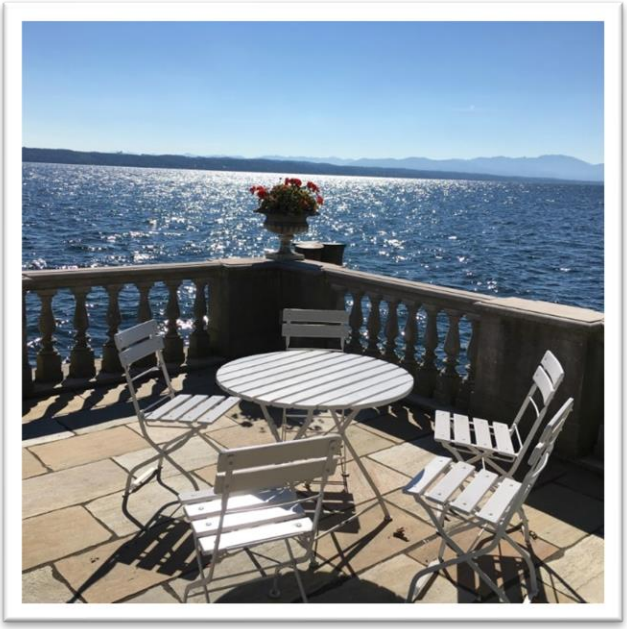
Program and Abstracts

European Lipoprotein Club

43rd Annual Scientific Meeting



September 07-10, 2020

Evangelische Akademie

Tutzing, Germany

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Contents

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Monday Sep 07

12:00 - 14:00	**Arrival, registration & snacks**
14:00 - 14:05	**Welcome** *Patrick Rensen (Leiden, The Netherlands)*
Session:	**Liver & Steatosis**
	Chairperson: Dagmar Kratky (Graz, Austria)
14:05 - 14:45	**Invited speaker: Henriette Uhlenhaut (Munich, Germany)** Genomic control of lipid metabolism by glucocorticoids
14:45 - 15:00	**Noam Zelcer (Amsterdam, The Netherlands)** Haploid genetic screens identify SPRING/C12ORF49 as a determinant of SREBP signaling and cholesterol metabolism
15:00 - 15:15	**Vinay Sachdev (Amsterdam, The Netherlands) *** The ERAD E3 ubiquitin ligase MARCH6 couples SREBP1-mediated hepatic lipid metabolism and fatty liver development
15:15 - 15:30	**Dyonne Vos (Groningen, The Netherlands) *** Retriever dictates selective endosomal recycling of lipoprotein receptors
15:30 - 15:45	**Andries Heida (Groningen, The Netherlands) *** Hyperactivation of the IKK-NFKB axis in hepatocytes exacerbates hepatic lipid accumulation
15:45 - 16:00	**Jan Albert Kuivenhoven (Groningen, The Netherlands)** SMLR1, a new player in hepatic VLDL metabolism
16:00 - 16:30	**Coffee break**
Session:	**Cells & CVD**
	Chairperson: Thorsten Hornemann (Zurich, Switzerland)
16:30 - 16:45	**Marit Westerterp (Groningen, The Netherlands)** Macrophage cholesterol efflux pathways suppress NETosis in atherosclerotic lesions
16:45 - 17:00	**Petra Frager (Barcelona, Spain) *** The role of hepatic TP53INP2 in cholesterol metabolism
17:00 - 17:15	**Lisa-Maria Pusch (Graz, Austria) *** ABHD13: a novel lysophospholipase

** Eligible for Young Investigator Oral Award*

Monday Sep 07

17:15 - 17:30	**Anouk La Rose (Groningen, The Netherlands) *** Smooth muscle cell cholesterol efflux pathways regulate vasoconstriction
17:30 - 17:45	**Magalie Lambert (Strasbourg, France) *** WNT5A signalling promotes cholesterol trafficking and protects against atherosclerosis
17:45 - 18:00	**Ivan Bradić (Graz, Austria) *** ATG7 and ATG3 are dispensible for LC3-II formation in thioglycolate-elicited peritoneal macrophages
18:00 - 18:15	**Robin Verwilligen (Leiden, The Netherlands) *** Absence of scavenger receptors stab1 and stab2 predisposes to macrophage foam cell formation without breaking the atherosclerosis resistance of zebrafish
18:15 - 18:30	**Venetia Bazioti (Groningen, The Netherlands) *** T-cell cholesterol efflux pathways are crucial for maintaining peripheral T-cell levels and do not affect atherogenesis
18:30 - 19:30	**Dinner**
20:00 - 21:00	**Keynote Lecture** *Chairperson: Patrick Rensen (Leiden, The Netherlands)* **Anne Tybjaerg-Hanssen (Copenhagen, Denmark)** Reducing residual cardiovascular risk beyond LDL cholesterol: targets, genes, and drugs
21:00 - late	**Bar open**

** Eligible for Young Investigator Oral Award*

Tuesday Sep 08

07:30 - 08:30	**Breakfast**
Session:	**Brown Fat & Other Tissues**
	Chairperson: Alexander Bartelt (Munich, Germany)
08:30 - 08:45	**Christian Wolfrum (Zurich, Switzerland)** GPR180, a novel TGFβ family receptor, is indispensable for brown adipocyte function
08:45 - 09:00	**Stefan Kotschi (Munich, Germany) *** Cold-activated mitohormesis in brown fat is mediated by NFE2L2
09:00 - 09:15	**Melanie Modder (Leiden, The Netherlands) *** A single day of high fat diet feeding induces lipid accumulation and insulin resistance in brown adipose tissue in mice
09:15 - 09:30	**Nienke Willemsen (Munich, Germany) *** Constructing the brown fat proteasome in non-shivering thermogenesis
09:30 - 09:45	**Renate Schreiber (Graz, Austria)** What ignites UCP1? Characterization of brown adipocyte-specific ATGL and HSL knockout mice
09:45 - 10:00	**Isabel Reinisch (Graz, Austria) *** p53 in the regulation of fructose metabolism in brown adipocytes under nutrient deprivation
10:00 - 10:15	**Robin van Eenige (Leiden, The Netherlands) *** Concomitant GIPR and GLP1R agonism stimulates VLDL-triglyceride turnover and attenuates atherosclerosis development
10:15 - 10:30	**Sander Kooijman (Leiden, The Netherlands)** Circadian control of brown adipose tissue activity by glucocorticoids
10:30 - 11:00	**Coffee break**
11:00 - 11:40	**Invited speaker: Zach Gerhart-Hines (Copenhagen, Denmark)** Noncanonical signaling pathways that control activation of thermogenic adipose

** Eligible for Young Investigator Oral Award*

Tuesday Sep 08

11:40 - 11:55	**Sajjad Khani (Munich, Germany) *** Epigenetic regulation of the Nfe2L1-proteasome pathway in oxidative metabolic tissues
11:55 - 12:10	**Imke Lemmer (Munich, Germany) *** Nfe2l1 drives proteostasis in myocytes and skeletal muscle
12:10 - 12:25	**Henrika Jodeleit (Munich, Germany)** Induction of Nfe2l1-mediated proteasomal activity in myocardial infarction
12:25 - 12:40	**Melanie Korbelius (Graz, Austria) *** Effects of intestinal ATGL overexpression: the good or the evil?
12:40 - 12:55	**Katharina Barbara Kuentzel (Graz, Austria) *** How does the loss of intracellular lipases affect mouse placenta and fetus?
13:00 - 14:00	**Lunch**
14:00 - 16:00	**Networking** (**OC Meeting**)

Session: **Lifestyle & Cardiometabolic Health**

Chairperson: Kevin Jon Williams (Philadelphia, USA)

16:00 - 16:15	**Maaike Schilperoort (Leiden, The Netherlands) *** Disruption of circadian rhythm by alternating light-dark cycles aggravates atherosclerosis development
16:15 - 16.30	**Pirkka-Pekka Laurila (Lausanne, Switzerland)** Inhibition of sphingolipid de novo synthesis counteracts age-related loss of fitness
16:30 - 16:45	**Borja Martinez-Tellez (Leiden, The Netherlands) *** Moderate exercise training decreases circulating endocannabinoids and n6-PUFA oxylipins in young healthy adults as associated with improved cardiometabolic health
16:45 - 17:00	**Pär Björklund (Stockholm, Sweden) *** Characterization of lipoproteins in interstitial fluid of in type 2 diabetes: indirect evidence of increased LDL aggregation capacity contributing to accelerated atherosclerosis

** Eligible for Young Investigator Oral Award*

Tuesday Sep 08

17:00 - 17:15	**Alexandre Motte (Paris, France) *** Reduced reverse cholesterol transport efficacy in healthy men with undesirable postprandial triglyceride response
17:15 - 17:30	**Vasily Sukhorukov (Moscow, Russian Federation) *** Mutational burden of mitochondrial DNA and atherosclerosis
17:30 - 17:45	**Liv Tybjærg Nordestgaard (Copenhagen, Denmark) *** Plasma levels of triglycerides and risk of dementia
17:45 - 18:00	**Bart van de Sluis (Groningen, The Netherlands)** Understanding the role of a liver-specific Hep-lncRNA in NASH progression
18:00 - 19:00	**Dinner**
19:00 - 21.00	**Wine and Science**
21:00 - late	**Bar open**

** Eligible for Young Investigator Oral Award*

Wednesday Sep 09

07:30 - 08:30	**Breakfast**
Session:	**Genes & Therapy**
	Chairperson: Mathilde Varret (Paris, France)
08:30 - 08:45	**Marie Wikström Lindholm (Berlin, Germany)** A novel short interfering ribonucleic acid to LPA induces potent and sustained reduction of serum lipoprotein (a) in cynomolgus monkeys
08:45 - 09:00	**Yara Abou Khalil (Paris, France) *** PCSK9 in the development of human atherosclerosis
09:00 - 09:15	**Yara Azar (Paris, France) *** Molecular spectrum of PCSK9-based FH in France, the French P.(Ser127Arg) founder variant
09:15 - 09:30	**Xavier Vanhoye (Lyon, France) *** Evaluation of polygenic scores in the diagnosis of familial hypercholesterolemia and hypobetalipoproteinemia
09:30 - 09:45	**Natalia Loaiza (Groningen, The Netherlands) *** Liver-specific Gpr146 downregulation attenuates dyslipidemia in APOE*3-Leiden.CETP mice
09:45 - 10:00	**Aldo Grefhorst (Amsterdam, The Netherlands)** Statins suppress hepatic secretion of ANGPTL3 via reduced liver X receptor (LXR) activation
10:00 - 10:15	**Michael Ploug (Copenhagen, Denmark)** ANGPTL4 regulates LPL activity by catalyzing the unfolding of LPL monomers
10:15 - 11:00	**Coffee break**

** Eligible for Young Investigator Oral Award*

Wednesday Sep 09

Session: **Adipogenesis & weight loss**

Chairperson: Stefan Nilsson (Umeå, Sweden)

11:00 - 11:15	**Maude Giroud (Munich, Germany)** HAND2 regulates adipogenesis via the glucocorticoid signaling pathway
11:15 - 11:30	**Cristy Verzijl (Groningen, The Netherlands) *** A role for GalNac-T2 dependent glycosylation in the regulation of insulin sensitivity
11:30 - 11:45	**Janina Caesar (Munich, Germany) *** NFE2L1 protects white adipocytes from cholesterol toxicity to maintain proteostasis
11:45 - 12:00	**Cong Liu (Leiden, The Netherlands) *** Pharmacological treatment with fibroblast growth factor 21 strongly improves plasma cholesterol metabolism to reduce atherosclerosis
12:00 - 12:15	**Antoine Rimbert (Nantes, France)** Genetic inhibition of GPR146 is associated with improved cardiovascular and metabolic health
12:15 - 12:30	**Kevin Jon Williams (Philadelphia, USA)** A randomized controlled trial of an innovative, user-friendly, interactive smartphone app-based lifestyle intervention for weight loss
12:30 - 13:30	**Lunch**
13:30 - 15.00	**Networking**

Session: **HDL & NMR**

Chairperson: Christina Christoffersen (Copenhagen, Denmark)

15:00 - 15:15	**Laurent Martinez (Toulouse, France)** Serum level of HDL particles are independently associated with long-term prognosis in patients with coronary artery disease

** Eligible for Young Investigator Oral Award*

Wednesday Sep 09

15:15 - 15:30	**Grigorios Panteloglou (Zurich, Switzerland)** The coatomer (COP I) complex limits uptake of both LDL and HDL in hepatocytes in vitro but regulates HDL-cholesterol levels only
15:30 - 15:45	**Núria Amigo (Reus, Spain)** NMR-based lipoprotein and glycoprotein profiling improves traditional cardiovascular risk-based prediction in type 2 diabetic subjects: the LIPOCAT study
15:45 - 16:00	**Ko Willems van Dijk (Leiden, The Netherlands)** Differential insulin sensitivity of NMR-based biomarkers in a two-step hyperinsulinemic euglycemic clamp protocol
16:00 - 17:00	**General Assembly & YI Awards**
18:30 - 22.00	**Fancy dinner**
22:00 - late	**Bar open**

Thursday Sep 10

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

Presenters eligible for YIA

In order of appearance in the program:

Vinay Sachdev (Amsterdam, The Netherlands)
Dyonne Vos (Groningen, The Netherlands)
Andries Heida (Groningen, The Netherlands)
Petra Frager (Barcelona, Spain)
Lisa-Maria Pusch (Graz, Austria)
Anouk La Rose (Groningen, The Netherlands)
Magalie Lambert (Strassbourg, France)
Ivan Bradić (Graz, Austria)
Robin Verwilligen (Leiden, The Netherlands)
Venetia Bazioti (Groningen, The Netherlands)
Stefan Kotschi (Munich, Germany)
Melanie Modder (Leiden, The Netherlands)
Nienke Willemsen (Munich, Germany)
Isabel Reinisch (Graz, Austria)
Robin van Eenige (Leiden, The Netherlands)
Sajjad Khani (Munich, Germany)
Imke Lemmer (Munich, Germany)
Melanie Korbelius (Graz, Austria)
Katharina Barbara Kuentzel (Graz, Austria)
Maaike Schilperoort (Leiden, The Netherlands)
Borja Martinez-Tellez (Leiden, The Netherlands)
Pär Björklund (Stockholm, Sweden)
Alexandre Motte (Paris, France)
Vasily Sukhorukov (Moscow, Russian Federation)
Liv Tybjærg Nordestgaard (Copenhagen, Denmark)
Yara Abou Khalil (Paris, France)
Yara Azar (Paris, France)
Xavier Vanhoye (Lyon, France)
Natalia Loaiza (Amsterdam, the Netherlands)
Cristy Verzijl (Groningen, The Netherlands)
Janina Caesar (Munich, Germany)
Cong Liu (Leiden, The Netherlands)

HAPLOID GENETIC SCREENS IDENTIFY SPRING/C12ORF49 AS A DETERMINANT OF SREBP SIGNALING AND CHOLESTEROL METABOLISM

Noam Zelcer

Academic Medical Center of the University of Amsterdam, Amsterdam, Netherlands.

Aim: The sterol-regulatory element binding proteins (SREBP) are central transcriptional regulators of lipid metabolism. In this study we aimed to identify novel physiologic regulators of SREBP signaling and cholesterol metabolism.

Method: To identify novel SREBP regulators we applied mammalian haploid genetic screens, a powerful genome-wide unbiased approach. Subsequently, gain- and loss- of function approaches were applied to identified candidates in cell models and in mice.

