SPONSOR OF YOUNG INVESTIGATOR AWARD 2018

AKCEA™ THERAPEUTICS

A subsidiary of Isis Pharmaceuticals, Inc.

24th Annual Scandinavian Atherosclerosis Conference

April 11-14, Krogerup Højskole, Humlebæk, Denmark

2018 Program

SCIENTIFIC COMMITEE	Tanja X. Pedersen (Denmark), Michael Davies (Denmark) Monique Mulder (The Netherlands), Katariina Öörnii (Finland) Marianne Benn (Denmark), Kirsten Holven (Norway) Stefano Romeo (Sweden), Vesa Olkkonen (Finland)
OFFICE	Christina Christoffersen Department of Clinical Biochemistry Rigshospitalet Blegdamsvej 9 2100 Copenhagen Denmark Phone: +45 3545 3011 Email: Christina.christoffersen@regionh.dk or abstract@ssar.dk
Organized by	
SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH:	Christina Christoffersen (Chairperson) Tuva Dahl (Secretary and Treasurer) Emil D. Bartels (Webmaster) Katariina Öörni Vesa Olkkonen Vilmundur Gudnason Gunnar Sigurdsson Trine Ranheim Stefano Romeo Paolo Parini
HOMEPAGE	www.ssar.dk (Emil D. Bartels, Webmaster)

Wednesday, April 11, 2018

16.00 – 18.00	Arrival, registration and coffee (dining room until 17.45)
18.00 – 19.30	Dinner
19.30 – 19.35	Welcome **Christina Christoffersen** *(Denmark)*
THE 2018 NIKKILÄ MEMORIAL LECTURES	
19.35 – 19.40	Introduction of the 2017 Nikkilä Lecturer **Katariina Öörni** *(Finland)* **and Anne Tybjærg-Hansen** *(Denmark)*
19.40 – 20.25	2018 Nikkilä Lecture: Shared risk factors for cardiovascular disease and dementia – epidemiologic, genetic and causal aspects **Ruth Frikke-Schmidt** *(Denmark)*
20.25 – 20.45	Discussion
20.45 –	Pub will be open

Thursday, April 12, 2018

08.00 – 09.00	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Chaired by **Tanja X. Pedersen** *(Denmark)* and **Michael Davies** *(Denmark)*
09.00 – 09.25	Human macrophages and their proinflammatory responses in atherosclerosis **Petri Kovanen** *(Finland)*
09.25 – 09.30	Discussion
09.30 – 09.45	CETP is produced by resting hepatic macrophages and modulated by lipopoly-saccharide: role of CETP in innate immunity? **Patrick Rensen** *(The Netherlands)*
09.45 – 10.00	Engineered regulatory T cell Adoptive Cell Therapy as a novel tool for the treatment of atherosclerosis **Fabrizia Bonacina** *(Italy)**
10.00 – 10.15	Diet-induced dyslipidemia alters the migration of regulatory T cells **Jacob Amersfoort** *(The Netherlands)**
10.15 – 10.30	The Antibody response in Atherosclerosis **Cristina Lorenzo Martín** *(Spain)**
10.30 – 11.15	Coffee, **poster walks (Session I)** and exhibitions
11.15 – 11.40	Inorganic nitrate and the control of oxygen supply and demand **Andrew Murray** *(United Kingdom)*
11.40 – 11.45	Discussion
11.45 – 12.00	Integrated precision proteomics as an effective way to identify novel pathophysiological mechanisms involved in atherosclerosis **Liam Ward** *(Sweden)**
12.00 – 12.15	Kidney-derived apolipoprotein M does not protect against acute kidney injury **Line Bisgaard** *(Denmark)**

12.15 – 12.30	Autoantibodies to Apolipoprotein A-1 as Independent Predictors of Cardiovascular Mortality in Renal Transplant Recipients **Josephine Anderson** *(The Netherlands)**
SESSION II	**CARDIOVASCULAR DISEASE** Chaired by **Marianne Benn** *(Denmark)* and **Kirsten Holven** *(Norway)*
12.30 – 12.55	Plasma ceramides predict cardiovascular death in patients with stable coronary artery disease and acute coronary syndromes beyond LDL-cholesterol **Reijo Laaksonen** *(Finland)*
12.55 – 13.00	Discussion
13.00 – 14.00	Lunch
14.00 – 15.00	**General meeting of the *Scandinavian Society for Atherosclerosis Research*** Open for all participants, decision on next year's topics and chairpersons
	Afternoon free for the Louisiana Museum of Modern Art (5 min walk), beach (5 min walk), Kronborg, the castle of Hamlet (12 min by train) or downtown Copenhagen (50 min by train)
16.30 – 17.30	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
17.45 – 18.45	Dinner
SESSION II	**CARDIOVASCULAR DISEASE – continued** Chaired by **Marianne Benn** *(Denmark)* and **Kirsten Holven** *(Norway)*
18.45 – 19.10	Lipoprotein (a) in cardiovascular disease **Børge Nordestgaard** *(Denmark)*
19.10 – 19.15	Discussion
19.15 – 19.30	High lipoprotein(a) and high risk of cardiovascular and all-cause mortality **Anna Langsted** *(Denmark)**

