpose we combined high-content imaging with automated tools for cell culture and molecular biology.

Results: We designed a cell system which allows us to quantify the activity for different *LDLR* variants at high precision. Variants are expressed as GFP fusion proteins in a LDLR knock out (KO) HepG2 cell line through stable genome integration using CRISPR/Cas9. This enables low-level and uniform expression of LDLR-GFP constructs in 95% of the cells. Expression of wild-type LDLR-GFP restored LDL uptake activity in LDLR KO cells and is regulated by lipoprotein starvation. We use high-content microscopy to determine LDL uptake and subcellular localization for each variant and normalize these activities to wild type LDLR-GFP. We set up an open-source robotics platform for large-scale generation of LDLR variant expression constructs, transfection into LDLR KO cells, automated cell culture and seeding into 384 well imaging plates. The activity and localization of each *LDLR* variant is quantified in lipid rich conditions and upon two lipid starvation challenges. So far, we generated more than 100 variant expression constructs and analyzed more than 50 variants, demonstrating that we can perform reliable assessment of *LDLR* variants across a large activity range.

Conclusions: Our detailed functional analysis of *LDLR* variants paves the way for improved characterization of FH patients, opens new avenues for rare-variant association studies and can guide new personalized medicine approaches for lipid lowering therapy.

A NOVEL GENE AFFECTING VLDL METABOLISM AND ATHEROSCLEROSIS

Willemien van Zwol, Jan Albert Kuivenhoven

University Medical Center Groningen, Groningen, Netherlands

Aim: Compromised secretion of very low-density lipoprotein (VLDL) results in hepatic lipid accumulation but on the other hand protects against atherosclerosis. Here, we present a novel gene, *SMLR1* identified through co-expression analysis with *APOC3*. The gene encodes for small leucin rich protein 1 of unknown function which is exclusively expressed in the small intestine and liver.

Method: Using somatic CRISPR/Cas9 gene editing, *Smlr1* expression was downregulated in livers of wild-type mice, fed a chow diet. Liver lipids were extracted, and VLDL secretion rate was assessed after poloxamer injection. Lipoprotein profiling was conducted by FPLC. Using the same mouse model, we studied the effect of 12-weeks high-fat diet (+0.2% cholesterol) on hepatic steatosis and atherosclerosis following liver-specific PCSK9 downregulation.

Results: Downregulation of *Smlr1* at the mRNA level (80%) and protein level (67%), resulted in 50% reduction of plasma levels of cholesterol and triglycerides ($p<0.001$ for both), and increased hepatic lipid levels (>2.9fold, $p<0.001$). In line, VLDL secretion rate was reduced by 45%. In the atherosclerosis experiment, the mice showed, compared to controls, 20-fold lower plasma lipid levels, reduced body weight gain, and hepatic steatosis. Atherosclerosis assessment in ongoing.

Conclusions: SMLR1 is a new regulator of plasma and hepatic lipid metabolism. Liver-specific loss of SMLR1 attenuates VLDL secretion and is associated with fatty liver, reduced plasma lipids, and decreased atherosclerosis. The latter has yet to be confirmed but taken that plasma cholesterol and triglycerides are hardly increased, we anticipate zero effect on atherosclerosis. SMLR1 is localized in the ER and Golgi complex and anticipated to play a role in the trafficking of lipoproteins.

THE GI-COUPLED P2Y13 RECEPTOR SIGNALING INHIBITS LIPOLYSIS AND PROTECTS FROM METABOLIC SYNDROME AND ASSOCIATED LIVER DISEASES

Thibaut Duparc, Emilia Gore, Diane Beuzelin, Guillaume Combes, Jules Merian, Souad Najib, Laurent Martinez

I2MC, Inserm, Toulouse, France

Aim: The G_i-coupled $P2Y_{13}$ receptor is a purinergic ADP-receptor that plays a role in lipid metabolism and protects from atherosclerosis. Given that $P2Y_{13}$ receptor is highly expressed in adipocytes, we aim to investigate its role in adipocyte lipolysis and its effect on metabolic syndrome and associated liver diseases such as fatty liver and steatohepatitis (NAFLD/NASH).