Results: Using haploid genetic screens we identified the SREBP Regulating Gene (SPRING/C12ORF49) as a determinant of the SREBP pathway. SPRING is a glycosylated Golgi-resident membrane protein and its ablation in Hap1 cells, Hepa1-6 hepatoma cells, and primary murine hepatocytes reduces SREBP signaling, cholesterol synthesis, and LDL uptake into cells. In mice, Spring deletion is embryonic lethal yet silencing of hepatic Spring expression also attenuates the SREBP response. Mechanistically, attenuated SREBP signaling in SPRING-KO cells results from reduced SREBP cleavage-activating protein (SCAP) and its mislocalization to the Golgi irrespective of the cellular sterol status. Consistent with limited functional SCAP in SPRING-KO cells, reintroducing SCAP restores SREBP-dependent signaling, cholesterol synthesis, and LDL uptake. Moreover, in line with the role of SREBP in tumor growth, a wide range of tumor cell lines display dependency on SPRING expression. Our ongoing studies on SPRING will be further presented.

Conclusions: Our study highlights the potential of using mammalian haploid genetic screens to interrogate lipid metabolism and identifies SPRING as a previously unrecognized physiologic modulator of SREBP signaling.

THE ERAD E3 UBIQUITIN LIGASE MARCH6 COUPLES SREBP1-MEDIATED HEPATIC LIPID METABOLISM AND FATTY LIVER DEVELOPMENT

Vinay Sachdev[1], Nienke van Loon[1], Jenina Kingma[1], Suzanne Duist[2], Jan F. de Boer[3], Marleen Van den Berg[1], Sander Kooijman[4], Folkert Kuipers[3], Patrick C.N. Rensen[4], Noam Zelcer[1]

[1]Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. [2]Tytgat Institute for Liver and Intestinal Research, ,Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands. [3]Departments of Pediatrics & Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, Netherlands. [4]Department of Medicine, Division of Endocrinology and Einthoven Laboratory for Experimental Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Netherlands.

Aim: The pathophysiology of fatty liver disease has many links with dyslipidemia. Using a genetic approach, we have recently identified the ER-resident E3 ubiquitin ligase membrane-associated ring-CH-type finger 6 (MARCH6) as a posttranscriptional regulator of the rate-limiting enzyme in cholesterol biosynthesis, Squalene epoxidase. The physiological role of MARCH6 in lipid and lipoprotein metabolism is unknown.

Method: We developed mouse models with constitutive or conditional ablation of March6 to study physiological role of MARCH6 *in vivo*. Metabolic characterization and functional studies were performed. To identify altered metabolic processes we combined global transcriptomic and lipidomic analysis, and studies in primary hepatocytes.

Results: March6$^{(-/-)}$ mice were born at a sub-Mendelian ratio precluding their study. We therefore studied mice with liver-specific ablation of March6 (HEP-M6) and their respective control (HEP-WT). Phenotypical characterization of these mice revealed the striking abundance of lipid droplets and TG accumulation in livers of HEP-M6 mice. Circulating plasma TGs were slightly reduced in HEP-M6 mice even though whole-body energy metabolism and hepatic VLDL turnover were comparable between two groups. RNAseq analysis of the hepatic transcriptome and lipidomic analysis revealed markedly increased fatty acid saturation and synthesis in livers of HEP-M6 mice. Stable 13-C isotope studies confirmed increased *de novo* lipogenesis in the livers of HEP-M6 mice. In line with these findings, March6 loss led to activation of the SREBP1-mediated fatty acid synthesis program in vivo and in isolated primary hepatocytes. Mechanistically, our results indicate a critical role of MARCH6 in regulating SREBP1 signaling.

Conclusions: Our study uncovers an unrecognized MARCH6-SREBP1 axis as a central determinant of hepatic lipid metabolism and highlights the multifaceted role of MARCH6 in regulation of whole-body lipid homeostasis.

Eligible for Young Investigator Oral Award

RETRIEVER DICTATES SELECTIVE ENDOSOMAL RECYCLING OF LIPOPROTEIN RECEPTORS

Dyonne Vos, Marieke Smit, Niels Kloosterhuis, Nicolette Huijkman, Ydwine van der Veen, Karin Wolters, Jan Albert Kuivenhoven, Bart van de Sluis

Department of Pediatrics, University of Groningen, University Medical Center Groningen, Groningen, Netherlands.

Aim: Recently, we showed that the CCC and WASH complexes coordinate the endosomal transport of LDLR, LRP1 and SR-BI. Hepatic loss of any of these two complexes results in impaired recycling of these lipoprotein receptors and, subsequently, in hypercholesterolemia in mice and humans. Retriever, a multiprotein complex, has recently been linked to CCC and WASH in order to regulate receptor recycling. However, the contribution of retriever to lipoprotein receptor recycling remains unknown. Here, we studied the role of retriever in endosomal transport of lipoprotein receptors and eventually in the regulation of plasma lipid levels.

Method: Retriever component *Vps26C* was ablated in Hepa1-6 cells using CRISPR/Cas9 technology. For *in vivo* studies, somatic CRISPR/Cas9 gene editing was used to generate hepatic VPS26C-deficient mice. To address the role of retriever specifically in regulating LRP1 functioning, we blunted LDLR levels by overexpressing a human gain-of-function variant of PCSK9 (D377Y) in livers of wildtype and VPS26C-deficient mice. Plasma cholesterol concentrations were measured and the lipoprotein profiles were determined by FPLC. Proteomics and western blot analysis were used to study the protein expression of the different protein complexes, LDLR, LRP1 and SR-BI.

Results: We found that ablation of VPS26C in Hepa1-6 cells significantly reduced total and cell surface LRP1 protein levels. Hepatic loss of retriever did not affect plasma lipid levels, but markedly increased hepatic LDLR protein levels without affecting LRP1 levels. Hepatic VPS26C editing resulted in increased plasma cholesterol levels in PCSK9-induced LDLR-deficient mice when compared with mice only lacking LDLR.

Conclusions: Our data indicate that retriever is a specific determinant in the WASH-CCC axis to coordinate selective endosomal recycling of lipoprotein receptors.

Eligible for Young Investigator Oral Award

HYPERACTIVATION OF THE IKK-NFKB AXIS IN HEPATOCYTES EXACERBATES HEPATIC LIPID ACCUMULATION

Andries Heida[1], Nanda Gruben[1], Marieke Smit[1], Niels Kloosterhuis[1], Rick Havinga[1], Theo van Dijk[1], Jan Freark de Boer[1], Alain de Bruin[2], Folkert Kuipers[3], Debby Koonen[1], Bart van de Sluis[1]

[1]UMCG, Groningen, Netherlands. [2]UU, Utrecht, Netherlands. [3]UMCG, Utrecht, Netherlands.

Aim: The pro-inflammatory signaling pathway NF-κB plays a central role in the progression of non-alcoholic steatohepatitis (NASH), but its contribution to the development of simple steatosis remains unclear. In this study, we investigated the role of the NF-κB signaling pathway in liver steatosis.

Method: To study the effect of NF-κB activation on hepatic lipid accumulation we used a mouse model expressing a constitutively active form of IKKβ in hepatocytes (*Ikkβca*^{Hep}). IKKβ is a component of the IKK complex, a central regulator of NF-κB activation. In addition, IKKβca was also expressed in a hepatic *A20* knockout background (*Ikkβca*^{Hep}*;A20*^{LKO}). *A20* is a NF-κB-target gene and negatively regulates NF-κB activation. These mouse models were fed a sucrose-rich diet for 8 weeks. Hepatic lipid levels were measured and using C^{13} labelled acetate de novo lipogenesis and cholesterol synthesis rate were determined. Gene expression analyses and immunoblotting were used to study the lipogenesis and cholesterol synthesis pathways.

Results: Expression of IKKβca augmented hepatic accumulation of triglycerides but, surprisingly, not the level of liver inflammation. Hepatic expression of *A20* was highly induced in *Ikkβca*^{Hep} mice and to assess whether elevated A20 levels impede hepatic inflammation, we ablated *A20* in hepatocytes of *Ikkβca*^{Hep} mice. Hepatic A20-deficiency in *Ikkβca*^{Hep} mice did not increase liver inflammation but it exacerbated the hepatic cholesterol and triglyceride concentrations. In addition to the elevated hepatic lipid levels, plasma cholesterol levels were increased in *Ikkβca*^{Hep}*;A20*^{LKO} mice when compared with wildtype mice. Although, the expression of lipogenic and cholesterolgenic genes were not markedly affected, we found that de novo lipogenesis and cholesterol synthesis rate were elevated upon NF-kB activation.

Conclusions: Hepatic activation of the NF-κB signaling pathway increases de novo lipogenesis and cholesterol synthesis implying that chronic activation of NF-κB in hepatocytes may contribute to early stage of NAFLD.

Eligible for Young Investigator Oral Award

SMLR1, A NEW PLAYER IN HEPATIC VLDL METABOLISM

Willemien van Zwol[1], Antoine Rimbert[2], Marieke Smit[1], Karin Wolters[1], Vincent Bloks[1], Niels Kloosterhuis[1], Nicolette Huykman[1], Bart van de Sluis[1], Philip Zimmermann[3], Jan Albert Kuivenhoven[1]

[1]UMCG, Groningen, Netherlands. [2]University of Nantes, Nantes, France. [3]Nebion Ltd, Zurich, Switzerland.

Aim: We identified a novel gene *SMLR1,* encoding for small leucin-rich protein 1, to be tightly co-regulated with *APOB*, *MTTP*, and *APOC3*, genes with central roles in the metabolism of apolipoprotein B-containing lipoproteins. There is no information on SMLR1 in the public domain. This study aims at validating *SMLR1* as a lipid gene.

Method: The effects of hepatic (CRISPR/Cas9-mediated) downregulation of *Smlr1* was studied in mice fed a chow diet. Protein levels were measured with LC-MS based proteomics. Liver lipids were extracted according to Bligh & Dyer. VLDL secretion rate was studied after intraperitoneally injection of poloxamer.

Results: Downregulation of *Smlr1* by approximately 70% (mRNA and protein level) in the liver resulted in a marked reduction of plasma cholesterol and triglycerides levels in plasma (50%, $p<0.001$), and increased hepatic cholesterol and triglyceride levels ($p<0.001$ for both). In line, a 45% reduction in the VLDL secretion rate was observed. Lipoprotein cholesterol profiling of plasma samples revealed reduced cholesterol in both LDL as HDL.

Conclusions: This study provides evidence that *SMLR1*, encoding for a protein of unknown function, plays a role in plasma and hepatic lipid metabolism. Hepatic downregulation in mice results in a phenotype that is remarkably close to the phenotype of liver-specific *Mttp-/-* mice (PMID: 10225972). We currently focus on the biological function of *Smlr1* through cellular localization studies and analysis of pathways involved in VLDL assembly and secretion.

MACROPHAGE CHOLESTEROL EFFLUX PATHWAYS SUPPRESS NETOSIS IN ATHEROSCLEROTIC LESIONS

Panagiotis Fotakis[1], Emily Dou[1], Sandra Abramowicz[1], Nan Wang[1], Alan Tall[1], Marit Westerterp[2,1]

[1]*Columbia University, New York, USA.* [2]*University of Groningen, Groningen, Netherlands.*

Aim: Neutrophils are the most abundant white blood cells and form the first line of host defense. To trap bacteria, neutrophils actively release extracellular net-like structures (NETosis), containing DNA, histones, and granular proteins. Recent studies have shown that NETs are also present in atherosclerotic plaques, where they increase plaque vulnerability. Mechanisms for NETosis in atherosclerotic plaques are poorly understood. *In vitro* studies have shown that inflammasome activation in neutrophils leads to NETosis. Inflammasomes are activated by excessive cholesterol accumulation and regulate secretion of pro-atherogenic interleukin-1b (IL-1b) and IL-18. ATP Binding Cassette A1 and G1 (ABCA1/ABCG1) mediate cholesterol efflux to HDL, and *Abca1/Abcg1* deficiency in neutrophils leads to excessive cholesterol accumulation. We examined the role of cholesterol efflux pathways in NETosis.

Method: Mice with neutrophil or macrophage *Abca1/Abcg1* deficiency were generated by crossbreeding *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice with *S100A8Cre* or *CX3CR1Cre* mice, respectively. We carried out bone marrow transplantation into *Ldlr*$^{-/-}$ mice, and fed mice Western-type diet (WTD). Neutrophils were isolated from bone marrow for *in vitro* experiments.

Results: Neutrophil *Abca1/Abcg1* deficiency increased NETosis in *in vitro* assays. However, it did not affect inflammasome activation or NETosis in atherosclerotic lesions of *Ldlr*$^{-/-}$ mice, suggesting that an additional stimulus may be required for neutrophil infiltration and NETosis in plaques. Interestingly, macrophage *Abca1/Abcg1* deficiency increased plasma IL-18 levels and caspase-1 cleavage in macrophages and neutrophils, reflecting inflammasome activation in both myeloid cell types. After 8 weeks of WTD, mice with macrophage *Abca1/Abcg1* deficiency, but not controls, showed prominent NETosis in atherosclerotic plaques. These data reveal a previously unrecognized role for macrophage cholesterol efflux pathways in suppressing NETosis in atherosclerotic plaques, likely downstream of inflammasome activation.

Conclusions: Increased cholesterol content in macrophages, but not neutrophils, promotes neutrophil inflammasome activation and NETosis in atherosclerotic plaques, which may enhance plaque vulnerability.

THE ROLE OF HEPATIC TP53INP2 IN CHOLESTEROL METABOLISM

Petra Frager

IRB Barcelona, Barcelona, Spain. University of Barcelona, Barcelona, Spain.

Aim: The liver, a central organ of cholesterol metabolism, is crucial for coordination of biosynthesis, uptake and secretion of cholesterol in form of lipoproteins, excretion and esterification in order to achieve homeostasis. This balance is progressively challenged by unhealthy lifestyles characterized by diminished physical activity as well as consumption of a "westernized diet" rich in calories and saturated fats. This development demands better understanding of the underlying pathological mechanisms. The objective of this study is to determine the impact of TP53INP2 in hepatic cholesterol metabolism.

Method: Liver-specific TP53INP2 knock-out mice were generated using the Cre/loxP system. Mice were subjected to a high fat diet-treatment. Body composition was analyzed using EchoMRI. Glucose tolerance tests and oral lipid tolerance tests were performed. Livers were examined histologically, and lipids were extracted for calorimetric quantification. Gene expression was measured by RNAseq and qPCR, protein expression by Western blot. Lipoprotein levels were determined biochemically and by 2D Nuclear Magnetic Resonance Spectroscopy. Autophagic flux was evaluated in primary hepatocytes using Bafilomycin A1. Cholesterol and neutral lipids of primary hepatocytes were stained with Filipin and Bodipy and analyzed using confocal microscopy.

Results: Liver-specific TP53INP2 knock-out mice showed increased body and liver weight upon high fat diet-treatment. Under these conditions, knock-out mice showed glucose intolerance and high plasma insulin levels coherent with enhanced insulin resistance. Hepatic cholesterol content and neutral lipid content were elevated. Plasma analysis by NMR showed an aberrant lipoprotein profile after 8 weeks of HFD and elevated HDL-Cholesterol levels after 16 weeks of high fat diet, corroborated by higher hepatic ApoA-I expression.