19.30 – 19.45	HDL Cholesterol Efflux Capacity is Associated with Incident Cardiovascular Disease in the General Population – a case-control study from PREVEND. **Uwe Tietge** *(The Netherlands)*
19.45 – 20.00	Unmet need for primary prevention in individuals with hypertriglyceridemia not eligible for statin therapy according to ESC/EAS guidelines: a contemporary population-based study **Christian Medom Madsen** *(Denmark)**
20.00 – 20.45	Coffee, **poster walks (Session II)** and exhibitions.
20.45 – 21.00	Barcoding in obese: who's at risk for the development of cardiovascular disease? **Margaux Fontaine** *(The Netherlands)**
21.00 – 21.15	Plasma transthyretin and risk of ischemic vascular disease in the general population: a prospective cohort study **Mette Christoffersen** *(Denmark)*
21.15 – 21.30	LDL cholesterol is more important for some forms of atherosclerotic disease than others. Using the Norwegian cohort of familial hypercholesterolemia as a model disease to generate new understanding of the role of LDL cholesterol. **Kjetil Retterstøl** *(Norway)*
21.30 – 21.45	Using metabolic profiling and gene expression analyses to explore molecular effects of replacing saturated fat with polyunsaturated fat- a randomized controlled dietary intervention study **Kirsten Holven** *(Norway)*
21.45 –	Pub will be open

Friday, April 13, 2018

08.00 – 09.00	Breakfast.
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Chaired by **Monique Mulder** *(The Netherlands)* and **Katariina Öörnii** *(Finland)*
09.00 – 09.25	Cellular immunity to LDL in atherosclerosis **Daniel Ketelhuth** *(Sweden)*
09.25 – 09.30	Discussion
09.30 – 09.45	Quercetin lowers plasma triglycerides accompanied by white adipose tissue browning in diet-induced obese mice **Eline Kuipers** *(The Netherlands)**
09.45 – 10.00	The ApoM/S1P axis controls triglyceride metabolism and brown fat activity **Anna Borup** *(Denmark)**
10.00 – 10.15	A diurnal rhythm in brown adipose tissue causes rapid clearance of plasma lipids at wakening **Wietse In het Panhuis** *(The Netherlands)**
10.15 – 10.30	Inhibition of the endocannabinoid system by treatment with a CB1R antagonist improves dyslipidemia **Robin van Eenige** *(The Netherlands)**
10.30 – 11.15	Coffee, **poster walks (Session III)** and exhibitions
11.15 – 11.40	The effect of LDL in monocyte-to-macrophage differentiation. **Lina Badimon** *(Spain)*
11.40 – 11.45	Discussion
11.45 – 12.00	Susceptibility of LDL particles to aggregate is reduced by PCSK9 inhibitor and by healthy diet **Maija Ruuth** *(Finland)**

12.00 – 12.15	Low LDL-cholesterol by PCSK9 variation and mortality in 109,800 individuals from the general population **Marianne Benn** *(Denmark)*
12.15 – 12.30	Metabolomic consequences of genetic inhibition of PCSK9 compared with statin treatment **Peter Würtz** *(Finland)*
12.30 – 12.45	Low HDL cholesterol as a "HbA1c" for long-term average elevated triglyceride-rich remnants: three Copenhagen studies including 120,828 individuals and patients **Børge Nordestgaard** *(Denmark)*
12.45 – 13.45	Lunch
SESSION IV	**OTHER TOPICS** Chaired by **Stefano Romeo** *(Sweden)* and **Vesa Olkkonen** *(Finland)*
13.45 – 14.10	Translational insights into Vascular Growth Factors **Kari Alitalo** *(Finland)*
14.10 – 14.15	Discussion
14.15 – 14.30	Intake of added sugars and all-cause and cardiovascular mortality **Stina Ramne** *(Sweden)****
14.30 – 14.45	Increased glucose and fatty acid metabolism and metabolic flexibility in myotubes from trained subjects **Nils Gunnar Løvsletten** *(Norway)****
14.45 – 15.00	OSBP-related protein 2 (ORP2) - a novel regulator of Akt signaling and cellular energy metabolism **Vesa Olkkonen** *(Finland)*
15.00 – 15.15	GPR120 agonism promotes metabolic health by stimulating calcium-dependent mitochondrial respiration in brown fat. **Maaike Schilperoort** *(The Netherlands)****
15.15 – 16.00	Coffee, **poster walks (Session IV)** and exhibitions

16.00 – 16.25	Human genetics of fatty liver disease **Stefano Romeo** (*Sweden)*
16.25 – 16.30	Discussion
16.30 – 16.45	Very high plasma levels of HDL cholesterol and risk of dementia in the general population. **Emilie Kjeldsen** *(Denmark)**
16.45 – 17.00	Vascular disease, related risk factors and development of dementia – a nationwide study of the Danish population. **Jesper Q. Thomassen** *(Denmark)*
17:00 – 17:15	Absolute 10 year risk of dementia by age, gender and APOE genotype for targeted prevention in 104,537 individuals from the general population. **Katrine L. Rasmussen** *(Denmark)**
17.15 – 17.30	Dietary Sargassum fusiforme improves memory and reduces amyloid plaque load in an Alzheimer's disease mouse model. **Monique Mulder** *(The Netherlands)*
17.30 – 19.00	Time free
19.00 – 19.30	**Cocktail**
19.30 –	**Banquet and dancing**