Method: Wild-type (WT) and $P2Y_{13}$ KO mice were fed a Western diet for 16- and 40-week. *In-vivo* lipolysis, glucose homeostasis and systemic inflammation were assessed. At sacrifice, lipolytic activity was measured in isolated adipocytes and explants from inguinal and epididymal white adipose tissue (iWAT and eWAT) by measuring glycerol and NEFAs release. Hepatic steatosis, inflammation and fibrosis were assessed by histology and biochemical measurements, and lipidomic and transcriptomic analyses were performed.

Results: $P2Y_{13}$ KO mice display increased β-adrenergic lipolytic response, impaired glucose homeostasis and insulin signaling and higher systemic inflammation. Adipocytes from $P2Y_{13}$ KO mice displayed higher intracellular cAMP levels than those isolated from WT mice, reflecting impaired G_i signaling. $P2Y_{13}$ deletion was associated to higher lipolysis in isolated adipocytes and explants from iWAT and eWAT. Same effects were observed in WT mice when $P2Y_{13}$ was pharmacologically inhibited with MRS2211 ($P2Y_{13}$ inhibitor) but not with MRS2179 (P2Y1 inhibitor). In the liver, $P2Y_{13}$ KO mice displayed higher steatosis and fibrosis (at 40-week) and increased expression of lipogenic and pro-inflammatory genes. Moreover, lipidomic and microarray analyses in the liver concomitantly revealed a NASH-related lipid signature in $P2Y_{13}$ KO mice and activation of inflammatory pathways mediated by lipid mediators such as arachidonic and hydroxyeicosatetraenoic acids.

Conclusions: These results support a protective role of $P2Y_{13}$ receptor against metabolic syndrome and NAFLD/NASH development through a mechanism involving Gi-mediated inhibition of lipolysis. For that reason, $P2Y_{13}$ activation may represent a potential pharmacological target to treat metabolic syndrome and associated diseases.

PRMT1 INHIBITOR TC-E 5003 REDUCES NON-ALCOHOLIC FATTY LIVER DISEASE AND ATHEROSCLEROSIS BURDEN IN WESTERN-TYPE DIET-FED LDL RECEPTOR KNOCKOUT MICE

Timothy Sijsenaar, Yiheng Zhang, Miranda Van Eck, Menno Hoekstra

Leiden Academic Centre for Drug Research, Leiden, Netherlands

Aim: Non-alcoholic fatty liver disease (NAFLD), characterized by increased lipogenesis and hepatic triglyceride accumulation, predisposes to atherosclerotic cardiovascular disease. In vitro studies have suggested that protein arginine methyltransferase 1 (PRMT1) is a transcriptional co-activator of the lipogenic program in hepatocytes. Here we evaluated the potential of PRMT1 inhibitor TC-E 5003 to lower NAFLD and atherosclerosis susceptibility in vivo.

Method: Low-density lipoprotein receptor knockout mice were fed a Western-type diet and injected intraperitoneally 3x/week with TC-E 5003 or solvent control for 8 weeks.

Results: TC-E 5003 treatment was associated with a 42% decrease ($P<0.01$) in hepatic fatty acid synthase mRNA expression levels and 42% lower ($P<0.01$) liver triglyceride stores, without a change in tissue cholesterol levels. Concomitant 33% ($P<0.05$), 37% ($P<0.05$), and 40% ($P<0.01$) reductions in plasma triglyceride, unesterified cholesterol, and cholesteryl ester levels were detected in TC-E 5003-treated mice as compared to controls, which could be attributed to a selective decrease in (very-)low-density lipoprotein levels. Aortic root Oil red O-positive atherosclerotic lesion areas tended to decrease upon PRMT1 inhibition ($153\pm17 \times 10^3$ μm^2 versus $205\pm23 \times 10^3$ μm^2; $P=0.11$). However, CD68 staining uncovered a significant decrease in aortic root macrophage content (-57%; $P<0.05$). Notably, the PRMT1 inhibition-associated decrease in atherosclerosis susceptibility coincided with a significant shift in monocyte polarization towards the less atherogenic $Ly6C^{low/med}$ phenotype. Studies in cultured murine peritoneal macrophages verified the anti-inflammatory effect of TC-E 5003.