Conclusions: Here we show a novel role of TP53INP2, a protein previously described as a positive regulator of autophagy, in hepatic cholesterol metabolism. Liver-specific depletion of TP53INP2 elevates hepatic cholesterol levels and alters plasma cholesterol levels. Interestingly, hepatic expression of TP53INP2 is regulated by nutritional status.

Eligible for Young Investigator Oral Award

ABHD13: A NOVEL LYSOPHOSPHOLIPASE

Lisa-Maria Pusch[1], Ulrike Taschler[1,2], Heimo Wolinski[1], Monika Oberer[1,2,3], Gernot Grabner[1], Robert Zimmermann[1,2,3]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria. [2]SFB Lipid Hydrolysis, Graz, Austria. [3]BioTechMed Graz, Graz, Austria.

Aim: The α/β hydrolase domain containing protein (ABHD) family comprises 22 members. Many of the family members are capable of hydrolyzing neutral or polar lipids. However, despite their highly conserved structure, several members of this family remained uncharacterized. Our aim was to identify and characterize new lipid hydrolases of the ABHD family.

Method: To identify new lipid hydrolases, we established activity screening assays with physiological lipid substrates. To characterize identified enzymes in more detail, we performed structural analysis, purified enzymes, optimized reaction conditions, determined substrate selectivity, and investigated its subcellular localization by laser scanning microscopy and membrane protection assays.

Results: Our screening assays identified the so far uncharacterized protein ABHD13 as lysophospholipid and monoacylglycerol hydrolase showing highest activity for lysophosphatidic acid. ABHD13 exhibits maximal activity at neutral pH and in the presence of the bipolar detergent CHAPS. Mutation of the active serine 193 completely depletes enzyme activity. ABHD13 co-localizes with the ER, and membrane protection assays indicated a sub-organelle localization in the ER lumen. Structural analysis predicts a short lipophilic cap domain that could be important for dimerization, for interaction with membranes, and/or for the recruitment of substrates. We observed that mutations in this domain lead to a reduced activity but retain its localization at the ER, suggesting that the cap domain is important for substrate binding but not for ER localization.

Conclusions: Altogether, our data suggest that ABHD13 acts as lysophospholipase in the ER lumen. Ongoing studies with ABHD13 knock out mice will reveal first insights into the physiological function of the enzyme.

Eligible for Young Investigator Oral Award

SMOOTH MUSCLE CELL CHOLESTEROL EFFLUX PATHWAYS REGULATE VASOCONSTRICTION

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Aim: Previous studies have shown that cholesterol efflux pathways mediated by ATP Binding Cassette A1 and G1 (ABCA1 and ABCG1) maintain vascular function by preserving endothelial vasorelaxation. ABCA1 and ABCG1 mediate cholesterol efflux to apolipoprotein A1 and HDL, respectively. Whether these pathways also regulate vasoconstriction is unknown. *In vitro* studies have shown that membrane cholesterol accumulation enhances signaling downstream of the α_1-adrenergic receptor. This receptor is highly expressed in smooth muscle cells (SMCs), and regulates vasoconstriction. We investigated the role of SMC cholesterol efflux pathways in vasoconstriction and atherogenesis.

Method: We generated *Myh11Cre^{ERT2}Abca1$^{fl/fl}$Abcg1$^{fl/fl}$Ldlr$^{-/-}$* mice, and *Myh11Cre^{ERT2}Ldlr$^{-/-}$* and *Abca1$^{fl/fl}$Abcg1$^{fl/fl}$Ldlr$^{-/-}$* controls. We induced SMC *Abca1/Abcg1* deficiency by tamoxifen-diet feeding. Mice were then fed Western-type diet (WTD) for 16 weeks. We studied vasoconstriction using myography.

Results: After WTD feeding, SMC *Abca1/Abcg1* deficient *Ldlr$^{-/-}$* mice, but not controls, showed prominent Oil Red O staining in the media of the thoracic aorta, reflecting SMC lipid accumulation. To examine the role of SMC *Abca1/Abcg1* deficiency in vasoconstriction, thoracic aortic rings were isolated, and subjected to increasing concentrations of phenylephrine (PE). SMC *Abca1/Abcg1* deficiency increased PE-induced vasoconstriction by 2-fold. Acetylcholine or sodium nitroprusside-induced vasorelaxation were not affected. Pre-incubation with cyclodextrin reversed the PE-induced vasoconstriction in aortic rings from mice of both genotypes, reflecting dependence on membrane cholesterol accumulation. SMC *Abca1/Abcg1* deficiency also induced severe enlargement of the urinary bladder. This may have been due to constriction of SMCs in the urethra or prostate where the PE-sensitive α_1-adrenergic receptor is highly expressed. The role of SMC cholesterol efflux pathways in atherogenesis is still under investigation.

Conclusions: These studies demonstrate that SMC cholesterol efflux pathways suppress PE-induced vasoconstriction in the aorta and likely also the prostate and urethra. These studies thus reveal a novel mechanism for regulation of vasoconstriction by membrane cholesterol accumulation in SMCs *in vivo*.

Eligible for Young Investigator Oral Award

WNT5A SIGNALING PROMOTES CHOLESTEROL TRAFFICKING AND PROTECTS AGAINST ATHEROSCLEROSIS

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Aim: Accumulation of cholesterol in the arterial wall is a key feature of atherosclerosis. Intimal infiltration of macrophages and vascular smooth muscle cells (VSMCs) loaded with cholesterol forms foam cells and arterial plaques. Severe complications as coronary artery disease or stroke can occur with the rupture of these plaques. We previously reported that Wnt5a, a Wnt ligand limit cholesterol accumulation in fibroblasts and adipose tissue of mice by reducing cholesterol synthesis and promoting its export (El Asmar *et al*, 2016, Zhou *et al*, 2009). Here we studied whether Wnt5a also protects against atherosclerosis.

Method: For this, we generated mice deleted for Wnt5a in VSMCs and fed them with a cholesterol-rich diet for 5 months.

Results: Mutant mice developed 100% more atherosclerotic lesions than controls. Histological and biochemical analysis showed that Wnt5a -/- VSMCs accumulated large amount of total cholesterol and cholesteryl-esters in enlarged endosome/lysosomes (LELs). The absence of Wnt5a in VSMCs also resulted in activation of the mechanistic target of rapamycin complex 1 (mTORC1), a pathway known to drive lysosomal function and promotes cholesterol trafficking. By decreasing mTORC1 activity, Wnt5a senses lysosomal cholesterol levels and promotes its trafficking to the endoplasmic reticulum (ER) where it can regulate its own biosynthesis. In addition, Wnt5a binds to cholesterol-enriched membranes, is associated with LDL particles, and interacts with Niemann-Pick C1 (NPC1) and Niemann-Pick C2 (NPC2), two lysosomal proteins that regulate cholesterol egress from LELs.

Conclusions: Thus, Wnt5a promotes lysosomal function, cholesterol trafficking and protects against atherosclerosis. These results reveal an unexpected function of the Wnt5a pathway and provide a conceptually new basis for future drug development to prevent cholesterol accumulation, atherosclerosis and lysosomal storage diseases.

Eligible for Young Investigator Oral Award

ATG7 AND ATG3 ARE DISPENSABLE FOR LC3-II FORMATION IN THIOGLYCOLATE-ELICITED PERITONEAL MACROPHAGES

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Aim: Autophagy is a complex cellular degradation process that requires the cooperation of many autophagy-related (ATG) proteins. Especially ATG5 (substrate) and ATG7, ATG3, and ATG10 (enzymes) are critical for the conjugation of microtubule-associated protein 1 light chain 3 (LC3) to phosphatidylethanolamine (PE), which is an important initial step in the formation of a sequestration membrane. To determine the impact of ATG5 and ATG7 on autophagy, we compared the formation of LC3-PE (LC3-II) in *Atg5* knock-out (-/-) and *Atg7*-/- murine macrophages.

Method: Experiments were performed using mice with a targeted deletion of *Atg5* or *Atg7* in myeloid cells, LysMCre mice were used as (wild-type) WT controls. Naive and thioglycolate-elicited peritoneal macrophages were cultured for 48 h in complete DMEM. Differentiation of bone marrow-derived cells was initiated with 10% of L929 conditioned medium after culturing the cells for 7 days in complete DMEM. Macrophages were lysed, protein and RNA were isolated and analyzed by Western blotting and quantitative real-time PCR experiments, respectively.

Results: Using *Atg5*-/- and *Atg7*-/- thioglycolate-elicited peritoneal macrophages, we discovered that loss of ATG5 but, unexpectedly, not of ATG7 resulted in LC3-II depletion. Whereas ATG10 expression was unaffected, we observed a complete loss of ATG3 protein expression in *Atg7*-/- thioglycolate-elicited murine macrophages, suggesting that ATG3 is redundant in the absence of ATG7. In contrast to thioglycolate-elicited peritoneal macrophages, neither naive peritoneal nor bone marrow-derived *Atg7*-/- macrophages exhibited LC3-II, indicating that inflammation triggers LC3-II formation in macrophages in the absence of ATG7.

Conclusions: We conclude that the metabolic status of macrophages dictates the level of LC3-PE conjugation with a supportive but nonessential role of ATG7 and ATG3. These data emphasize the important role of macrophage LC3 during inflammation and claim the necessity for modification of the currently accepted model of autophagy.

Eligible for Young Investigator Oral Award

ABSENCE OF SCAVENGER RECEPTORS STAB1 AND STAB2 PREDISPOSES TO MACROPHAGE FOAM CELL FORMATION WITHOUT BREAKING THE ATHEROSCLEROSIS RESISTANCE OF ZEBRAFISH

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Aim: Zebrafish have been proposed as novel animal model for studying atherosclerosis. In the current proof-of-principle study we evaluated the impact of high cholesterol diet (HCD) feeding on cholesterol metabolism and atherogenesis in zebrafish.

Method: Zebrafish larvae were challenged with 4% HCD labeled with 10 µg/g cholesteryl ester Bodipy FLC12 for 10 days to visualize lipid accumulation in the caudal vein.

Results: Feeding wild-type zebrafish (ABTL *mpeg1:rfp*, 5dpf) with HCD (4% green fluorescently labeled cholesteryl ester) for 10 days did not increase total-body cholesterol levels or induce development of atherosclerotic lesions in the caudal vein. More specifically, no co-localization of red fluorescent macrophages with green fluorescently labeled cholesterol was observed. However, we did note compensatory downregulation of genes involved in cholesterol acquisition, i.e. HMG-CoA reductase (-47%; P=0.05) and the low-density lipoprotein (LDL) receptor (-85%; P=0.003). Interestingly, expression of the endothelial scavenger receptor stabilin-2 (*stab2*) also tended to decrease (-82%; P=0.06) upon HCD feeding, whereas stabilin-1 (*stab1*) expression remained the same. In accordance with a role for *stab2* in cholesterol metabolism, uptake of oxidized LDL by endothelial cells of the caudal vein was reduced in *stab2* deficient zebrafish and absent in *stab1*$^{-/-}$*stab2*$^{-/-}$ mutants. As a result, gene expression levels of the scavenger receptors *cd36*, *srb1* as well as the *ldl* receptor remained significantly higher (P<0.05) in *stab1*$^{-/-}$*stab2*$^{-/-}$ mutants after 10 days HCD feeding. The impaired clearance of oxLDL by *stab1*$^{-/-}$*stab2*$^{-/-}$ endothelial cells was paralleled by a redistribution of oxLDL to macrophages in the caudal vein. However, despite the observed increase in foam cell formation, *stab1*$^{-/-}$*stab2*$^{-/-}$ mutant zebrafish still did not develop atherosclerotic lesions in response to the HCD feeding.

Conclusions: Our data show that wild-type zebrafish are protected against HCD-induced atherosclerosis. Furthermore, our findings highlight that stabilins are an important player in total-body cholesterol homeostasis as its absence predisposes to macrophage foam cell formation.

Eligible for Young Investigator Oral Award

T-CELL CHOLESTEROL EFFLUX PATHWAYS ARE CRUCIAL FOR MAINTAINING PERIPHERAL T-CELL LEVELS AND DO NOT AFFECT ATHEROGENESIS

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Aim: T-cell receptor stimulation, which commonly occurs during antigen presentation by dendritic cells, macrophages or B-cells, decreases expression of the cholesterol transporters ATP Binding Cassette A1 and G1 (ABCA1 and ABCG1) in T-cells by >90%. ABCA1 and ABCG1 mediate cholesterol efflux to apolipoprotein A1 and high-density-lipoprotein, respectively. T-cell *Abcg1* deficiency induces formation of anti-inflammatory T regulatory cells (T_{regs}), and suppresses atherogenesis. Excessive cholesterol accumulation in T-cells however leads to conversion of T_{regs} into pro-atherogenic T helper 1 (T_h1) and T-follicular helper (T_{FH}) cells. We investigated the role of combined T-cell *Abca1* and *Abcg1* deficiency in T-cell subsets and atherogenesis.

Method: We generated *CD4CreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$*Ldlr*$^{-/-}$ mice and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$*Ldlr*$^{-/-}$ controls. These mice were fed chow or Western type diet (WTD). T-cells were analyzed by flow cytometry.

Results: *CD4CreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$*Ldlr*$^{-/-}$ mice showed >90% deletion of *Abca1* and *Abcg1* in $CD4^+$ and $CD8^+$ T-cells compared to controls. Unexpectedly, T-cell *Abca1/Abcg1* deficiency decreased $CD4^+$ and $CD8^+$ T-cell numbers by 50% in blood, spleen, and lymph nodes, without affecting thymic T-cells or thymus size. We also observed increased $T_{memory/effector}$ and $T_{central\ memory}$ cells in blood, spleen, and lymph nodes, reflecting T-cell activation. These observations suggested an activation induced T-cell death phenotype. Consistent with this hypothesis, T-cell *Abca1/Abcg1* deficiency enhanced CD3/IL-2-induced T-cell activation and apoptosis *in vitro*. On WTD, T-cell *Abca1/Abcg1* deficiency increased pro-atherogenic T_h1 and T_{FH} cells as well as anti-atherogenic T_{regs} in aortic lymph nodes by 2-fold. After 10 weeks of WTD, T-cell *Abca1/Abcg1* deficiency did not affect atherosclerotic lesion size or severity, perhaps being the consequence of a 50% reduction of T-cells in atherosclerotic plaques.

Conclusions: T-cell Abca1/Abcg1 mediated cholesterol efflux pathways suppress T-cell activation induced cell death, as such maintaining peripheral T-cell numbers. T-cell *Abca1/Abcg1* deficiency does not affect atherosclerosis, likely as a consequence of a decrease in T-cells in atherosclerotic plaques.

Eligible for Young Investigator Oral Award

GPR180, A NOVEL TGFB FAMILY RECEPTOR, IS INDISPENSABLE FOR BROWN ADIPOCYTE FUNCTION

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Aim: Obesity and the associated metabolic diseases such as type 2 diabetes, dyslipidaemia or cardiovascular complications represent a major health burden therefore effective strategies to combat the disease must target food intake and/or energy expenditure. In contrast to white adipose tissue , which stores excessive energy in the form of lipids, brown adipose tissue is a highly metabolically active tissue capable of dissipating chemical energy in the form of heat Thus, activation of brown adipose tissue represents a prospective strategy to improve metabolic control. The aim of the current study was to identify novel molecular pathways that regulate formation and activation of brown adipocytes.