Saturday, April 14, 2018

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

24th Annual Scandinavian Atherosclerosis Conference

April 11-14, Krogerup Højskole, Humlebæk, Denmark

2018 Posters

Thursday, April 12, 2018

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

SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY**
No 21	Pathways of endothelial dysfunction during inflammation in atherosclerosis **Clare Hawkins** *(Denmark)*
No 22	Does hypochlorous acid (HOCl) induced modification of extracellular matrix (ECM) of the arterial wall contribute to atherosclerosis? **Cai Huan** *(Denmark)*
No 25	Hypercholesterolemia affects macrophage metabolism and function **Sanne Verberk** *(The Netherlands)*
No 26	Characterisation of extracellular matrix materials in human atherosclerotic lesions **Christine Chuang** *(Denmark)*
No 30	ATP citrate lyase as a negative regulator of inflammatory macrophage responses **Jeroen Baardman** *(The Netherlands)*
No 36	White Blood Cell Differential Count and Screening-Detected Abdominal Aortic Aneurysms in Men **Åsa Tivesten** *(Sweden)*
YIA Poster walk **10.30 – 11.15**	**Selected abstracts (2 min presentation + 1 min discussion)**
No 27	Association between plasma omega 6 fatty acids and expression of inflammation-related genes in peripheral blood mononuclear cells: a cross-sectional study in healthy children **Jacob Juel Christensen** *(Norway)**
No 29	IFNy-stimulated B cells effectively inhibit T follicular helper cells and reduce atherosclerosis by expression of high levels of PD-L1 **Hidde Douna** *(The Netherlands)**
No 31	Mcl-1 deficient macrophages lead to a pro-atherogenic, giant-cells enriched plaque phenotype **Lieve Temmerman** *(The Netherlands)**

No 32	Vaccination with ApoB100 derived HLA-A2 restricted CD8 T cell epitopes did not reduce atherosclerosis in male LDLrKO hApoB100tg HLA-A2tg mice **Frank Schaftenaar** *(The Netherlands)**
No 33	Increment of Chondrocyte-like Cells in Aortic Valves in Hypercholesterolaemic Mice **Jonna Weisell** *(Finland)**
No 35	Effects of a novel selenosugar on primary human vascular cells, mouse aortic rings and apoE-/- mice **Triantafyllos Zacharias** *(Denmark)**
SESSION II	**CARDIOVASCULAR DISEASE**
No 19	Intake of SFA compared to PUFA induce lower postprandial LDL receptor gene expression in PBMC in subjects with and without FH **Linn K. L. Øyri** *(Norway)*
No 91	Statin use is associated with carotid plaque composition: The Rotterdam Study **Blerim Mujaj** *(The Netherlands)*
No 92	Risk of nocturnal dipping status for coronary heart disease is driven by nighttime blood pressure level ***Wen-Yi Yang*** *(Belgium)*
YIA Poster walk 20.00 – 20.45	**Selected abstracts (2 min presentation + 1 min discussion)**
No 1	Lifestyle related cancer deaths in patients with familial hypercholesterolemia **Henriette Krogh** *(Denmark)**
No 5	Sex-specific analysis on efficacy and safety of the “Big-Five” cardiovascular drugs: systemic reviews and quantitative meta-analyses **Michelle Schreuder** *(The Netherlands)**
No 6	High lipoprotein (a) levels do not exacerbate aortic valve velocity in a group of FH subjects **Ingunn Narverud** *(Norway)**
No 7	Predilection of Low Protein C-induced Spontaneous Atherothrombosis for the Right Coronary Sinus in Apolipoprotein E-deficient mice **Amber Ouweneel** *(The Netherlands)**

No 9	Plasma methionine and risk of myocardial infarction: effect modification by established risk factors **Indu Dhar** *(Norway)**
No 14	Clinical familial hypercholesterolaemia and risk of peripheral arterial disease and chronic kidney disease: The Copenhagen General Population Study **Frida Emanuelsson** *(Denmark)**
No 16	Increased levels of the cysteine protease legumain in plasma and atherosclerotic plaques from patients with carotid stenosis **Ngoc Nguyen Lunde** *(Norway)**
No 88	Lipoprotein(a) levels are associated with stenosis in the carotid artery in patients with a mild-to-moderate stenosis **Dianne H.K. van Dam-Nolen** *(The Netherlands)**