Conclusions: PRMT1 inhibition impacts both inflammatory and metabolic processes to reduce NAFLD and atherosclerosis burden in Western-type diet-fed LDL receptor knockout mice.

PCSK9 DEFICIENCY REWIRES HEART METABOLISM AND DRIVES HEART FAILURE WITH PRESERVED EJECTION FRACTION

Lorenzo Da Dalt, Andrea Baragetti, Monika Svecla, Alberico Catapano, Danilo Norata

University of Milan, Milan, Italy

Aim: PCSK9 in involved the degradation of several receptors of the LDL-R family and of CD36 by favoring their routing to the lysosome. As a consequence, PCSK9 deficiency results in increased tissue expression of lipoprotein receptors and we aimed to test whether this is could affect lipid accumulation in the heart and affect cardiac metabolism and function.

Method: WT, Pcsk9 KO, Albumin CRE-PCSK9LoxP/LoxP conditional KO (lacking PCSK9 production selectively in the liver and no PCSK9 in the circulation) and double LDLr-Pcsk9 KO male mice were fed for 20 weeks with SFD (Standard Fat Diet – 10% Kcal fat). The heart was collected and an extensive metabolomics and proteomic analysis was performed. Mitochondrial respiration was investigated under resting conditions and following maximal coupling and uncoupling conditions. Heart function was profiled by echography and by running endurance tests.

Results: Pcsk9 KO presented reduced running resistance coupled to echocardiographic abnormalities suggestive of heart failure with preserved ejection fraction (HFpEF). Heart mitochondrial activity, following maximal coupled and uncoupled respiration, was reduced in Pcsk9 KO compared to WT and was coupled to major changes in cardiac metabolism together with increased expression of LDLR and CD36 and lipid accumulation. A similar phenotype was observed in Pcsk9/Ldlr DKO, thus excluding a contribution for LDLR on cardiac impairment observed in Pcsk9 KO mice. Heart function profiling of the liver selective Pcsk9 KO model further excluded the involvement of circulating PCSK9 in the development of HFpEF, pointing to a possible role for local produced PCSK9. Concordantly, carriers of the R46L loss of function variant for PCSK9 presented increased left ventricular mass but similar ejection fraction compared to matched control subjects.

Conclusions: These data suggest that PCSK9 deficiency impacts cardiac lipid metabolism in a LDLR independent manner and promotes the development of HFpEF.

Poster presenters eligible for YIA

In order of appearance in the program:

Alena Akhmetshina (Graz, Austria)	01
Lara Coppi (Milan, Italy)	03
Veronica Dahik (Paris, France)	04
Emilie Kjeldsen (Copenhagen, Denmark)	08
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Imke Lemmer (Munich, Germany)	11
Rumei Li (Groningen, The Netherlands)	12
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Olivia Sveidahl Johansen (Copenhagen, Denmark)	21
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Susanne van Wouw (Amsterdam, The Netherlands)	24
Yong Wang (Munich, Germany)	25
Nienke Willemsen (Munich, Germany)	26
Yiheng Zhang (Leiden, The Netherlands)	28

Posters

01 Alena Akhmetshina (Graz, Austria) *

IMPACT OF LAL DEFICIENCY IN SKELETAL MUSCLE FORMATION AND METABOLISM

02 Núria Amigó (Reus, Spain)

NMR METABOLOMICS, A SUCCESSFUL STRATEGY TO IDENTIFY A TYPE 2 DIABETES MELLITUS MOLECULAR SIGNATURE BEYOND GLUCOSE IN THE DI@BETES STUDY