Method: We used different in vitro and in vivo approaches to study the role of the newly identified Cthrc1/Gpr180 axis in the regulation of TGFβ signaling as well as the influence on brown adipocyte functionality.

Results: We identified GPR180 as a novel receptor, whose expression in humans is associated with improved metabolic phenotype, and which regulates brown adipocyte function and whole-body glucose homeostasis. We demonstrate that GPR180 is not a GPCR but a novel component of TGFβ signalling pathway, which regulates the activity of the TGFβ receptor complex to modulate SMAD3 phosphorylation. In addition, we identify CTHRC1 as a potential ligand for GPR180, which controls UCP1 activity in brown adipocytes in vitro and improves glucose tolerance in mice, in vivo. The positive effects of CTHRC1 on metabolism are dependent on the presence of GPR180.

Conclusions: In conclusion, the newly identified components of TGFβ receptor signalling, CTHRC1 and GPR180, represent an alternative axis mediating the low-grade activation of the TGFβ signalling pathway to prevent pathophysiological response and important for the control of glucose and energy metabolism.

COLD-ACTIVATED MITOHORMESIS IN BROWN FAT IS MEDIATED BY NFE2L2

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Aim: Brown adipose tissue (BAT) is a metabolically highly active tissue with beneficial effects on metabolic health when activated by cold exposure. The high respiration found in BAT results in high levels of reactive oxygen species (ROS), which are known to cause cellular damage but also to stimulate the anti-oxidative stress response. Nuclear factor, erythroid-2, like-2 (Nfe2l2) is a transcription factor that is activated by ROS and serves as a key regulator of the anti-oxidative stress response. However, the regulation and biological significance of Nfe2l2 for oxidative stress and brown fat function remain unknown.

Method: Mice exposed to different housing temperatures and BAT was collected. We engineered immortalized brown adipocytes with RNAi and CRISPR-Cas9-SAM to silence and overexpress endogenous Nfe2l2, respectively. Cold, adrenergic activation and oxidative stress responses by H_2O_2 were studied in these mice and cells by qPCR, Western Blot, Glycerol Release Assay, Oil Red O Staining and measurement of intracellular ROS via DCFDA / H2DCFDA Staining and protein carbonylation. Respiration was measured by Seahorse extracellular flux analysis.

Results: In mice, cold acclimatization increased Nfe2l2 and it downstream targets, which was replicated in cell culture by adrenergic stimulation. Silencing of Nfe2l2 led to increased levels of expression of Ucp1, indicating improved brown fat function. Opposite results were found after overexpressing Nfe2l2. However, adipogenesis and lipolysis were not affected by the manipulation of Nfe2l2. However, brown adipocytes, in which Nfe2l2 was silenced, consumed more oxygen.

Conclusions: Nfe2l2 and its downstream effectors modify brown fat function without disturbing adipogenesis and emerge as pharmacological targets in regard to metabolic health. While Nfe2l2 is required to mitigate oxidative stress, acute loss of Nfe2l2 presumably results in mitohormesis, defined as a low-level stress response that is beneficial and improves BAT mitochondrial function.

Eligible for Young Investigator Oral Award

A SINGLE DAY OF HIGH FAT DIET FEEDING INDUCES LIPID ACCUMULATION AND INSULIN RESISTANCE IN BROWN ADIPOSE TISSUE IN MICE

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Aim: Brown adipose tissue (BAT) catabolizes glucose and fatty acids to produce heat and thereby contributes to energy expenditure. Long-term high fat diet (HFD) feeding results in so-called 'whitening' of BAT characterized by increased lipid deposition, mitochondrial dysfunction and reduced fat oxidation. The aim of the current study was to unravel the rate and related mechanisms by which HFD induces BAT whitening and insulin resistance.

Method: Wild-type mice were fed a HFD for 0, 1, 3 or 7 days. After termination, insulin-stimulated [^{14}C]deoxyglucose uptake and uptake of glycerol tri[^{3}H]oleate-labeled TG-rich lipoprotein-like particles was determined, organs were weighed, and histology was performed.

Results: Within one day of HFD BAT weight and lipid content were increased. HFD also immediately reduced insulin-stimulated deoxyglucose uptake and fatty acid uptake by BAT. Mitochondrial mass and Ucp1 expression were unaltered, while after 3 days of HFD a more fused mitochondrial network was induced accompanied by increased macrophage markers in BAT. Counterintuitively, the switch to HFD was accompanied by an acute rise in core body temperature.

Conclusions: A single day of HFD feeding is sufficient to induce the first signs of whitening and insulin resistance in BAT, which reduces the uptake of glucose and triglyceride-derived fatty acids. BAT whitening and insulin resistance is likely sustained by reduced mitochondrial oxidation due to changes in mitochondrial dynamics and macrophage infiltration, respectively. Likely, the switch to HFD swiftly induces thermogenesis in other metabolic organs, which allows attenuation of BAT thermogenesis.

Eligible for Young Investigator Oral Award

CONSTRUCTING THE BROWN FAT PROTEASOME IN NON-SHIVERING THERMOGENESIS

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Aim: In response to cold, brown adipose tissue (BAT) can shift from a low-energy, metabolically resting state to a high-energy, metabolically active state, and vice versa in response to thermoneutrality. Presumably, these dynamic, adaptive processes place a high burden on cellular proteostasis, including the ubiquitin-proteasome system (UPS). Recently, it was discovered that in BAT, proteasomal activity is strongly induced after cold adaptation. Here, we investigate the hypothesis that brown adipocytes are equipped with a "specialized" proteasome to meet the specific demands of non-shivering thermogenesis.

Method: We used an *in vitro* model of immortalized mouse pre-brown adipocytes. The catalytic activities of the proteasome were studied during differentiation as well as after treatment with sympathomimetics that raise intracellular cAMP levels, including norepinephrine and CL-316,243. For this we used a fluorometric peptide degradation assay. We also studied the levels of genes important for proteasome function by qPCR and Western blot.

Results: Brown adipocyte differentiation induced gene expression of proteasomal subunits, in particular proteasome 20S subunit alpha 1 (*Psma1*). Proteasome activity was markedly increased in differentiated cells compared to undifferentiated cells. Remarkably, this increase in activity is mainly observed in the chymotrypsin-like and, to a lesser extent, in caspase-like activity. Acute treatment with sympathomimetics increased glycerol levels in differentiated cells; but it did not increase proteasome activity.

Conclusions: Our findings point towards a specialized proteasome in brown adipocytes, as differentiation induced proteasomal activity of a subset of the catalytic processes. While our data suggest that brown adipocytes upregulate proteasome content, there are likely also posttranslational mechanisms independent of sympathetic activation. This project will add to our fundamental understanding of proteasome biology in the context of brown adipocytes.

Eligible for Young Investigator Oral Award

WHAT IGNITES UCP1? CHARACTERIZATION OF BROWN ADIPOCYTE-SPECIFIC ATGL AND HSL KNOCKOUT MICE

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Aim: In mammals, brown adipose tissue (BAT) is the key organ for thermoregulation. BAT is enriched with mitochondria expressing uncoupling protein 1 (UCP1) that allows heat generation. UCP1 is activated by fatty acids (FAs) that are considered to derive from intracellular triglyceride (TG) hydrolysis. The first and rate-limiting step in TG hydrolysis is catalyzed by adipose triglyceride lipase (ATGL). However, BAT-specific ATGL knockout mice showed normal cold tolerance challenging the longstanding hypothesis. Next to ATGL, hormone-sensitive lipase (HSL) shows some TG hydrolase activity that might be sufficient to release FAs for UCP1 activation. Here we studied the impact of BAT-specific loss of ATGL and HSL on thermoregulation.

Method: We characterized mice lacking ATGL and HSL in brown adipocytes (BAT-iDAKO). We assessed body temperature and whole-body energy metabolism using indirect calorimetry upon cold exposure.

Results: Compared to wildtype mice (WT), BAT-iDAKO mice had a ~5-fold higher BAT mass that contained large unilocular adipocytes. Immunoblots of BAT lysates from BAT-iDAKO revealed residual 5% ATGL and 17% HSL protein expression compared to WT but unaltered protein expression in inguinal WAT (ingWAT). TG hydrolase activities were 75% lower in BAT lysates of BAT-iDAKO than in WT but unaltered in ingWAT. Expression of classical BAT marker and fat oxidation genes was blunted in BAT-iDAKO mice. Mitochondrial and UCP1 protein content were 63% and 82% lower in BAT of cold exposed BAT-iDAKO than in WT mice, respectively. Yet, BAT-iDAKO mice maintained normal body temperature and exhibited similar energy expenditure as WT mice during cold. UCP1 protein levels were markedly increased in ingWAT of cold-exposed BAT-iDAKO mice.

Conclusions: Our data show that BAT-iDAKO mice have reduced mitochondrial content and UCP1 protein but normal body temperature or whole-body energy expenditure during cold. Increased UCP1 protein levels in ingWAT of BAT-iDAKO mice may compensate for impaired BAT to allow normal thermoregulation.

P53 IN THE REGULATION OF FRUCTOSE METABOLISM IN BROWN ADIPOCYTES UNDER NUTRIENT DEPRIVATION

Isabel Reinisch[1], Birgit Galle[2], Ingeborg Klymiuk[2], Michael Karbiener[3], Sandra Blass[1], Roland Malli[1,4], Fangrong Zhang[1], Tobias Madl[1,4], Elisabeth Moyschewitz[1], Martina Auer[1], Jelena Krstic[1], Renate Schreiber[5,4], Markus Galhuber[1], Andreas Prokesch[1,4]

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Aim: Cold-activated brown adipose tissue (BAT) dissipates energy to drive thermogenesis, acting as a "metabolic sink" by taking up large amounts of glucose and fatty acids from the circulation. How this highly energy consuming process is regulated under fasting, when energy substrates need to be conserved, remains unclear. The aim of this study was to elucidate molecular mechanisms that prevent futile energy dissipation of BAT under fasting.

Method: We investigated BAT from fasted mice that were kept below thermo-neutrality using transcriptomics, miRNA-sequencing and bioinformatic-tools (UCSC, JASPAR, TargetScan). In vitro, we used gain-of function approaches to induce p53 and miRNA-92a-1-5p expression. Additionally, to study fructose uptake and utilization in brown adipocytes, we used NMR and SEAHORSE-measurements.

Results: Transcriptomics and miRNA-seq of BAT of fasted mice under mild cold-stress revealed p53 signalling as top upregulated pathway and miRNA-92a-1-5p as most upregulated miRNA, respectively. Bioinformatics analyses predicted a p53 binding site in the miRNA-92 locus and identified the fructose transporter Glut5 as miRNA-92a-1-5p target. Glut5 was strongly downregulated in fasted, cold-challenged BAT and the p53/miRNA-92a-1-5p/Glut5 axis was confirmed in a series of in vitro experiments. To further understand the role of regulation of Glut5 and fructose metabolism in brown adipocytes we supplemented medium with fructose and found that fructose is taken up vividly. The intracellular fate of fructose was shown to contribute to glycolysis. Further experiments aim to delineate regulatory action of fructose via Chrebp in brown adipocytes.

Conclusions: Our data suggest p53 as an inducer of miRNA-92a-1-5p expression in fasted BAT, leading to reduced Glut5 levels. Furthermore, our study is first to indicate a role of fructose metabolism in regulation of brown adipocyte activity.

Eligible for Young Investigator Oral Award

CONCOMITANT GLUCOSE-DEPENDENT INSULINOTROPIC RECEPTOR (GIPR) AND GLUCAGON-LIKE PEPTIDE-1 RECEPTOR (GLP1R) AGONISM STIMULATES VLDL-TRIGLYCERIDE TURNOVER AND ATTENUATES ATHEROSCLEROSIS DEVELOPMENT

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Aim: Tirzepatide, a dual GIP/GLP-1 receptor agonist was recently shown to cause robust weight loss in patients with Type II Diabetes (Frias *et al.* 2018). Since GIPR agonism stimulates lipolysis in white adipose tissue and GLP1R agonism promotes brown adipose tissue (BAT) thermogenesis, we hypothesized that GIPR agonism combined with GLP1R agonism enhances the fatty acid (FA) flux to BAT to facilitate thermogenesis, thereby alleviating dyslipidemia and attenuating atherosclerosis development.

Method: Dyslipidemic female APOE*3-Leiden.CETP mice were fed a Western-type diet (containing 16% fat and 0.15% cholesterol) and received a daily subcutaneous injection with either vehicle, a GIPR agonist (GIPFA-085; 300 nmol/kg/day), a GLP1R agonist (GLP-140; 30 nmol/kg/day) or both agonists for 4 weeks. Body weight and body composition were monitored throughout the study by echoMRI. At the end of the study plasma triglycerides (TGs) and cholesterol were measured in 4h-fasted plasma samples, and clearance of VLDL-TG and remnants was assessed upon injection of glycerol tri[^{3}H]oleate and [^{14}C]cholesteryl oleate-labeled VLDL-like particles. In the aortic valve region, atherosclerotic lesions were scored.

Results: GLP1R agonism lowered body weight (-2.0 g) and fat mass (-1.8 g), while it increased the uptake of VLDL-TG-derived FA by BAT (+157%) compared to vehicle. On all of these parameters, concomitant GIPR and GLP1R agonism outperformed GLP1R agonism alone (body weight -2.8 g; fat mass -2.3 g; VLDL-TG derived FA uptake by BAT +191%, compared to vehicle). Concomitant GIPR and GLP1R agonism, but not GLP1R agonism or GIPR agonism alone, tended to lower plasma TG levels (-46%) and markedly increased hepatic VLDL-remnant uptake (+67%). Importantly, concomitant GIPR and GLP1R agonism decreased atherosclerotic lesion progression (-35% severe lesions).

Conclusions: Concomitant GIPR and GLP1R agonism stimulates VLDL-TG turnover and hepatic remnant clearance more than the individual agonists and correspondingly attenuates atherosclerosis development. Current studies evaluate the effects of co-treatment in diet-induced obesity.

Eligible for Young Investigator Oral Award

CIRCADIAN CONTROL OF BROWN ADIPOSE TISSUE ACTIVITY BY GLUCOCORTICOIDS

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Aim: Brown adipose tissue (BAT) displays a strong circadian rhythm in metabolic activity, which is reflected in rhythmicity of circulating lipoprotein levels (Van den Berg, Cell Rep 2018), but it is unclear how this rhythm is regulated. The aim of the current study was to investigate the role of the superimposed rhythm in the glucocorticoid corticosterone in the metabolic activity of BAT.

Method: Wildtype and hyperlipidemic APOE*3-Leiden.CETP mice were subcutaneously implanted with pellets releasing a continuous low dose of corticosterone to flatten corticosterone rhythm. Alternatively, daily corticosterone injections were given to study the effect of hypercortisolism. Adipose-specific GR KO mice and brown adipocyte cell cultures were used to investigate underlying mechanisms.

Results: Implantation of corticosterone-containing pellets resulted in constant and flattened circulating corticosterone, with slight hypercortisolism. Strikingly, flattened corticosterone rhythm caused a complete loss of circadian rhythm in BAT activity of both male and female mice as measured by triglyceride-derived fatty acid uptake from VLDL-like particles. In line with these data, Lpl mRNA and LPL protein were highly rhythmic in BAT of vehicle-implanted mice, but were blunted in mice with flattened corticosterone rhythm. All described effects were independent of glucocorticoid receptor expression in (brown) adipocytes and not caused by dysregulation of clock gene expression or hypercortisolism, but rather mediated by reduced sympathetic outflow to BAT as evidence by a blunted rhythm in norepinephrine production and reduced adrenergic signaling. In APOE*3-Leiden.CETP mice, long-term experimental flattening of corticosterone - and thus BAT activity rhythm - resulted in increased lipid deposition in adipose tissue depots and as a consequence weight gain.