Friday, April 13, 2018

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

SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT**
No 39	Treat-To-Target Familial Hypercholesterolemia (TTT-FH): A 8-10 years prospective study in adult patients with familial hypercholesterolemia (FH) treated in a specialized Lipid Clinic **Kjell-Erik Arnesen** *(Norway)*
No 41	Interrelation level of leptin, postprandial hypertriglyceridemia, endothelial dysfunction and body mass index in patients with coronary heart disease combined with non-alcoholic fatty liver disease **Mariya Grechanyk** *(Ukraine)*
No 44	Characterization of MDA-LDL-specific Antibody Response in Atherosclerosis **María Inmaculada Martos Folgado** *(Spain)*
No 54	Antioxidant capacity of HDL is strongly influenced by size and cholesterol level of this particle and cholesterol ester transfer activity **Maria Camila Pruper de Freiras** *(Brazil)*
No 56	Oxidized LDL stimulates production of inflammatory extracellular vesicles by platelets **Maarit Neuvonen** *(Finland)*
YIA Poster walk **10.30 – 11.15**	**Selected abstracts (2 min presentation + 1 min discussion)**
No 42	Genetic variants in APOM revealed no association with diabetes mellitus and ischemic heart disease in the general population **Stefan Hajny** *(Denmark)**
No 49	USF1 silencing promotes cholesterol efflux, prevents cholesterol uptake and alleviates inflammation in macrophages **Maija Ruuth** *(Finland)**
No 51	Treat-To-Target Familial Hypercholesterolemia (TTT-FH): A prospective study in adult patients with FH. Pregnant women have many years off lipid lowering medication **Karoline Randsborg** *(Norway)**

No 52	Testosterone reduces brown fat activity in male mice **Marta Lantero Rodriguez** *(Sweden)**
No 53	HDL Cholesterol Efflux Predicts New Onset Diabetes After Transplantation (NODAT) in Renal Transplant Recipients Independent of HDL Cholesterol Levels **Tamas Szili-Torok** *(The Netherlands)**
No 60	Effects of fish and krill oil on gene expression in peripheral blood mononuclear cells and circulating markers of inflammation: a randomised controlled trial **Amanda Rundblad** *(Norway)**
No 65	Added sugar intake and micronutrient dilution on a Swedish population **Esther Gonzalez-Padilla** *(Sweden)**
SESSION IV	**OTHER TOPICS**
No 69	A preliminary report of lipid levels and selected biomarkers of vascular changes in children with idiopathic headaches **Ilona Kopyta** *(Poland)*
No 71	Novel Biomarker discovery in Non-Alcoholic steatihepatitis (NASH) – translation of complex data into clear results **Tanja X. Pedersen** *(Denmark)*
YIA Poster walk **15.15 – 16.00**	**Selected abstracts (2 min presentation + 1 min discussion)**
No 57	Development of a novel rat model of diabetic cardiomyopathy **Louise Thisted** (*Denmark*)****
No 58	Proteoglycan 4 deficiency improves obesity phenotype in mice **Joya Nahon** *(The Netherlands)**
No 61	Adipokines affect PCSK9 expression – in vitro and in vivo evidence **Margherita Botta** *(Italy)**
No 63	Reduced Wnt signaling in South Asian compared with white Caucasian pre-diabetic men **Laura Janssen** *(The Netherlands)**

No 64	Anti-oxidative function of follicular fluid high density lipoproteins predicts outcomes of modified natural cycle IVF. **Ruxandra Nagy** *(The Netherlands)**
No 66	Cardiovascular risk of children and adolescents with epilepsy is reduced by modified ketogenic diet **Prudencio Mariana Baldini** *(Brazil)**
No 67	Dietary Supplementation of Galactooligosaccharides reduces intestinal fat absorption leading to lower fat accumulation and improved insulin sensitivity **Rima Mistry** *(The Netherlands)**

Oral Presentations – Abstracts –

Inflammation and Vascular Biology

SESSION I

CETP is produced by resting hepatic macrophages and modulated by lipopolysaccharide: role of CETP in innate immunity?

Zhuang Li[1,2], Sam J.L. van der Tuin[1,2], Jimmy F.P. Berbée[1,2], Sander S. Rensen[6], Jingyuan Fu[5,7], Albert K. Groen[4,5], Ko Willems van Dijk[1,2,3], Yanan Wang[1,2,5], Patrick C.N. Rensen[1,2]