03 Lara Coppi (Milan, Italy) *

ROLE OF HISTONE DEACETYLASE 3 (HDAC3) AND NEURONATIN (NNAT) IN PATHOPHYSIOLOGY OF ADIPOSE TISSUE

04 Veronica Dahik (Paris, France) *

MEMBRANE PHOSPHATIDYLETHANOLAMINE REMODELING BY PLA2G2D CONTRIBUTES TO THE ANTI-INFLAMMATORY PHENOTYPE OF ADIPOSE TISSUE MACROPHAGES

05 Aldo Grefhorst (Amsterdam, The Netherlands)

ANGIOPOIETIN-LIKE 3 (ANGPTL3) RESIDES ON HDL

06 Henrika Jodeleit (Munich, Germany)

CARDIOMYOCYTE NFE2L1 LIMITS MYOCARDIAL INFARCTION-INDUCED HYPERTROPHY

07 Sander Kersten (Wageningen, The Netherlands)

SAFETY AND EFFECTIVENESS OF LIVER-SPECIFIC INACTIVATION OF ANGPTL4 VIA ANTISENSE OLIGONUCLEOTIDES

08 Emilie Kjeldsen (Copenhagen, Denmark) *

ADHERENCE TO DIETARY GUIDELINES AND RISK OF DEMENTIA: A PROSPECTIVE COHORT STUDY OF 94,184 INDIVIDUALS

09 Katharina Kuentzel (Graz, Austria) *

IMPAIRED ACID CHOLESTERYL ESTER HYDROLYSIS AFFECTS IN UTERO PLACENTA AND FETAL DEVELOPMENT

10 Folkert Kuipers (Groningen, The Netherlands)

GENETIC AND MICROBIAL ASSOCIATIONS TO PLASMA AND FECAL BILE ACIDS RELATE TO PLASMA LIPIDS IN HUMANS

11 Imke Lemmer (Munich, Germany) *

CRITICAL ROLE FOR MYOCYTE NFE2L1 IN MUSCLE PROTEOSTASIS AND ENERGY METABOLISM

12 Rumei Li (Groningen, The Netherlands) *

INTRODUCING A HUMAN-LIKE BILE ACID COMPOSITION IN MICE PREVENTS HIGH-FAT DIET INDUCED HEPATIC STEATOSIS

13 Cong Liu (Leiden, The Netherlands) *

HEPATIC FIBROBLAST GROWTH FACTOR 21 OVEREXPRESSION ATTENUATES DIET-INDUCED HEPATIC STEATOSIS AND INFLAMMATION

14 Monique Mulder (Rotterdam, The Netherlands)

24(S)-SARINGOSTEROL PREVENTS COGNITIVE DECLINE IN A MOUSE MODEL FOR ALZHEIMER'S DISEASE

15 Marion Mussbacher (Graz, Austria)

ROLE OF ADIPOSE TRIGLYCERIDE LIPASE IN THE MODULATION OF VASCULAR CONTRACTILITY BY PERIVASCULAR ADIPOSE TISSUE

16 Andreas Prokesch (Graz, Austria)

TARGETING METABOLIC FLEXIBILITY CONFERRED BY P53 IN HCC THERAPY

17 Isabel Reinisch (Graz, Austria) *

ADIPOCYTE p53 COORDINATES THE SYSTEMIC RESPONSE TO INTERMITTENT FASTING IN OBESE MICE

18 Frederike Sass (Copenhagen, Denmark) *

ADIPOSE DEPOT-SPECIFIC CONTROL OF LIPOLYSIS BY THE METABOLITE RECEPTORS GPR81 AND GPR109A

19 Gabriele Schoiswohl (Graz, Austria)

ADVANCED LIPODYSTROPHY REVERSES FATTY LIVER IN MICE LACKING ADIPOCYTE HSL

20 Maaike Straat (Leiden, The Netherlands) *

MILD COLD EXPOSURE CAUSES A UNIQUE DYNAMIC PATTERN IN CIRCULATING TRIACYLGLYCEROL LIPID SPECIES IN EUROPID AND SOUTH ASIAN INDIVIDUALS