Conclusions: A physiological glucocorticoid rhythm is essential for rhythmic BAT activity and metabolic health. We anticipate that disruption of glucocorticoid rhythm, and thereby BAT activity rhythm, could partially underlie the relationship between rhythm disturbances and metabolic disease in humans.

EPIGENETIC REGULATION OF THE NFE2L1-PROTEASOME PATHWAY IN OXIDATIVE METABOLIC TISSUES

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Aim: Nfe2l1 is a transcriptional master regulator of proteasomal protein quality control with very high expression in oxidative tissues like brown adipose tissue (BAT), heart and skeletal muscle for reasons unknown. Trimethylation of Histone H3 at Lysine 4 (H3K4me3) is a chromatin modification that broadly marks the transcription start sites of essential genes, thus conferring enhanced transcriptional consistency. However, the methylation map of the Nfe2l1 promoter in metabolically active tissues such as thermogenic adipocytes and muscle remains unclear. Here, we studied the methylation profile of Nfe2l1 promoter as a possible post-translational regulation by which these tissues might secure proteostasis under demanding metabolic conditions.

Method: ChIP-Seq and downstream bioinformatic analysis were used to globally map the profile of H3k4me3 in tissues of mice including BAT, muscle and heart. We are using CRISPR/Cas technology to study the effect of truncated promoter with reduced H3K4me3 breadth in proteostasis and adipocyte function.

Results: Nfe2l1 but not its sister molecule Nfe2l2 was broadly methylated (> 8kb) specifically in BAT, muscle and heart of mice under physiologic conditions. Transcriptional activation of Nfe2l1 is required for cold adaptation of mice, however this transcriptional response upon cold exposure was not reflected in the methylation of the Nfe2l1 promoter.

Conclusions: Our analysis demonstrates broad H3k4me3 methylation in the promoter of Nfe2l1 specifically in the metabolically active oxidative tissues. Suggesting that this epigenetic signature signals the relevance of Nfe2l1 in securing protein quality control in oxidative tissues. Currently, we are studying the upstream regulators of this process including transcription factors which may drive tissue specific methylation profile.

Eligible for Young Investigator Oral Award

NFE2L1 DRIVES PROTEOSTASIS IN MYOCYTES AND SKELETAL MUSCLE

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Aim: Physical activity and exercise have beneficial effects on fitness and health. Particularly, exercise-induced increases in metabolism require specific molecular adaptation of myocytes. A critical component of healthy muscle function is proteostasis, the proper production and degradation of proteins, the latter being mediated by the Ubiquitin Proteasome System (UPS). We hypothesize that the transcription factor nuclear-factor erythroid-2, like-1 (Nfe2l1), which is a master regulator of proteasomal activity, plays a significant role in myocytes and muscle function.

Method: We used differentiated C2C12 mouse myocytes, in which Nfe2l1 was silenced by RNAi and skeletal myocyte-specific knock-out (KO) mice (Acta1-Cre) to investigate the impact of Nfe2l1. We also explored the effect of impaired proteostasis induced by chemical proteasome inhibition in myocytes. Baseline whole-body energy metabolism of KO mice and controls was analyzed by indirect calorimetry in vivo.

Results: We found that Nfe2l1 was induced upon myocyte differentiation and highly expressed in muscle tissue on mRNA and protein level. Silencing of Nfe2l1 in differentiated C2C12 myocytes reduced proteasomal subunits, especially when the proteasome was inhibited by epoxomicin or bortezomib treatment. Also *in vivo*, in muscle of mice lacking myocyte Nfe2l1 proteasome activity was compromised. This was associated with an aberrant respiratory exchange ratio during the day-night cycle, indicating altered fuel utilization in KO mice.

Conclusions: We conclude that Nfel21 has an important role in proteostasis in myocytes and muscle tissue and therefore could be a key player in metabolic fitness and exercise biology.

Eligible for Young Investigator Oral Award

INDUCTION OF NFE2L1-MEDIATED PROTEASOMAL ACTIVITY IN MYOCARDIAL INFARCTION

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Aim: Myocardial infarction and ischemic heart disease are highly prevalent cardiovascular diseases (CVD) associated with high mortality. During chronic and acute myocardial dysfunction, cardiomyocytes are exposed to a variety of stress factors, including hypoxia, reactive oxygen species, as well as inflammation. Nuclear factor erythroid-2-like-1 (Nfe2l1) is a transcription factor and key regulator of proteasomal protein quality control and highly expressed in cardiomyocytes. However, the regulation and biological significance of Nfe2l1 for heart function and CVD remains unknown. Here we investigate Nfe2l1-mediated proteasomal activity in cardiometabolic function.

Method: To mimic myocardial infarction (MI), ligation of the left anterior descending artery (LAD) in C57BL/6J mice was performed causing ischemic injury of the heart. Mice were sacrificed 4 hours, 24 hours and 7 days after surgery. Inflammation, Nfe2l1 levels, and proteasomal activity were determined via Western Blot, qPCR and flourometric peptide assays. To validate the results, cardiomyocyte specific knock-out mice of Nfe2l1 using Myh6-Cre-loxP system were generated and analyzed as described above.

Results: LAD surgery led to an increase of heart Nfe2l1 and proteasomal subunits after 4 hours and 24 hours respectively which declined after 7 days. The proteasomal activity assay revealed an increase upon LAD surgery, which was Nfe2l1-dependent as cardiomyocyte-specific deletion resulted in decreased proteasomal subunit expression and activity.

Conclusions: Our results indicate that Nfe2l1-mediated proteasomal activity is linked to cardiomyocyte stress during myocardial infarction. Targeting this pathway might help to boost stress resistance and improve cardiometabolic outcomes.

EFFECTS OF INTESTINAL ATGL OVEREXPRESSION: THE GOOD OR THE EVIL?

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Aim: The increased frequency of metabolic and cardiovascular diseases is mainly linked to an increased intake of dietary lipids. Enterocytes of the small intestine (SI) play a crucial role in maintaining whole body lipid homeostasis by controlling lipid uptake and lipoprotein secretion. Our previous studies have shown that lack of adipose TG lipase (ATGL) leads to massive accumulation of lipid droplets (LDs) within enterocytes, thus affecting TG and cholesterol homeostasis. Hence, we hypothesize that ATGL overexpression might positively affect intestinal and whole body lipid metabolism.

Method: To investigate the role of intestinal ATGL on systemic lipid levels, lipoprotein metabolism, and intestinal lipid absorption, we generated mice expressing a FLAG-tagged *Atgl* CDS under the control of the enterocyte-specific villin promotor (Atgl iTg).

Results: Notwithstanding that *Atgl* mRNA expression was drastically elevated (80-fold), enzymatic activity of ATGL in the jejunum of Atgl iTg mice was only mildly induced. Interestingly, Atgl iTg mice displayed an even higher concentration of intracellular TG in the early absorption phase, however, dietary TG absorption *per se* remained comparable between the genotypes. Of note, we observed accelerated cholesterol absorption in Atgl iTg mice, which was mainly mediated by an upregulation of PPARa target genes.

Conclusions: This study demonstrates that intestine-specific overexpression of ATGL only mildly ameliorates high fat/high cholesterol diet-induced intestinal steatosis. In line with results from mice lacking intestinal ATGL, unaltered lipoprotein secretion indicates that ATGL does not provide free fatty acids as substrates for chylomicron assembly. Unexpectedly, our *in vivo* studies evidence that intestinal ATGL rather regulates cholesterol homeostasis via activation of PPARa than TG metabolism.

Eligible for Young Investigator Oral Award

HOW DOES THE LOSS OF INTRACELLULAR LIPASES AFFECT MOUSE PLACENTA AND FETUS?

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Aim: Fatty acids (FAs) are critically involved in energy production, biosynthesis of membranes, and fetal development. Intracellular lipases release FAs either from cytosolic triglycerides (TG) through the consecutive action of adipose triglyceride lipase, hormone-sensitive lipase (HSL), and monoacylglycerol lipase, or from lysosomal cholesteryl esters (CE) and TG by lysosomal acid lipase (LAL). Defective lipolysis provokes severe human pathologies manifested by massive accumulation of TG and/or CE in various organs. The role of intracellular lipases in the placenta and developing fetus is poorly understood. Therefore, we aim to investigate how loss of intracellular lipases affects placental and fetal lipid metabolism.

Method: Placental and fetal liver tissues were dissected on day 19 of pregnancy from HSL- and LAL-deficient ($Lal^{-/-}$) mice. Tissue sections were stained with Oil Red O to detect neutral lipids, and counterstained with cytokeratin-8 or CD31 antibodies. Tissue lipids were extracted according to the method of Folch, and lipase activity was determined using radioactive tracers.

Results: We observed that cytosolic lipid droplets are located predominantly in trophoblasts of the labyrinth zone in the wildtype mouse placenta. The loss of HSL had no impact on placental or fetal weight and lipid content. In contrast, $Lal^{-/-}$ placentae showed a tendency towards elevated lipid levels and decreased acid CE hydrolase activity. Interestingly, $Lal^{-/-}$ embryos accumulated CE but not TG in the liver, in line with reduced hepatic CE hydrolase activity.

Conclusions: Comparable to the human situation, lipids accumulate in the mouse placenta mainly in the region of nutrient exchange and trophoblasts (the labyrinth). In summary, the observed CE accumulation in $Lal^{-/-}$ placenta and embryo liver indicates that consequences of defective lysosomal lipolysis already affect the developing fetus.

Eligible for Young Investigator Oral Award

DISRUPTION OF CIRCADIAN RHYTHM BY ALTERNATING LIGHT-DARK CYCLES AGGRAVATES ATHEROSCLEROSIS DEVELOPMENT

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Aim: Disruption of circadian (i.e. 24 h) rhythm by means of shift work has been associated with obesity, dyslipidemia and cardiovascular disease in humans. However, causality and underlying mechanisms have not yet been established. Therefore, we aimed to investigate in mice whether mimicking shift work by weekly shifts in the light-dark cycle directly affects atherosclerosis development, and elucidate underlying mechanisms.

Method: Female APOE*3-Leiden.CETP mice on a Western-type diet, a humanized mouse model of dyslipidemia and atherosclerosis, were exposed to either regular light-dark cycles, weekly 6 h phase advances or delays, or weekly alternating-light dark cycles (12 h shifts). After 15 weeks, histological analysis of the aortic root area was performed to evaluate atherosclerotic lesion development.

Results: Mice that were exposed to weekly alternating light-dark cycles displayed a striking increase in atherosclerosis, with an approximately two-fold increase in lesion size (+84%; P<0.01) and severity (+117%; P<0.001). Phase advances also tended to increase lesion severity (+59%; P=0.06), while phase delays did not. Alternating light-dark cycles increased lesion macrophage content (+105%; P<0.001) without obvious changes in plasma lipids, suggesting involvement of the immune system. Mechanistically, we found no changes in the overall number or activation status of circulating monocytes and other immune cells. Instead, we identified increased gene expression of markers for inflammation (*Tnfa*, *F4/80*, *iNos*), oxidative stress (*Sod1*, *Gpx1*, *Nrf1*) and chemoattraction (*Icam1*, *Vcam1*, *Ccr2*) in the aortic vessel wall. Further histological evaluation revealed increased expression of the monocyte chemoattractant protein-1 (MCP-1/CCL2; +50%; P<0.05) within atherosclerotic lesions, which correlated positively with the lesion macrophage content (P<0.001).

Conclusions: We are the first to show that mimicking shift work directly aggravates atherosclerosis development, most likely via increased attraction and migration of monocytes into the stressed vessel wall.

Eligible for Young Investigator Oral Award

INHIBITION OF SPHINGOLIPID *DE NOVO* SYNTHESIS COUNTERACTS AGE-RELATED LOSS OF FITNESS

Pirkka-Pekka Laurila[1], Peiling Luan[1], Martin Wohlwend[1], Tanes Imamura de Lima[1], Sebastien Herzig[1], Maroun Bou-Sleiman[1], Johanna Tuhkanen[2], Thomas Eichmann[3], Jari Lahti[2], Johan Eriksson[2], Johan Auwerx[1]

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Aim: Sphingolipids are important players in cardiometabolic disease. Here, we studied the role sphingolipids in healthy aging by comparing their tissue-distribution in young and aged individuals.

Method & Results

While sphingolipids were reduced in liver and plasma upon aging, there was a global accumulation of intermediates of the sphingolipid-de-novo-synthesis-pathway in aged skeletal muscle(SKM), accompanied by increased transcript abundance of enzymes of sphingolipid-de-novo-synthesis-pathway in aging. These transcript levels, especially serine-palmitoyltransferase, the rate-limiting enzyme of sphingolipid-de-novo-synthesis-pathway, were inversely correlated with muscle weight, muscle function, and exercise capacity in BXD mouse population. Systemic treatment of aged 18-month-old mice with serine-palmitoyltransferase-inhibitor myriocin for 17 weeks reduced sphingolipid levels in SKM, and increased muscle mass, strength, coordination, and exercise capacity. Unbiased gene-set-enriched-analysis of SKM-transcriptome pointed that serine-palmitoyltransferase-inhibition improved SKM-regeneration and muscle-stem-cell activation. Mechanistically, transplantation of sphingolipid-depleted stem-cells into aged mice improved their muscle-regeneration and exercise capacity. We next demonstrated the cell-autonomous effect of sphingolipid-de-novo-synthesis inhibition on myofiber differentiation. Depletion of sphingolipids in *ex-vivo*-cultured muscle-stem-cells boosted the production of Myogenin, a master-regulator of myotube differentiation, leading to accelerated differentiation of large myotubes. While CRISPR-Cas9-mediated silencing of enzymes of the sphingolipid-de-novo-synthesis-pathway, including serine-palmitoyltransferase and ceramide-synthase, activated myogenesis program, inactivation of DEGS1, the enzyme converting dihydroceramides to ceramides by inserting Δ4,5-*trans*-double bond, reduced myoblast-differentiation. Indeed, loading serine-palmitoyltransferase-deficient myoblasts with dihydroceramides reduced Myogenin and abrogated the promyogenic effect of serine-palmitoyltransferase-ablation. To validate our findings in humans, we identified genetic loci reducing mRNA-expression of *SPTLC1*. In a Finnish cohort of aged individuals, the *SPTLC1*-mRNA-decreasing allele was associated with improved performance in force and fitness tests, consistent with benefits of pharmacological serine-palmitoyltransferase-inhibition in mice. This association was replicated in aged UK-biobank participants (n=26,000).

Conclusions: Our study discovers a novel role for sphingolipid-metabolism in muscle regeneration, points dihydroceramides to negatively regulate myogenic differentiation, and identifies inhibition of sphingolipid-de-novo-synthesis-pathway as an attractive therapeutic strategy to promote healthy aging.