[1]Dept. Medicine, Div. Endocrinology, Leiden University Medical Center, Leiden; [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden; [3]Dept. Human Genetics, Leiden University Medical Center, Leiden; [4]Amsterdam Diabetes Center, Academic Medical Center, University of Amsterdam, Amsterdam; [5]Dept. Pediatrics, University Medical Center Groningen, University of Groningen, Groningen; [6]Dept. Surgery, Maastricht University Medical Center, Maastricht; [7]Dept. Genetics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Background: We previously showed using mouse and human studies that CETP is primarily produced by hepatic macrophages rather than hepatocytes (Wang, Hepatology 2015). We now aimed to elucidate the subsets of hepatic macrophages that produce CETP, and to get insight into the biological function of CETP. **Methods & Results:** After ablation of hepatic macrophages in APOE*3-Leiden.CETP mice via injection with liposomal clodronate, the reappearance of hepatic gene and protein expression of CETP coincided with the resting Kupffer cell markers Clec4f and Vsig4, but not with the infiltrating monocyte marker Ly6C. Double-immunofluorescence staining showed that CETP co-localized with Clec4f+ macrophages and not Ly6C+ monocytes, suggesting that hepatic expression of CETP is confined to resting macrophages. Injection of from Gram-negative bacterial lipopolysaccharide (LPS) into these mice markedly decreased hepatic CETP expression and plasma CETP concentration without affecting hepatic macrophage number. This was paralleled by decreased hepatic expression of Clec4f and Vsig4, and an increase in Ly6C. Simultaneously, LPS transiently increased the ratio of plasma HDL-cholesterol over nonHDL-cholesterol. Likewise, in humans, microarray gene-expression analysis of liver biopsies revealed that hepatic CETP expression and plasma level of CETP both correlated with hepatic VSIG4 expression, and LPS administration decreased plasma CETP concentration. In vitro experiments with human bone-marrow-derived macrophages revealed that LPS reduces CETP expression dependent on LXR. **Conclusion:** Hepatic expression of CETP is exclusively confined to resting F4/80+Clec4f+Vsig4+Ly6C- Kupffer cells. LPS activates resting Kupffer cells to reduce Clec4f, Vsig4 and CETP expression, consequently decreasing plasma CETP and raising HDL. This sequence of events is consistent with a proposed role of CETP in determining the levels and function of HDL to combat invading Gram-negative bacteria.

Engineered regulatory T cell Adoptive Cell Therapy as a novel tool for the treatment of atherosclerosis

F. Bonacina[1], E. Martini[2], S. Garetto[2], F. Sala[1], S. Locatelli[1], A.L. Catapano[1,4], M. Kallikourdis[2] and G.D. Norata[1,3,5]

[1]Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy; [2]IRCCS Humanitas Research Foundation, Rozzano, Italy; [3]Centro SISA, Ospedale Bassini, Cinisello Balsamo, Italy; [4]IRCSS Multimedica, Milan, Italy; [5]School of Biomedical Sciences, Curtin Health Innovation Research Institute, Faculty of Health Science, Curtin University, Perth, Western Australia.

Aim: Inflammation is a major contributor of atherogenesis. As loss of anti-inflammatory activity of regulatory Tcells (Treg) is a pathogenic feature of (auto)immune mediated-disease, Treg-Adoptive Cell Therapy (ACT) is emerging as a therapeutic strategy to specifically modulate impaired immune responses. Although ACT has produced encouraging results in animal models, targeting site of action of transferred cells is still lacking. We aim at developing plaque-homing Treg for atherosclerosis treatment in animal models. **Methods:** Treg were retrovirally (IRES-EGFP vector) transfected with chemokine receptors or an empty vector and i.v. injected (about 2x105 GFP+ cells/mouse) in 8-week WTD male LDLR KO. Homing of transfected Treg to atherosclerotic plaque and, after 4-week WTD, its progression and composition was analysed by flow cytometry and histology (H&E, picro Sirius staining). **Results:** The chemokine CX3CL1 is selectively expressed in the aorta, but not in other tissues (lymph nodes, spleen and liver) ($p<0,01$) of 8-week WTD LDLR KO, contrary to CCL2, usually associated with inflammation during atherosclerosis. Therefore, we compared homing of CCR2- and CX3CR1-transfected Treg to the aorta. Although CCR2-tranfected Treg migrated to the aorta, they didn't show tissue specificity. Conversely, CX3CR1-Treg showed a specific homing to atherosclerotic plaques (2,5% of GFP+ out of lived cell compared to 0.6% of control) while no significant difference was observed in lymph nodes, spleen and liver. Next we investigated whether CX3CR1-Treg reduced atherosclerosis by performing plaque analysis 4 weeks after ACT. Altough levels of plasma cholesterol were comparable (about 560 mg/dL), plaque area was decreased by 72% (5,12% vs 18,78%, $p<0,01$) and stability, measured as collagen content, increased by 26% (9,87% vs 7,81%, $p<0,01$) in CX3CR1- compared to control-Treg treated mice. **Conclusion:** Overexpressing CX3CR1 appears a promising ACT to selectively home Treg into the plaque and limit atherosclerosis progression.

Diet-induced dyslipidemia alters the migration of regulatory T cells

J. Amersfoort[1], H. Douna[1], F.H. Schaftenaar[1], P.J. van Santbrink[1], G.H.M. van Puijvelde[1], I. Bot[1], A. Harms[2], T. Hankemeier[2], Y. Wang[3], H. Chi[3], J. Kuiper[1]

[1]Division of BioTherapeutics, LACDR, Leiden University, The Netherlands; [2]Division of Biomedicine and Systems Pharmacology, LACDR, Leiden University, The Netherlands; [3]Department of Immunology, St. Jude Children's Research Hospital, Memphis TN, USA