21 Olivia Sveidahl Johansen (Copenhagen, Denmark) *

EXPLORING LIPOLYSIS-DEPENDENT EXPRESSION OF THE CONSTITUTIVELY ACTIVE RECEPTOR *GPR3*

22 Ulrike Taschler (Graz, Austria)

THE TISSUE-SPECIFIC CONTRIBUTION OF MONOGLYCERIDE LIPASE TO WHOLE BODY ENERGY HOMEOSTASIS

23 Robin van Eenige (Leiden, The Netherlands) *

INHIBITION OF ENDOCANNABINOID SYNTHESIS ENZYMES AS A NOVEL STRATEGY TO DECREASE CARDIOVASCULAR DISEASE RISK

24 Susanne van Wouw (Amsterdam, The Netherlands) *

MULTI-OMIC PROFILING OF LXR ACTIVATION IDENTIFIES SLPG AS A STEROL-INDUCIBLE LYSOPLASMALOGENASE IN MACROPHAGES

25 Yong Wang (Munich, Germany) *

ROLE OF MYELOID CANNABINOID RECEPTOR CB1 IN ATHEROGENESIS AND PROLIFERATION

26 Nienke Willemsen (Munich, Germany) *

REMODELLING OF THE PROTEASOME AS A KEY FEATURE OF BROWN ADIPOCYTE DIFFERENTIATION AND COLD ADAPTATION

27 Noam Zelcer (Amsterdam, The Netherlands)

THE MARCH6-SQLE AXIS CONTROLS ENDOTHELIAL CHOLESTEROL HOMEOSTASIS AND ANGIOGENESIS

28 Yiheng Zhang (Leiden, The Netherlands) *

PRMT4 INHIBITOR TP-064 IMPACTS BOTH METABOLIC AND INFLAMMATORY PROCESSES WITHOUT CHANGING ATHEROSCLEROSIS SUSCEPTIBILITY IN APOE-KNOCKOUT MICE

29 Francesca Zimetti (Parma, Italy)

PCSK9 IMPAIRS HDL-MEDIATED BRAIN CHOLESTEROL TRANSPORT FROM ASTROCYTES TO NEURONS, WITH POTENTIAL IMPLICATIONS IN ALZHEIMER'S DISEASE