MODERATE EXERCISE TRAINING DECREASES CIRCULATING ENDOCANNABINOIDS AND N6-PUFA OXYLIPINS IN YOUNG HEALTHY ADULTS AS ASSOCIATED WITH IMPROVED CARDIOMETABOLIC HEALTH

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Aim: Exercise is a non-pharmacological tool inducing a myriad of physiological adaptations and enhancing human cardiometabolic health, although the mechanisms mediating the protective effects of exercise remain poorly understood. Interestingly, endocannabinoid (ECs) and oxylipins have recently been suggested to play an important role in the development of cardiovascular diseases. Therefore, the aim of this study was to investigate the dose-dependent effect of exercise (i.e., no exercise, moderate exercise intensity and vigorous exercise intensity) on circulating levels of endocannabinoids and oxylipins in young healthy adults.

Method: A total of 87 young healthy adults (22.1±2.2 years; BMI: 24.6±4.1 kg/m^2; 30% male) were randomized to receive no exercise (control group), moderate exercise intensity (60% of the Heart Rate Reserve [HRR]) or vigorous exercise intensity (80% of the HRR) for 24 weeks of concurrent training. Before and after the intervention period, blood samples were collected in fasting conditions at 8.00 am. Plasma endocannabinoids and oxylipins were assessed using LC-MS/MS, whereas body composition was determined via DEXA.

Results: Both exercise intensities significantly reduced fat body mass, increased lean body mass and slightly improved the cardiometabolic profile compared with the control group (all $P<0.05$). However, only moderate exercise intensity decreased the plasma concentration of anandamide (AEA), 2-arachidonylglycerol (2-AG), dihomo-gamma-linolenoyl (DGLEA), palmitoleoyl ethanolamide (POEA) and oleoyl ethanolamide (OEA) ($P<0.05$), as well as arachidonic acid (AA) and derived hydroxyeicosatetraenoic acids (HETEs), including 5-HETE, 11-HETE and 15-HETE, compared with control and vigorous intensity groups. The change in ECs was related with the change in LDL-C, whereas the change in oxylipins was related with the change in fat body mass.

Conclusions: Both exercise intensities reduce fat body mass and slightly improve the overall cardiometabolic profile. However, only moderate exercise intensity decreases circulating levels of endocannabinoids and n6-PUFA oxylipins, which may reduce systemic inflammation and improve vascular dysfunction.

Eligible for Young Investigator Oral Award

CHARACTERIZATION OF LIPOPROTEINS IN INTERSTITIAL FLUID IN TYPE 2 DIABETES – INDIRECT EVIDENCE OF INCREASED LDL AGGREGATION CAPACITY CONTRIBUTING TO ACCELERATED ATHEROSCLEROSIS

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Aim: The increased frequency of cardiovascular disease in type 2 diabetes (T2D) cannot be fully explained from currently established risk factors. In particular, the LDL-cholesterol-related risk is disproportionally elevated in such patients. The transvascular transport of LDL particles is believed to be increased in T2D patients, which should lead to higher interstitial fluid (IF) to serum ratios of LDL-cholesterol and apoB in such individuals. Unexpectedly, these ratios were instead reduced by 18% and 60%, respectively, in T2D patients (Apro J. et al., J Lipid Res 2015). We hypothesized that the finding of the reduced IF-to-serum ratios of LDL-cholesterol and apoB in T2D is caused by an increased aggregation of LDL particles, leading to a subsequent binding and cellular uptake in the intercellular compartment. This was tested in a new, larger cohort of patients with T2D which was compared to a group of matched control subjects.

Method: IF from skin blisters generated by vacuum and skin biopsies were obtained from 50 T2D patients and 50 healthy controls. Serum and IF cholesterol and apoproteins were determined by FPLC and ELISA.

Results: A 20% lower IF-to-serum ratio of LDL-C was confirmed in T2D patients; the findings were even more pronounced in T2D with nephropathy, a group known to have more severe vascular disease. Analyses of skin cholesterol and apoB, as well as functional properties of isolated lipoprotein fractions are currently performed.

Conclusions: The presence of lowered IF-to-serum ratios of LDL-cholesterol and apoB in T2D are in tune with recent findings of increased aggregation and subsequent cellular binding of LDL particles in this condition. Disturbances in the metabolism of lipoprotein cholesterol within the intercellular compartment may be of major importance for the increased frequency of cardiovascular complications in T2D.

Eligible for Young Investigator Oral Award

REDUCED REVERSE CHOLESTEROL TRANSPORT EFFICACY IN HEALTHY MEN WITH UNDESIRABLE POSTPRANDIAL TRIGLYCERIDE RESPONSE

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Aim: Elevation of non-fasting triglyceride (TG) levels above 1.8 g/l (2 mmol/l) is associated with increased risk of cardiovascular disease. Exacerbated postprandial hypertriglyceridemia (PP–HTG) and metabolic context both modulate the overall efficacy of the reverse cholesterol transport (RCT) pathway, however the specific contribution of exaggerated PP–HTG on RCT efficacy remains indeterminate.

Method: Healthy male volunteers (n=78) exhibiting no clinical features of metabolic disorder underwent a postprandial exploration following consumption of a typical Western meal providing 1200 kcal. Subjects were stratified according to maximal non-fasting TG levels reached after ingestion of the test meal into subjects with desirable PP–TG response (G_{Low}, TG <1.8 g/l, n=47) and subjects with undesirable PP–TG response (G_{High}, TG>1.8 g/l, n=31). The impact of the degree of PP–TG response on major steps of RCT pathway, including cholesterol efflux from human macrophages, cholesteryl ester transfer protein (CETP) activity, and hepatic HDL-cholesteryl ester (CE) selective uptake, was evaluated.

Results: Cholesterol efflux from human macrophages was not significantly affected by the degree of PP–TG response. Postprandial increase in CETP-mediated CE transfer from HDL to triglyceride-rich lipoprotein particles, and more specifically to chylomicrons, was enhanced in G_{High} vs G_{Low}. The hepatic HDL-CE delivery was reduced in subjects from G_{High} in comparison with those from G_{Low}.

Conclusions: Undesirable PP–TG response induces an overall reduction in RCT efficacy that contributes to the onset elevation of both fasting and non-fasting TG levels and to development of cardiometabolic diseases.

Eligible for Young Investigator Oral Award

MUTATIONAL BURDEN OF MITOCHONDRIAL DNA AND ATHEROSCLEROSIS

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Aim: By epidemiological studies, genetic factors may explain up to 15% variability of atherosclerotic diseases. Considerable attention was paid to the role of mitochondrial DNA (mtDNA) damage in the pathogenesis of atherosclerosis. The hypothetical mechanism of atherogenic effect of mtDNA mutations may be due to oxidative stress, development of mitochondrial dysfunction and inflammatory reaction, and cell death. Several basic and clinical studies were performed to reveal the association of mtDNA mutations with atherosclerosis.

Method: The heteroplasmy level in human leucocytes was determined by pyrosequencing method adopted for conditions where both mutant and normal alleles were present in the same specimen. The blood was taken from apparently healthy persons, CHD patients, and MI survivors.

Results: At least 10 mutations in 8 mitochondrial genes encoding the 12S subunit of ribosomal RNA, leucine t-RNA, cytochrome B, and NADH dehydrogenase subunits were significantly associated with atherosclerotic lesions. The associations of the same mutations with the extent of subclinical carotid atherosclerosis assessed by carotid intima-media thickness were revealed. The mtDNA next generation sequencing demonstrated significant correlation of mtDNA mutations with CHD and myocardial infarction. The most common proatherogenic and antiatherogenic haplotypes of mtDNA mutations were identified. A panel of several mtDNA variants associated with atherosclerosis was obtained. The ongoing research is aimed to the studies of precise mechanisms whereby mtDNA mutations can lead to atherosclerosis development at the cellular level. The methodological approaches are based on creation of cytoplasmic hybrids, and on the direct editing of mtDNA, in order to reproduce the pathogenic mitochondrial genotype.

Conclusions: We consider mtDNA mutations as the mechanistic biomarkers of atherosclerosis. We need precise cellular models created by the means of mtDNA editing to study pathogenic role of deleterious mtDNA mutations, and to find plausible molecular targets for prevention and treatment of atherosclerotic pathology.

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Eligible for Young Investigator Oral Award

PLASMA LEVELS OF TRIGLYCERIDES AND RISK OF DEMENTIA

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Aim: The impact of cardiovascular risk factors for dementia pathogenesis remains largely unknown. Accumulating evidence suggests, however, that cardiovascular disease and dementia have shared risk factors, including smoking, midlife hypertension, physical inactivity, and diabetes. Whether high levels of atherogenic triglyceride-rich lipoproteins also is a shared risk factor between cardiovascular disease and dementia is not known. We aimed to investigate whether high levels of plasma triglycerides were associated with high risk of non-Alzheimer's dementia, vascular dementia, and Alzheimer's dementia.

Method: The association between plasma levels of triglycerides on a continuous scale and risk of non-Alzheimer's dementia (dementia with vascular components: vascular dementia and unspecified dementia), vascular dementia, Alzheimer's dementia, and ischemic stroke as a positive control, was examined in the Copenhagen City Heart Study (CCHS) and the Copenhagen General Population Study (CGPS) using Cox regression restricted cubic splines. The association between the highest plasma triglyceride percentile and risk of any of the four endpoints were displayed as Hazard Ratios (HRs) using Cox proportional hazard models with age as time scale and delayed entry (left truncation) in the CCHS and the CGPS.

Results: Higher concentrations of plasma triglycerides were associated with higher risk of non-Alzheimer's dementia and ischemic stroke on a continuous scale. No significant association was found for Alzheimer's dementia. The highest plasma triglyceride percentile was associated with a HR of 1.52 (95% confidence interval: 1.03-2.26) for non-Alzheimer's dementia. Corresponding HRs for vascular dementia, Alzheimer's dementia and ischemic stroke were 1.78 (0.74-4.31), 1.22 (0.74-1.99), and 1.75 (1.39-2.21), respectively.

Conclusions: Very high levels of plasma triglycerides were associated with high risk of non-Alzheimer's dementia, highlighting that atherogenic triglyceride-rich lipoproteins are contributing factors to the dementia syndromes with vascular components.

Eligible for Young Investigator Oral Award

UNDERSTANDING THE ROLE OF A LIVER-SPECIFIC HEP-LNCRNA IN NASH PROGRESSION

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Aim: Non-alcoholic steatohepatitis (NASH) is characterized by steatosis, lobular inflammation and are predisposed to other complications, such as fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Although the mechanism by which NASH progress to these severe liver pathologies is not well understood accumulating data implicate a role for long non-coding RNAs (lncRNAs) in lipogenesis, inflammation and hepatic proliferation and thus might contribute to the disease progression of NASH. Recently, we have identified a liver-specific lncRNA (Hep-lncRNA) whose expression is negatively correlated with NASH grade in humans and mice. In this study, we aim to elucidate the contribution of Hep-lncRNA to NASH development.

Method: Using somatic CRISPR/Cas9 gene editing, Hep-lncRNA was specifically targeted in mouse livers. Wildtype and hepatic deficient Hep-lncRNA mice were fed either a chow or a high-fat high cholesterol diet (HFC), subsequently, liver inflammation and hepatic lipid accumulation were determined. In addition, Hep-lncRNA expression was determined in other preclinical liver disease models.

Results: Hepatic Hep-lncRNA deficiency did not lead to changes in body weight or liver weight when compared to wild-type mice. Histological and biochemical analyses suggest that loss of Hep-lncRNA does not affect the development of steatosis. However, the expression of several inflammatory markers, such as TNF-a and CD68, were slightly increased upon Hep-lncRNA ablation. Interestingly, in other liver disease models, we found that Hep-lncRNA expression was positively correlated with markers for hepatic differentiation, and negatively correlated with proliferation markers. Furthermore, preliminary data show that the lncRNA is highly upregulated during the acute phase response after partial hepatectomy (PH) but strongly down-regulated during the hepatic proliferating phase.

Conclusions: Although we did not find a strong evidence for a role of lncRNA in NASH development, we hypothesize that Hep-lncRNA regulates hepatocyte proliferation and might be involved in NASH-related HCC development and, currently, additional experiments are being performed to test our hypothesis.

PCSK9 IN THE DEVELOPMENT OF HUMAN ATHEROSCLEROSIS

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Aim: PCSK9 is the third gene involved in familial hypercholesterolemia, a major cause of atherosclerosis in humans. Its role in the regulation of liver LDL receptors and plasma cholesterol level has been widely studied and anti-PCSK9 drugs are developed. Nevertheless, the interactions between PCSK9 and atherosclerotic pathology in human are not yet studied. Our **aim** is to demonstrate that PCSK9 could promote atherosclerosis through smooth muscle cells (SMC) involved in the formation of foam cells and the initiation and progression of atherosclerosis.

Method: Healthy and atheromatous human arteries (fatty streaks or fibrolipidic lesions, aorta and coronary arteries) are collected in accordance with ethical guidelines (Biobanque Cardiovasculaire BRIF: BB-0033-00029 / BBMRI-EU / Infrastructure BIOBANQUE – CODECOH 2018-3141). Various techniques of histochemistry, immunofluorescence, immunochemistry, confocal microscopy and molecular biology were used.

Results: Real time RT-PCR performed on RNA extracted from tissues demonstrates that PCSK9 is not synthesized in the arterial wall. Histochemical studies and immunostaining show specific signal of PCSK9 in fatty streaks and fibrolipidic lesions when compared to healthy tissues. PCSK9 is intracellularly localized particularly in foam cells. An ELISA assay shows that PCSK9 concentration is significantly higher in atheromatous aortas compared to healthy ones. These data demonstrate that the circulating PCSK9 synthesized by the liver penetrates the arterial wall by inward convection, especially when the wall is largely contaminated by LDL. The mechanism of PCSK9 endocytosis by human SMC is under exploration.

Conclusions: The identification of PCSK9 within the arterial wall and a potential implication in the progression of atherosclerosis will contribute in better understanding of the underlying mechanisms of the disease and the discovery of new indications for PCSK9 inhibitors.

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Eligible for Young Investigator Oral Award

MOLECULAR SPECTRUM OF *PCSK9*-BASED FH IN FRANCE, THE FRENCH P.(SER127ARG) FOUNDER VARIANT

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Aim: *PCSK9* is the third gene involved in familial hypercholesterolemia (FH). The first FH-causing *PCSK9* variant reported in 2003, p.(Ser127Arg), is almost exclusively found in French patients and represents 67% of the *PCSK9* variants in France. This study aims at characterizing the molecular spectrum of *PCSK9*-based FH in France and identifying potential genotype/phenotype correlations and the founder effect of p.(Ser127Arg).

Method: *PCSK9* variants were searched through a diagnostic approach using Targeted next-generation sequencing genes panels. Five French families and 22 probands carrying p.(Ser127Arg) were selected for genotyping. Haplotypes were constructed with microsatellites (D1S200, D1S417, D1S2742) spanning *PCSK9* (GeneMapper® Software determined the alleles length (pb)) and SNPs in exon 1: p.(Leu21dup), exon 4: c.524-68G>C; c.524-90G>C; c.657+82A>G (Sanger sequencing) in two families. SNPs in exon 3: c.400-201A>G and exon 9: c.1664G>A will be genotyped with TaqMan® SNP Genotyping Assays.