A hallmark of advanced atherosclerosis is a decrease in regulatory T cell (Treg) abundance inside atherosclerotic lesions. Cellular metabolism facilitates Treg migration towards sites of inflammation. As dyslipidemia drives atherosclerosis and creates a metabolically aberrant environment, we hypothesized that dyslipidemia inhibits the migration of Tregs towards lesions by changing their metabolism. To examine this, we compared Tregs isolated from mice with Western-type diet (WTD)-induced dyslipidemia to mice with normolipidemia which were fed a normal chow diet (NCD). We found that WTD induced cholesterol accumulation in Tregs (NCD 1252±21 vs WTD 1395±20 MFI, $p<0.001$). We examined mTOR activity as this is affected by cholesterol levels and mTOR regulates fatty acid oxidation (FAO). mTOR activity as measured by p-S6 levels was decreased (NCD 5888±209 vs WTD 5140±199 MFI, $p<0.05$) and FAO was elevated during a WTD accordingly (NCD 3215±1226 vs WTD 5907±2541, $p<0.05$). Next, we performed a diet-switch experiment in which we switched WTD-fed mice to an NCD to restore normolipidemia. The cholesterol- and FAO levels in Tregs normalized but mTOR activity remained diminished indicating that another factor mainly enhanced FAO during WTD. As this factor was sensitive to lipids, we postulated that the nuclear receptor PPARd enhanced FAO during a WTD. Treatment of Tregs with the PPARd agonist GW501516 (GW) increased their FAO levels (DMSO 1498±650 vs GW 3358±570, $p<0.05$) and their migratory capacity (DMSO 10±4 vs GW 16±4 cells, $p<0.01$). GW treatment increased the expression of Cpt1a and Slc25a20 which regulate FAO in Tregs and their expression was also increased by a WTD. A WTD also made Tregs migrate more efficiently in vitro towards atherosclerotic plaques (NCD 20±8.5 vs WTD 35±20, $p<0.01$). Altogether, WTD-induced dyslipidemia increases FAO in Tregs via modulation of mTOR and PPARd and increases their migration.

The antibody response in atherosclerosis

Cristina Lorenzo[1], Pilar Delgado[1], Inmaculada Martos[1], Sonia Mur[1], Christian Busse[2], Hedda Wardemann[2], Almudena Ramiro[1].

[1]Centro Nacional de Investigaciones Cardiovasculares, Madrid; [2]Deutsches Krebsforschungszentrum, Heidelberg.

Atherosclerosis is considered a chronic inflammatory disease with a strong autoimmune component. Both innate and adaptive immunity are involved in disease initiation and progression most likely through their response to endogenously modified structures, including oxidized lipoproteins. However, the B cell response during atherosclerosis development is very far from being understood, and the extent, quality and function of the antibody repertoire associated to atherosclerosis remain unknown. We aim at the characterization of the B cell response in atherosclerosis and at the analysis of the antibody repertoire associated with the disease, antigen specificity and their protective or atherogenic function. We have addressed the antibody response associated to atherosclerosis using low density lipoprotein-receptor deficient (LDLR-/-) mice fed with high fat diet (HFD). We found that the proportion of germinal center, plasma and memory B cells was significantly increased in the spleen of LDLR-/- HFD, revealing that atherosclerosis promotes a B cell response. This was accompanied by a progressive accumulation of antibodies against prototypic atherosclerosis antigens in the serum. In order to study the atherosclerosis-associated antibody repertoire at the single cell level we have performed high-throughput single cell sequencing of heavy and light chains of antibody genes. We found that atherosclerotic mice express an enriched fraction of IgM+, mutated antibodies and detected a distinct group of expanded clones associated to atherosclerosis. Cloning and expression of these antibodies has revealed reactivity against prototypic atherosclerosis antigens and atheroma plaque reactivity. Surprisingly, a great proportion of these antibodies displayed different plaque reactivity pattern compared with modified LDL particles, suggesting that additional antigens are triggering a humoral response. Ongoing pull down experiments followed by proteomics analysis will determine their antigen specificity. Moreover, the functional characterization of these antibodies in vivo will provide evidence for their atherogenic or protective properties.

Integrated precision proteomics as an effective way to identify novel pathophysiological mechanisms involved in atherosclerosis

Liam J. Ward[1,2], Wei Li[2], and Xi-Ming Yuan[1]

[1]Occupational and Environmental Medicine, Dept. of Clinical and Experimental Medicine, Faculty of Medicine and Health Sciences, Linköping, University, Linköping, Sweden; [2]Obstetrics and Gynaecology, Dept. of Clinical and Experimental Medicine, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden.

The heterogeneity of atherosclerotic tissue is a prominent obstacle for experimental analysis and understanding of atherosclerosis. Using proteomic techniques and the introduction of a novel intra-plaque sampling procedure, using biopsies from distinct lesion regions, we have developed a thorough depiction of the atheroma proteome. Resolution of individual proteomic patterns from 5 lesion regions (internal control, fatty streak, plaque shoulder, plaque centre and fibrous cap) was successful in reducing the effect of heterogeneity by revealing 15 previous unmapped proteins by using 2-DE and MALDI-TOF mass spectrometry (MS). This method also revealed specific region- and sex-specific alterations in the proteomes, including the sex-specific increases of ferritin light chain and transthyretin in men and women respectively. Moving forward, by means of tandem-MS and multivariate modelling, we identified 16 protein groups discriminating the atheroma proteome between sexes, most notably greater abundances of inflammatory response proteins in men, and blood coagulation and complement activation proteins in women. A greater abundance of iron metabolism proteins, hemopexin and serotransferrin, were also present in women. We next employed similar proteomic techniques to develop the stressed THP-1 macrophage proteome, by treatment with an atheroma-relevant mixture of oxysterols, similar to those that may be found in atherosclerotic plaques. The macrophages presented an imbalance in proteins associated with cell death and cellular longevity, lipid uptake and metabolism, and inflammatory response. In conclusion, we have set about an integrated proteomics approach in understanding atherosclerosis using precision proteomics from the advanced tissue level down to the simple cellular model in hopes of further elucidating the mechanisms behind atherosclerosis.