** Eligible for Young Investigator Poster Award*

List of participants

Akhmetshina	Alena	alena.akhmetshina@medunigraz.at
Amigó	Núria	namigo@biosferteslab.com
Amor	Melina	melina.amor@medunigraz.at
Angelin	Bo	bo.angelin@ki.se
Bartelt	Alexander	alexander.bartelt@med.uni-muenchen.de
Bianco	Valentina	valentina.bianco@medunigraz.at
Bici	Ana	ana.bici@med.uni-muenchen.de
Bradić	Ivan	ivan.bradic@stud.medunigraz.at
Chen	Bingni	bingni.chen@med.uni-muenchen.de
Christoffersen	Christina	christina.christoffersen@regionh.dk
Coppi	Lara	lara.coppi@unimi.it
Corban	Carlotta	7032211@stud.uke.uni-hamburg.de
Dahik	Veronica	veronica.dahik@inserm.fr
Deng	Lei	lei.deng@wur.nl
Evangelakos	Giannis	john.evangelakos@gmail.com
Ferri	Nicola	nicola.ferri@unipd.it
Finger	Fabian	fabian.finger@sund.ku.dk
Frikke-Schmidt	Ruth	ruth.frikke-schmidt@regionh.dk
Gerhart-Hines	Zach	zgerhar2@gmail.com
Grabner	Gernot	gernot.grabner@uni-graz.at
Grefhorst	Aldo	a.grefhorst@amsterdamumc.nl
Heine	Markus	ma.heine@uke.de
Hendrix	Sebastian	s.hendrix@amsterdamumc.nl
Herzig	Stephan	stephan.herzig@helmholtz-muenchen.de
Hlushchenko	Iryna	iryna.hlushchenko@helsinki.fi
Hodson	Leanne	leanne.hodson@ocdem.ox.ac.uk
Hoekstra	Menno	hoekstra@lacdr.leidenuniv.nl
Hornemann	Thorsten	thorsten.hornemann@usz.ch
Jaeckstein	Michelle	m.jaeckstein@uke.de
Jodeleit	Henrika	henrika.jodeleit@med.uni-muenchen.de
Jouffe	Céline	celine.jouffe@helmholtz-muenchen.de
Karavaeva	Iuliia	iuliia@sund.ku.dk
Kersten	Sander	sander.kersten@wur.nl
Kjeldsen	Emilie	emilie.westerlin.kjeldsen@regionh.dk
Kooijman	Sander	s.kooijman@lumc.nl
Korbelius	Melanie	m.korbelius@medunigraz.at
Kratky	Dagmar	dagmar.kratky@medunigraz.at
Kuipers	Folkert	f.kuipers@umcg.nl
Kuivenhoven	Jan Albert	j.a.kuivenhoven@umcg.nl
Küntzel	Katharina Barbara	katharina.kuentzel@medunigraz.at
Laurila	Pirkka-Pekka	
Le Goff	Wilfried	wilfried.le_goff@sorbonne-universite.fr
Lemmer	Imke	imke.lemmer@med.uni-muenchen.de
Li	Rumei	r.li@umcg.nl
Liu	Cong	c.liu@lumc.nl
Maestri	Alice	alice.maestri@ki.se

List of participants

Martinez	Laurent	lorent.martinez@gmail.com
Metz	Sophia	sophia.metz@sund.ku.dk
Meurs	Amber	a.meurs@amsterdamumc.nl
Meyer	Simon	simomeyer.93@googlemail.com
Moustafa	Tarek	tarek.moustafa@medunigraz.at
Mulder	Monique	m.t.mulder@erasmusmc.nl
Muley	Carolin	carolin.muley@googlemail.com
Mussbacher	Marion	marion.mussbacher@uni-graz.at
Nilsson	Stefan	stefan@lipigon.se
Norata	Giuseppe Danilo	danilo.norata@unimi.it
Panteloglou	Grigorios	grigorios.panteloglou@usz.ch
Pedrelli	Matteo	matteo.pedrelli@ki.se
Pfisterer	Simon	simon.pfisterer@helsinki.fi
Ploug	Michael	m-ploug@finsenlab.dk
Prokesch	Andreas	andreas.prokesch@medunigraz.at
Reinisch	Isabel	isabel.reinisch@medunigraz.at
Rensen	Patrick	p.c.n.rensen@lumc.nl
Sardjoe Mishre	Aashley	asd.sardjoe@gmail.com
Sass	Frederike	frederike.sass@sund.ku.dk
Schoiswohl	Gabriele	gabriele.schoiswohl@uni-graz.at
Schönke	Milena	m.schoenke@lumc.nl
Shaul	Philip W.	philip.shaul@utsouthwestern.edu
Straat	Maaike	m.e.straat@lumc.nl
Sveidahl Johansen	Olivia	olivia.johansen@sund.ku.dk
Taschler	Ulrike	ulrike.taschler@uni-graz.at
Turri	Marta	marta.turri@unimi.it
Tybjærg Nordestgaard	Liv	liv_nordestgaard@hotmail.com
van de Sluis	Bart	a.j.a.van.de.sluis@umcg.nl
van Eenige	Robin	r.van_eenige@lumc.nl
van Wouw	Suzanne	s.a.vanwouw@amsterdamumc.nl
Van Zwol	Willemien	w.van.zwol@umcg.nl
Varret	Mathilde	mathilde.varret@inserm.fr
Verkade	Esther	e.verkade@umcg.nl
Verwilligen	Robin	r.a.f.verwilligen@lacdr.leidenuniv.nl
Vesely	Paul	paulwvesely@gmail.com
Vos	Dyonne	d.y.vos@umcg.nl
Wang	Yong	yong.wang@med.uni-muenchen.de
Willems van Dijk	Ko	k.willems_van_dijk@lumc.nl
Willemsen	Nienke	nienke.willemsen@live.nl
Wolfrum	Christian	christian-wolfrum@ethz.ch
Zeigerer	Anja	anja.zeigerer@helmholtz-muenchen.de
Zelcer	Noam	nzelcer@gmail.com
Zhang	Monica	monica1125zyh@gmail.com
Zimetti	Francesca	francesca.zimetti@unipr.it