Results: We identified new *PCSK9* rare variants: p.(Arg215Cys), p.(Asp367His), p.(Glu410Lys), p.(Arg495Trp), p.(Gly516Val), p.(Ala676Gly), all predicted deleterious. However, functional studies are needed to establish their real causative effect. The L11 allele p.(Leu21tri) reported associated with familial combined hyperlipidemia was found in 17 probands with varying phenotypes. Most of the L11 carriers present normal triglycerides levels indicating that this *PCSK9* variant can also lead to *bona fide* FH. The haplotype carrying p.(Ser127Arg) variant is the same in both families: 5'-188/190-L9-G-G-G-169-248-3'.The difference for D1S417 alleles, 188/190, can be due to the loss of one CA repeat in the microsatellite over generations or can mark the limit of the common haplotype and will help us to find the common ancestor. Genotyping of exons 3&9 SNPs and other subjects is ongoing.

Conclusions: These preliminary results show that the p.(Ser127Arg) gain-of-function variant in *PCSK9* is probably due to founder effect in France.

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A NOVEL SHORT INTERFERING RIBONUCLEIC ACID TO *LPA* INDUCES POTENT AND SUSTAINED REDUCTION OF SERUM LIPOPROTEIN (A) IN CYNOMOLGUS MONKEYS

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Aim: SLN360 is a liver targeted GalNAc-conjugated siRNA developed for the treatment of cardiovascular disorders associated with elevated lipoprotein (a) [Lp(a)], a key independent, genetically determined, causal cardiovascular risk factor that is not specifically addressed by current lipid lowering therapies. Targeting the *LPA* transcript in the liver with SLN360 will lead to sustained reductions in serum Lp(a).

Method: SLN360 was tested in vitro for *LPA* knockdown in primary hepatocytes. Healthy female cynomolgus monkeys (n=4/group) were dosed subcutaneously with SLN360 at 3 and 9mg/kg (single dose) or 3 weekly doses at 3mg/kg. Potency and duration of action were determined through mRNA analysis of liver biopsies taken 2, 4 and 6 weeks after final dosing and serial serum samples up to day 63 for peripheral biomarkers.

Results: In vitro, SLN360 specifically reduces *LPA* mRNA expression in human and cynomolgus hepatocytes. In vivo, serum SLN360 is almost entirely cleared 24 hours after s.c. injection. Maximal liver *LPA* reduction was observed two weeks after final subcutaneous injection regardless of treatment schedule (up to 91%), which was maintained 6 weeks after dosing in the 9mg/kg or 3x3mg/kg group (85% and 88% knockdown, respectively). Peak serum Lp(a) reductions (85->95%) were seen at day 21. Serum Lp(a) was still reduced by approximately 50% and 88% at day 63 after single 3 or 9mg/kg doses respectively, while repeated 3mg/kg dosing induced a >95% reduction until day 63. Additionally, no consistent dose- or time-dependent effect on the expression of *PLG* or a panel of sensitive markers of altered liver lipid or lipoprotein metabolism was observed in vivo.

Conclusions: SLN360 reduces *LPA* mRNA in vitro and in vivo resulting in a sustained reduction in serum Lp(a) levels in cynomolgus monkeys following s.c. dosing. SLN360 has potential to address the unmet need of Lp(a) reduction in cardiovascular diseases.

EVALUATION OF POLYGENIC SCORES IN THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA AND HYPOBETALIPOPROTEINEMIA

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Aim: Identification of causal mutation leads to diagnosis of monogenic hypobêtalipoproteinemia (FHBL) or hypercholesterolemia (FH). Nevertheless, in patients in whom no genetic origin could be identified, accumulation of common small-effect affecting LDL-cholesterol level alleles may result in polygenic hypobetalipoproteinemia or hypercholesterolemia phenotype. Here we aim to compare different weighted polygenic risk scores (wPRS) in order to improve the diagnosis of polygenic dyslipidemia.

Method: 2 new wPRS were set up: 1) 102 SNPs previously described as modulators of LDL-C level (PMID: 24097068, 24097064, 20686565, 19060906), and 2) 338 SNPs of GLGC GWAS 2013 reaching genome significance genotyped by our routine NGS diagnostic strategy. These wPRS were tested in 906 FH, 185 FHBL and 503 controls (1000 Genome Europe) and compared with other wPRS previously described: the 6 SNPs (PMID 25414277), 10 SNPs (PMID 27765764), 12 SNPs (PMID 23433573) and 33 SNPs (PMID 20686565) scores. ROC curves were used to assess the ability of wPRS to discriminate mutation negative patients (M-) vs healthy individuals.

Results: In FH, the 102 SNPs wPRS shows the best diagnosis abilities (AUC : 0.754, significantly superior to all others). A threshold > 1.5 (97.5th percentile) allows the detection of 18.1% of polygenic form within M- hypercholesterolemic patients. In FHBL, the 6 SNPs wPRS shows the best diagnosis abilities (AUC : 0.8, significantly superior to score 102 and 10). A threshold < 0.20 (3.5th percentile), allows the detection of 40.7% of polygenic form within M- hypocholesterolemic patients.

Conclusions: The incorporation of these genetic risk scores into molecular diagnosis improves Precision Medicine by the diagnosis of polygenic hypocholesterolemia and hypercholesterolemia. Moreover, increasing the number of SNPs in wPRS, using NGS strategies, allows best diagnosis ability.

Eligible for Young Investigator Oral Award

LIVER-SPECIFIC *GPR146* DOWNREGULATION ATTENUATES DYSLIPIDEMIA IN APOE*3-LEIDEN.CETP MICE

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Aim: GPR146 is an orphan G-protein coupled receptor that has been recently demonstrated to regulate plasma cholesterol in humans and mice. Genetic ablation of *Gpr146* in mice reduces LDL-c and atherosclerosis, supporting its further exploration as a novel promising target for lipid lowering. As a first step to learn whether these findings could translate to humans, we evaluate here the impact of liver-specific *Gpr146* down-regulation on plasma lipid levels and atherosclerosis using a humanized mouse model for lipoprotein metabolism.

Method: APOE*3-Leiden.CETP female mice were injected intravenously with Adeno-Associated virus 8 carrying a short-hairpin-RNA which targets, specifically in the liver, *Gpr146* (shRNA-GPR146) or a Scrambled-control. The mice were fed a Western-type diet for 20 weeks and plasma samples were collected every 4 weeks to monitor plasma lipid levels. Histological analysis of the aortic root will be performed to determine atherosclerotic plaque size.

Results: Compared to controls, mice treated with shRNA-GPR146 showed marked reductions in total plasma cholesterol (-34%; 11.3 vs 17.1 mM; $p=1.6x10^{-7}$), non-HDL-cholesterol (-35%; 10.4 vs 16.1 mM; $p=1.1x10^{-7}$) and triglycerides (-54%; 3.8 vs 8.3 mM; $p=9.9x10^{-8}$), while HDL-cholesterol levels did not show differences between treatments (0.93 vs 0.98 mM; p=0.7). The atherosclerosis plaque analysis is still in progress.

Conclusions: Liver-specific downregulation of *Gpr146* induces a protective shift of the lipoprotein profile in APOE*3-Leiden.CETP transgenic mice. As this mouse model has been shown to be highly predictive of the human response to an array of lipid-lowering drugs, our findings suggest that hepatic GPR146 downregulation, constitutes a promising strategy to treat dyslipidemia and to potentially attenuate atherosclerosis.

Eligible for Young Investigator Oral Award

STATINS SUPPRESS HEPATIC SECRETION OF ANGIOPOIETIN-LIKE 3 (ANGPTL3) VIA REDUCED LIVER X RECEPTOR (LXR) ACTIVATION

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Aim: Angiopoietin-like 3 (ANGPTL3) is a key regulator of plasma lipid concentrations and is a potential novel therapeutic target to lower LDL. It has been suggested that statins suppress hepatic *ANGPTL3* mRNA expression in humans, but the exact mechanism remains unknown. We investigated the effect of statins on *ANGPTL3* mRNA expression and ANGPTL3 secretion *in vitro* and established the effect of statin treatment on plasma ANGPTL3 concentrations in hypercholesterolemic subjects.

Method: Human hepatoma Huh7 cells were incubated with simvastatin, the squalene synthase inhibitor TAK475, the liver X receptor (LXR) agonist T0901317 and/or the LXR agonist GSK2230. Plasma ANGPTL3 concentrations were measured in genetically confirmed familial hypercholesterolemia (FH) patients of whom 91 received statins and 63 were statin naïve. In addition, the effect of stopping statin treatment was evaluated in 14 hypercholesterolemic subject.

Results: Both simvastatin and TAK475 reduced *ANGPTL3* mRNA expression and ANGPTL3 secretion in a dose dependent manner. T0901317 increased *ANGPTL3* mRNA expression and ANGPTL3 secretion by 6- and 3-fold, respectively, but simvastatin addition did not mitigate this effect. Co-incubation with GSK2230 diminished simvastatin-induced reductions in *ANGPTL3* mRNA expression and ANGPTL3 secretion. Both simvastatin and TAK475 reduced intracellular concentrations of oxysterols, known endogenous LXR ligands, implying that simvastatin and TAK475 suppress *ANGPTL3* mRNA expression via reduced oxysterol-mediated LXR activation. Plasma ANGPTL3 concentrations were 13% lower in FH patients on statin therapy compared to statin naïve FH patients (155 ± 49 vs. 178 ± 52 ng/ml, p=0.0057). In the 14 hypercholesterolemic subjects, plasma ANGPTL3 concentrations increased by 21% (p=0.0003) after 4-week statin cessation.

Conclusions: Simvastatin lowers hepatic *ANGPTL3* mRNA expression and ANGPTL3 secretion by decreasing oxysterol-mediated LXR activation. In hypercholesterolemic subjects, statins lower plasma ANGPTL3 concentrations which might imply that the effects of ANGPTL3 lowering therapies depend on whether a patient is using a statin or not.

ANGPTL4 regulates LPL activity by catalyzing the unfolding of LPL monomers

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Aim: Intravascular processing of triglyceride-rich lipoproteins is mediated by lipoprotein lipase (LPL) bound to GPIHBP1, a membrane protein of endothelial cells. In the absence of GPIHBP1, LPL remains mislocalized within the subendothelial spaces, causing severe hypertriglyceridemia (chylomicronemia). The intravascular activity of LPL is controlled angiopoietin like proteins 3, 4 and 8 (ANGPTL). We have previously shown that ANGPTLs inactivate LPL by catalyzing the irreversible unfolding its α/β-hydrolase domain (Mysling et al 2016, eLife 20958; Kristensen et al 2018 PNAS 115, E6020).Dogma has held that these ANGPTLs inactivate LPL by converting LPL homodimers into monomers, rendering them highly susceptible to spontaneous unfolding and loss of enzymatic activity. We now report that the functional unit of LPL is the monomer state—not the head-to-tail dimer.

Method: With highly purified proteins and contemporary biophysical methods (SPR, HDX-MS, SAXS and X-ray crystallography), we study the molecular interplay between LPL, GPIHBP1 and ANGPTL4.

Results: Some of our key observations are: *1)* we were able to isolate LPL monomers using an anti-LPL monoclonal antibody (5D2); *2)* this LPL monomer is as stable as LPL dimers; *3)* the inactivation by ANGPTL4-catalyzed unfolding of LPL is targeting the monomer state of LPL directly and not via promoting LPL dimer dissociation into unstable LPL monomers; and *4)* binding of GPIHBP1 to these LPL monomers mitigates the ANGPTL4 inhibition.

Conclusions: Our findings thus necessitate changes to long-standing dogma on mechanisms for LPL inactivation by ANGPTL proteins. Understanding the molecular mechanism by which ANGPTL4 binding initiates the chain of events leading to the irreversible unfolding of the α/β-hydrolase domain will most likely identify the Achilles heel in LPL structure and provide possible ways to stabilize LPL (Kristensen et al 2020 PNAS 117, 4337).

HAND2 REGULATES ADIPOGENESIS VIA THE GLUCOCORTICOID SIGNALING PATHWAY

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Aim: While adipocyte dysfunctions induced by obesity are associated with systemic metabolic disturbances, the formation of new adipocytes and healthy adipose tissue expansion is associated with metabolic benefits. Understanding the molecular mechanisms governing adipogenesis is of great clinical importance to restore metabolic health in obesity. Here we investigate the role of Heart- and neural crest derivatives-expressed protein 2 (HAND2) in adipogenesis.

Method: For mechanistical cell culture experiment, we used cultured primary adipocytes from human and mice and human multipotent adipose derived stem (hMADS) cells. RNAi were used to silence *HAND2* and adipogenesis efficiency was measured by Oil Red O staining, qPCR and microarray. RNASeq approach was used to identify gene clusters regulated by HAND2. *In vivo*, we created a conditional adipocyte *Hand2* deletion mouse model using Cre under control of the *Adipoq* promoter (HAND2^{AdipoqCRE}).

Results: HAND2 is a transcriptional factor link to obesity, is enriched in white adipocytes, induced early in differentiation and responds to dexamethasone, a typical glucocorticoid receptor (GR, encoded by *NR3C1*) agonist. Silencing of NR3C1 *in vitro* results in diminished *HAND2* expression, establishing that HAND2 is regulated by glucocorticoids via GR *in vitro* and *in vivo*. Silencing HAND2 *in vitro* impairs adipocyte differentiation. However, *in vivo* HAND2^{AdipoqCRE} mice did not show similar effects.

Conclusions: HAND2 plays an important role in adipocyte differentiation and highlight new mechanisms of regulation of GR dependent adipogenesis common to human and mice.

A ROLE FOR GALNAC-T2 DEPENDENT GLYCOSYLATION IN THE REGULATION OF INSULIN SENSITIVITY

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Aim: Previously, a role for o-linked glycosylation mediated by GalNac-T2 has been established in HDL metabolism. Loss of *GALNT2* has been shown to decrease HDL-cholesterol in humans, mice, rats and non-human primates. Recent human studies, however, have also suggested a role for *GALNT2* in type 2 diabetes and obesity. In line with this we also observed increased bodyweight, liver weight and fasting blood glucose levels in *Galnt2*$^{-/-}$ mice compared to WT littermates in response to a 12 week high fat diet (HFD). In the current study we aimed to investigate the mechanism by which GalNac-T2 affects the development of metabolic disease independent of bodyweight differences.

Method: *Galnt2*$^{-/-}$ and WT mice were fed a low fat diet prior to a 6 weeks HFD challenge. Indirect calorimetry, glucose homeostasis and o-glycosylation of (potential) target proteins were analyzed.

Results: In response to the LFD, *Galnt2*$^{-/-}$ mice displayed a decreased respiratory exchange ratio, indicative of more lipid oxidation compared to WT mice. This difference, however, disappeared upon HFD feeding, where both groups of mice completely relied on lipid oxidation. *Galnt2*$^{-/-}$ mice also displayed decreased glycosylation of the insulin receptor (INSR) in liver, muscle and adipose tissue. In line with this observation *Galnt2*$^{-/-}$ mice were more insulin resistant and exhibited higher fasting blood glucose levels than WT mice, conjoined by increased plasma non-esterified free fatty acid levels and decreased gonadal white adipose tissue weight. This suggests a reduced insulin-mediated suppression of adipose lipolysis. Finally, increased ectopic lipid accumulation was found as reflected by increased hepatic steatosis, liver mass and liver-to-BW ratio.

Conclusions: Together, our findings suggest a novel role for GalNac-T2 in glucose and energy homeostasis through glycosylation of the INSR and ultimately affecting insulin sensitivity.