Mission of ELC

The European Lipoprotein Club (ELC) was established in 1977 in order to "promote active participation, collaboration and exchange of ideas concerning basic and clinical research on the structure, function and metabolism of lipoproteins in health and disease".

The ELC currently includes more than 500 scientists from about 40 countries, and the number grows every year. ELC is thus a Europe-wide network of basic scientists and clinicians, with world-wide collaborations with colleagues actively involved in lipoprotein research. The network is strengthened and expanded by contacts established at the ELC meetings, and fostered by the exchange of ideas, clinical material, techniques, and young visiting investigators.

The ELC organizes one scientific meeting every year in September, with particular emphasis on an active participation and in-depth discussion in an informal atmosphere. The meetings have been hosted since 1980 at the Evangelische Akademie in Tutzing, Germany. An organizing committee comprising 11 members, representing as many different European countries as possible, arranges the meetings. These representatives are elected by the members and serve for 5 years. One representative acts as the chairman of the ELC, and has the major responsibility for the meeting organization. From 1980 to 2011, the local organization was in the hands of the late Dr. Joachim Ziegenhorn and his colleagues from Boehringer Mannheim/Roche. From 2012 to 2018 the local organization has been undertaken by Dr. Joachim Siedel.

The current chairman is Prof. Patrick Rensen, Leiden, The Netherlands. Prof. Dagmar Kratky, Graz, Austria, acts as the treasurer. Several topics, selected by the organizing committee, are discussed at each meeting, in working sessions spread over 4 days. Anyone interested and involved in research in the lipoprotein field is invited to submit an abstract for the meeting. Participating individuals are selected for oral or poster presentation based on the relevance and scientific merit of their abstracts. Because of the size of the auditorium, and in order to maintain an atmosphere conducive to active discussion, participants are limited to approximately 100.

For many years, the ELC has been generously supported by Boehringer Mannheim, to the extent that all housing, dining and other conference facilities were being taken care of. At the 1998 meeting, after the take-over of Boehringer Mannheim by Hoffmann-LaRoche, representatives from Roche have generously continued the support of the ELC until 2012. Since 2013 the European Atherosclerosis Society (EAS) has been a sustained and major sponsor of the meeting, which has also been supported generously by the Deutsche Forschungsgemeinschaft (DFG) approximately every other year. A limited participation fee - covering registration, housing, and full board throughout the meeting - is required provided acceptance of participation.

Contact information

ELC Website:	www.eas-society.org/elc_main
ELC Contact:	European Lipoprotein Club c/o EAS Office Tel: +46 (0) 31 760 24 27 Email: info@eas-elc.org
ELC Chairman:	Patrick Rensen, PhD Head Endocrinology Research Laboratory Leiden University Medical Center Dept. Medicine, Div. Endocrinology and Einthoven Laboratory for Experimental Vascular Medicine Room C-07-47 Albinusdreef 2 / 2333 ZA Leiden P.O. Box 9600 / 2300 RC Leiden The Netherlands

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