Eligible for Young Investigator Oral Award

NFE2L1 PROTECTS WHITE ADIPOCYTES FROM CHOLESTEROL TOXICITY TO MAINTAIN PROTEOSTASIS

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Aim: Cholesterol is an important nutrient that is heavily regulated both in biogenesis as well as in elimination. However, how adipocytes buffer cholesterol, particularly in obesity, remains largely unclear. The cholesterol-sensing transcription factor Nuclear factor erythroid-2, like-1 (Nfe2l1) regulates proteostasis by stimulating protein degradation via the proteasome. Also, Nfe2l1 binds and senses excess of cholesterol in the endoplasmic reticulum (ER) membrane, which influences the transcriptional activity of Nfe2l1. Here, we investigated the role of cholesterol-sensing by Nfe2l1 for proteostasis and adipocyte health.

Method: For mechanistic cell culture experiments, we used human mesenchymal stem cells engineered with telomerase that were differentiated into adipocytes. Nfe2l1 was silenced using RNAi and the adipocyte stress levels were measured by qPCR after treatment with cholesteroland and the proteasome inhibitor epoxomicin. In vivo, we studied a Cre-loxP transgenic mouse model, in which Nfe2l1 was deleted in adipocytes using Adipoq-Cre.

Results: Cholesterol treatment did not induce adipocyte stress unless Nfe2l1 was silenced or the cells were treated with epoxomicin. Under these conditions, cholesterol treatment markedly and specifically induced ATF3, which is a surrogate stress marker for ER stress and inflammation. In vivo, loss of adipocyte Nfe2l1 was associated with ER stress, inflammation in white adipose tissue as well as with systemic insulin resistance.

Conclusions: Adipocytes represent a cholesterol reservoir, but this function requires Nfe2l1 as an excess cholesterol sensor. In the absence of Nfe2l1, cholesterol causes significant toxicity and disturbed proteostasis, ultimately resulting in ER stress and adipose tissue inflammation possibly via activation of ATF3. Our results identify a novel relay system integrating nutrient sensing and metabolic health.

Eligible for Young Investigator Oral Award

PHARMACOLOGICAL TREATMENT WITH FIBROBLAST GROWTH FACTOR 21 STRONGLY IMPROVES PLASMA CHOLESTEROL METABOLISM TO REDUCE ATHEROSCLEROSIS

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Aim: Fibroblast growth factor 21 (FGF21), a key regulator of energy metabolism, is currently evaluated in humans for treatment of type 2 diabetes and nonalcoholic steatohepatitis. However, the effects of FGF21 on cardiovascular benefit, particularly on lipoprotein metabolism in relation to atherogenesis, remain elusive.

Method: Female APOE*3-Leiden.CETP mice, a well-established model mimicking atherosclerosis initiation and development in humans, were used. These mice were fed a Western-type diet, and subcutaneously injected with either FGF21 (1 mg/kg body weight) or vehicle three times per week for 16 weeks. At the end of the study, kinetic lipid clearance was studied and atherosclerotic lesion area, severity and composition were assessed in the aortic root.

Results: FGF21 treatment reduced plasma total cholesterol, explained by a reduction in non-HDL-cholesterol. Mechanistically, FGF21 promoted brown adipose tissue (BAT) activation and white adipose tissue (WAT) browning, thereby enhancing the selective uptake of fatty acids from triglyceride-rich lipoproteins into BAT and into browned WAT, consequently accelerating the clearance of the cholesterol-enriched remnants by the liver. In addition, FGF21 reduced body fat and markedly reduced hepatic steatosis, related to increased hepatic expression of genes involved in fatty acid oxidation and triglyceride secretion. Ultimately, FGF21 largely decreased atherosclerotic lesion area, which was mainly explained by the reduction in non-HDL-cholesterol as shown by linear regression analysis, and improved lesion severity and increased the atherosclerotic plaque stability index.

Conclusions: FGF21 improves hypercholesterolemia by accelerating triglyceride-rich lipoprotein turnover as a result of activating BAT and browning of WAT, thereby reducing atherosclerotic lesion severity and increasing atherosclerotic lesion stability index. We have thus provided mechanistic insight and support for the clinical use of FGF21 in the treatment of atherosclerotic cardiovascular disease.

Eligible for Young Investigator Oral Award

GENETIC INHIBITION OF GPR146 IS ASSOCIATED WITH IMPROVED CARDIOVASCULAR AND METABOLIC HEALTH

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Aim: Reducing the burden of atherosclerotic cardiovascular disease (ASCVD) remains a challenge as its prevalence is exacerbated by metabolic disorders linked to the global pandemic of obesity. Additional efficacious and affordable treatment options are needed to prevent harmful adverse effects from existing treatments and further reduce the risk of ASCVD in high risk patients or in patients refractory to existing therapies. Through studies in mice, the G Protein-Coupled Receptor 146 (encoded by GPR146 gene) was recently suggested to be a promising drug target. However, studies in humans remain limited.

Method: In large cohort studies (eQTLgen; 31,684 samples, UK Biobank; 361,194 individuals, CARDIoGRAMplusC4D; 184,305 individuals, DIAGRAM; 159,208 individuals), we found and used common genetic variants affecting the expression of the *GPR146* gene (eQTL) as instruments to investigate associations with plasma lipid traits, risk of coronary artery disease, metabolic traits and type 2 diabetes.

Results: Individuals with reduced *GPR146* expression present with: i) lower plasma levels of plasma total cholesterol (P=2.3e-08), HDL cholesterol (P=9.9e-10) and LDL cholesterol (P=1.3e-05); ii) lower risk of coronary artery disease (P=0.03); iii) lower levels of circulating liver enzymes (alkaline phosphatase (P=3.4e-05), aspartate aminotransferase (P=9.5e-04) gamma-glutamyltransferase (P=1.2e-06), iv) lower plasma Hb1Ac levels (7.8e-03) and lower risk of type 2 diabetes (P=6.5e-03). Individuals with increased *GPR146* expression exhibit opposite phenotypes.

Conclusions: This study shows that the genetic inhibition of *GPR146* is associated with an unusual and unique favorable phenotype which combines reduced plasma lipids levels and protection against ASCVD with, unexpectedly, reduced risk of Type 2 diabetes and favorable effects on liver function. These data support GPR146 as a possible new target for pharmacological intervention in cardiometabolic diseases.

A RANDOMIZED CONTROLLED TRIAL OF AN INNOVATIVE, USER-FRIENDLY, INTERACTIVE SMARTPHONE APP-BASED LIFESTYLE INTERVENTION FOR WEIGHT LOSS

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Aim: Most electronic lifestyle interventions for weight loss are labor-intensive, requiring logging onto websites and manually recording activity and diet. Minimal weight loss, or even weight gain, seen with electronic lifestyle interventions could be attributed to the cumbersome technology used, and lack of a human coach. We created a comprehensive electronic lifestyle intervention that was delivered on a user-friendly, interactive smartphone app-based platform with both professional and peer coaching. We evaluated its effectiveness in a randomized controlled trial.

Method: Adults with BMI 25-42 kg/m^2, with sedentary jobs and smartphones, were randomized to receive the app-based intervention for six months, along with conventional outpatient weight-management visits with a physician every three months, or to a wait-listed control group that received only the conventional outpatient weight-management visits. The intervention included apps, wearable activity trackers, smartscales, food photography logs, physician-driven app-based behavioral coaching, and peer support via the app. The pre-specified primary outcome was a comparison of the changes in body weight in kg, from baseline to six months, in the intervention versus control groups.

Results: At six months, we found a statistically significant, clinically meaningful difference in weight change of -4.16±2.01 kg (mean±SE, 95% CI -8.29 to -0.02, $p<0.05$, intervention versus control, pre-specified primary outcome). Waist circumference and hemoglobin A_{1c} significantly improved ($p<0.01$ and $p<0.05$, respectively, intervention versus control, pre-specified secondary outcomes). Weight change in the intervention group significantly correlated with numbers of food photographs participants shared (rho=-0.8681, $p<0.01$), numbers of their text messages (rho=-0.8077, $p<0.01$), number of times and days each participant stepped on the smartscale (rho= -0.736, $p<0.01$; rho= -0.608, $p<0.05$, respectively), and mean daily step counts (rho= -0.5549, $p<0.05$).

Conclusions: Our app-based lifestyle intervention produced statistically significant, clinically meaningful weight loss and improved metabolic health. Engagement with the app-based intervention correlated strongly with successful weight loss.

SERUM LEVEL OF HDL PARTICLES ARE INDEPENDENTLY ASSOCIATED WITH LONG-TERM PROGNOSIS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Aim: HDL-Cholesterol (HDL-C) is not an accurate surrogate marker to measure the cardioprotective functions of HDL in coronary artery diseases (CAD) patients. Hence, measurement of other HDL-related parameters may have prognostic superiority over HDL-C. In this work, we examined the predictive value of HDL particles profile for long-term mortality in CAD patients and to compare its informative value to that of routinely available HDL markers, HDL-C and apoA-I.

Method: HDL particles profile were measured by nuclear magnetic resonance (NMR) spectroscopy in a cohort of 214 male patients (45-74 years) with established, angiographically documented, CAD. The patients' vital status was yearly assessed and mortality was recorded, distinguishing all-cause mortality, cardiovascular mortality and other causes of death. The predictive value of HDL particles concentration and distribution for long term prognosis was evaluated, taking into account an extended panel of potential confounders related to cardiovascular risk and heart condition, including LVEF and Gensini score.

Results: Median follow up was 12.5 years with a 36.4% mortality rate. Cardiovascular mortality accounted for 64.5 %. Mean concentrations of total HDL particles (HDL-P), small-sized HDL (SHDL-P) and apoA-I were lower in deceased than in surviving patients whereas no difference was observed according to HDL-C and large HDL particles. All NMR-HDL measures were correlated between themselves and with other HDL markers (HDL-C, apoA-I and LpA-I). In a multivariate model adjusted for cardiovascular risk factors and bioclinical variables (age, smoking, treatment for dyslipidemia, eGFR, LVEF, duration of CAD and Gensini score), HDL-P and SHDL-P displayed the strongest inverse association with all-cause and cardiovascular mortality. Weaker associations were recorded for apoA-I.

Conclusions: Based on our results, we conclude that the concentration of total HDL particles and small-sized HDL particles may serve as a better prediction tool than HDL-C and apoA-I to assess long term prognosis in population at high risk.

THE COATOMER (COP I) COMPLEX LIMITS UPTAKE OF BOTH LDL AND HDL IN HEPATOCYTES IN VITRO BUT REGULATES HDL-CHOLESTEROL LEVELS ONLY

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Aim: The liver removes both LDL and HDL from the circulation. The LDL receptor (LDLR) takes up LDL-holoparticles. Scavenger-receptor SR-BI mediates selective uptake of HDL associated lipids. The mechanisms mediating or regulating the endocytosis of HDL-holoparticles are little understood.

Method: We performed a genome wide RNA interference screening for genes limiting uptake of fluorescent HDL or LDL into Huh7 hepatocarcinoma cells. We used other siRNAs to knock-down individual candidate genes in Huh7 cells to measure their effect on the uptake of HDL or LDL and the cell surface abundance of LDLR or SR-BI with FACS, on the mRNA expression of LDLR and SCARB1 by RT-PCR, and the glycosylation of LDLR or SR-BI by Western Blotting. We explored UK biobank data for the association of mutations in candidate genes with LDL-c and HDL-c levels.

Results: Three and six out of nine members, respectively of the COP I complex were among the top hits limiting both LDL and HDL uptake into Huh7 cells. Knock down of each COP I subunit markedly reduced the uptake of radioiodinated LDL but not radioiodinated HDL. However, if recorded by FACS, RNA interference with COP I genes reduced the uptake of both fluorescent lipids and proteins of HDL particles as well. Loss of COP I genes reduced the cell surface abundance and interfered with the normal glycosylation of both LDLR and SR-BI. SNPs and rare variants of several COP I genes are associated with differences in HDL-C but not LDL-C.

Conclusions: Our findings suggest that the COP I genes regulate the uptake of both LDL and HDL, the glycosylation and trafficking of LDLR and SR-BI as well as the plasma levels of HDL-C.

NMR-BASED LIPOPROTEIN AND GLYCOPROTEIN PROFILING IMPROVES TRADITIONAL CLASSICAL CARDIOVASCULAR RISK-BASED PREDICTION IN TYPE 2 DIABETIC SUBJECTS: THE LIPOCAT STUDY

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Aim: Patients with type 2 diabetes mellitus (DM2) are at higher cardiovascular disease (CVD) risk. ^{1}H-Nuclear Magnetic Resonance (NMR) based-on methods for lipoprotein and glycoprotein profiling have been proposed for CVD risk assessment in this population, where atherogenic dyslipemia and inflammation are concomitant conditions. The primary objective of this study was to characterize the NMR-lipoprotein and glycoprotein profiles in a combined analysis of DM2 subjects from prospective population-based cohorts of 831 subjects; we also aimed to evaluate novel NMR- molecular panels compared to traditional risk factors (including age, gender, BMI, physical activity, hypertension, dyslipemia, standard lipids, HbA1c and smoking status) to predict CVD at follow-up.

Method: DM2 patients were selected from 3 different prospective cohorts (N= 272 ARTPER followed 10 years; N= 292 FIBROSCAN, 6 years; N= 267 LLEIDA, 8 years). Advanced NMR analysis included lipoprotein composition, size and subclass particle number characterization, and NMR-glycoprotein profiling including the concentration of the sugar-protein bonds -and their aggregation state- characterization. A cross-validated logistic regression (LR) analysis was performed to determine the reclassification index (RI) of NMR-parameters to predict CVD in DM2 patients.

Results: Inclusion of NMR-assessed parameters in the LR analysis showed a modest -but significant- higher discrimination ability to identify DM2 patients with future CVD RI=7%, compared to traditional parameters alone (AUC-ROC= 0.70 vs 0.68, $p<0.001$). The incidence of CVD was associated with several pro-atherogenic and pro-inflammatory lipoprotein and glycoprotein abnormalities in DM2 patients.

Conclusions: The use of ^{1}H-NMR-derived lipoprotein and glycoprotein profile improves the capability of traditional models for CVD prediction in DM2 subjects.

DIFFERENTIAL INSULIN SENSITIVITY OF NMR-BASED BIOMARKERS IN A TWO-STEP HYPERINSULINEMIC-EUGLYCEMIC CLAMP PROTOCOL

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Aim: Blood metabolomics is gaining wide spread attention for its potential to deliver biomarkers for cardiometabolic and other diseases. The hormone insulin is a main regulator of metabolism, but the effects of insulin levels on many metabolomics measures under euglycemic conditions are poorly characterized.

Method: Here, we set out to assess the insulin sensitivity of plasma biomarkers measured using 1H-NMR spectroscopy in apparently healthy insulin-sensitive individuals undergoing a two-step hyperinsulinemic-euglycemic clamp analysis. A total of 24 subjects (50% female) were included and plasma biomarkers were measured at 12 time points before and during the clamp study. The effects of low and high dose insulin infusion at constant glucose level on plasma biomarkers were analyzed using linear mixed effect models.

Results: Insulin dose dependently increased the number and size of LDL particles, increased the size of HDL particles and decreased the number of medium sized HDL particles. In addition, insulin dose dependently decreased the concentration of beta-hydroxybutyrate and branched amino acids.

Conclusions: These data indicate that hyperinsulinemia-euglycemia results in a lipoprotein profile that is unfavorable for CVD risk. In addition, plasma biomarkers are differentially insulin sensitive and this may have biomarker-specific implications for the development of insulin resistance.

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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.

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