Kidney-derived apolipoprotein M does not protect against acute kidney injury.

Bisgaard LS[1,2], Christensen PM[1,2], Füchtbauer E[3], Nielsen LB[4], and Christoffersen C[1,2]

[1]Dept. of Clinical Biochemistry, Rigshospitalet, DK; [2]Dept. of Biomedical Sciences, University of Copenhagen, DK; [3]Dept. of Molecular Biology and Genetics and [4]The Faculty of Health Sciences, Aarhus University, Denmark.

Apolipoprotein M (apoM) is mainly expressed in liver and kidney proximal tubular epithelial cells. In plasma, apoM is associated with HDL particles via a retained signal peptide. ApoM is a carrier of sphingosine-1-phosphate (S1P), a small bioactive lipid involved in e.g. angiogenesis, lymphocyte trafficking, and vascular barrier function. Recently, it was shown that apoM/S1P also protects against development of liver fibrosis. In urine, apoM is normally undetectable in both wild type mice and healthy humans. However, lack of megalin receptors in proximal tubuli induces loss of apoM into the urine. The biological function of kidney-derived apoM is unknown. The purpose of this study was to unravel the role of apoM in kidney biology and in acute kidney injury. A Novel kidney specific human apoM transgenic mouse was generated by expressing human apoM under the control of the Sglt2 promoter (Sglt2-hapoM). Surprisingly, Sglt2-hapoM mice had human apoM in plasma (mean 0.18 µM), indicating that kidney-derived apoM can be secreted to plasma. The effect of kidney specific apoM overexpression on acute kidney injury was accessed using two experimental models, either cisplatin injection or ischemia/reperfusion injury. We did not observe any differences in kidney injury markers (urea and creatinine) between Sglt2-hapoM and wild type (WT) mice subjected to cisplatin injections. Also, kidney injury score determined by histological evaluation was similar in the two groups. Finally, genexpression of inflammatory markers (i.e. IL6, MCP-1) was similar in kidneys from apoM-deficient, WT and Sglt2-hapoM mice subjected to ischemia/reperfusion injury. In conclusion, kidney-derived apoM does not protect against acute kidney injury but contributes to plasma apoM levels.

Autoantibodies to Apolipoprotein A-1 as Independent Predictors of Cardiovascular Mortality in Renal Transplant Recipients

Josephine L.C. Anderson[1], Sabrina Pagano[2,3], Julien Virzi,[2,3] Robin P.F. Dullaart[4], Folkert Kuipers[1], Stephan J.L. Bakker[5], Nicolas Vuilleumier[2,*] and Uwe J.F. Tietge[1,*]

[1]Department of Paediatrics, [4]Endocrinology, [5]Internal Medicine, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; [2]Division of Laboratory Medicine, Department of Genetics and Laboratory Medicine, Geneva University Hospital, Geneva, Switzerland; [3]Department of Human Protein Sciences, Faculty of Medicine, Geneva University, Switzerland;
**These authors contributed equally to this work*

Aims: To study i) the prognostic accuracy of autoantibodies against apoA-1 (anti-apoA-1 IgG) for incidence of cardiovascular disease (CVD) specific and overall mortality in renal transplant recipients (RTR), known to have a high CVD burden only partly explained by traditional CVD risk factors, and ii) a possible relationship of anti-apoA-1 IgG with HDL functionality. **Methods and Results:** 462 prospectively included RTR were followed for 7.0 years. Baseline anti-apoA-1 IgG were determined and associations with incidence of CVD mortality (n=48), all-cause mortality (n=92) and graft failure (n=39) were tested. HDL functionality assessed in vitro by measuring anti-oxidative and cholesterol efflux capacity was not associated with anti-apoA-1 IgG. Kaplan –Meier analyses demonstrated significant associations of anti-apoA-1 IgG with CVD (log rank test among tertiles: P = 0.048). Cox regression showed that for each standard deviation increase of log transformed anti-apoA-1 IgG values, there was a 4-fold increase (Hazard Ratio[HR]: 4.00; 95% Confidence intervals [95%CI]:1.32-12.11; p=0.01) in risk for CVD mortality, and a more than 2-fold increase for all-cause mortality (HR:2.69, 95%CI:1.25-5.83; p=0.01), independent of adjustment for CVD risk factors, renal and HDL function. The association with all-cause mortality disappeared after excluding cases of CVD specific mortality. The sensitivity, specificity, positive predictive value and negative predictive value of anti-apoA-1 positivity for CVD mortality were 18.0%, 89.3%, 17.0% and 90.0% respectively. **Conclusion:** In conclusion, this prospective study demonstrates that in RTR, anti-apoA-1 IgG are independent predictors of CVD mortality. In RTR, anti-apoA-1 IgG are not associated with HDL functionality.

Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION II

High lipoprotein(a) and high risk of cardiovascular and all-cause mortality

Anne Langsted, MD, PhD[1,2], Pia R. Kamstrup, MD, PhD[1,2], Børge G. Nordestgaard, MD, DMSc[1,2,3,4]

[1]Department of Clinical Biochemistry and [2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark; [3]Faculty of Health and Medical Sciences, University of Copenhagen, Denmark; [4]Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Denmark.

Background: Several lipoprotein(a)-lowering therapies are currently being developed with the long term goal of reducing cardiovascular disease and mortality; however, the relationship between lipoprotein(a) and mortality is unclear. We tested the hypothesis that lipoprotein(a) levels are associated with risk of cardiovascular and all-cause mortality. **Methods:** We studied individuals from two prospective studies of the Danish general population, of which 69,764 had information on lipoprotein(a) concentrations, 98,810 on LPA KIV-2 number of repeats, and 119,094 on LPA rs10455872 genotype. **Results:** Observationally, lipoprotein(a) >93mg/dL(199nmol/L; 96th-100th percentiles) versus <10mg/dL(18nmol/L; 1st-50th percentiles) were associated with a hazard ratio of 1.43(95% confidence interval: 1.21-1.69) for cardiovascular mortality and of 1.21(1.10-1.32) for all-cause mortality. The median survival for individuals with lipoprotein(a) >93mg/dL(199nmol/L; 96th-100th percentiles) and 93mg/dL(199nmol/L; 1st-95th percentiles) were 83.9 and 85.1 years(log rank p=0.005). For cardiovascular mortality, a 50mg/dL(105nmol/L) increase in lipoprotein(a) levels was associated observationally with a hazard ratio of 1.14(1.08-1.21), and genetically with causal risk ratios of 1.22(1.08-1.40) based on LPA KIV2 and of 0.98(0.88-1.08) based on LPA rs10455872. For all-cause mortality, corresponding values were 1.06(1.03-1.09), 1.10(1.04-1.17), and 0.96(0.91-1.01), respectively. **Conclusions:** High levels of lipoprotein(a) through corresponding low LPA KIV-2 number of repeats were causally associated with high risk of cardiovascular and all-cause mortality. This suggests that lipoprotein(a)-lowering therapies has the potential to decrease cardiovascular and all-cause mortality.

HDL Cholesterol Efflux Capacity is Associated with Incident Cardiovascular Disease in the General Population – a case-control study from PREVEND

Sanam Ebtehaj[1], Eke G. Gruppen[2], Stephan J.L. Bakker[3], Robin P.F. Dullaart[2], and Uwe J.F. Tietge[1]

[1]Department of Paediatrics, [2]Department of Endocrinology, [3]Department of Internal Medicine University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

The usefulness of HDL cholesterol (HDL-C) as predictive biomarker for cardiovascular disease (CVD) has been questioned after disappointing outcomes of genetic and pharmacological intervention studies. Subsequently, the focus of attention is shifting towards evaluating anti-atherogenic functional properties of HDL. However, still limited data are available on the prospective association of HDL function metrics with CVD events. Therefore, the aim of the current work was to determine, if baseline HDL cholesterol efflux capacity (CEC) is associated with future CVD events in the general population. We carried out a prospective study among participants of the Prevention of REnal and Vascular End stage Disease (PREVEND) cohort (follow-up,12 years). The whole study population consists of n=8,592 subjects, of which 325 were excluded for having a previous CVD event; of the remaining 8,267 eligible participants all subjects with a new CVD event during follow-up (n=369) were selected and matched to controls for age, sex and HDL-C levels. CEC at baseline was quantified using incubation of human macrophage foam cells with apolipoprotein B-depleted plasma. Despite identical HDL-C and apoA-I levels between cases and controls CEC was significantly lower in cases ($p<0.001$). In all subjects combined, CEC was positively correlated with HDL-C and apoA-I and negatively with BMI, hsCRP and urinary albumin excretion. CEC was inversely associated with incident CVD events, both expressed per quartile and per 1 SD change ($p<0.001$); this association remained significant after adjustments for HDL-C, hsCRP, kidney function and several other clinical covariates. Combined these data demonstrate that in the general population baseline CEC is significantly associated with the future development of CVD events independent of HDL-C and apoA-I plasma levels.

Unmet need for primary prevention in individuals with hypertriglyceridemia not eligible for statin therapy according to ESC/EAS guidelines: a contemporary population-based study

Christian M. Madsen[1,2,3], Anette Varbo[1,2], Børge G. Nordestgaard[1,2,3]

[1]Department of Clinical Biochemistry and [2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark; [3]Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Aims: To identify individuals at high risk of atherosclerotic cardiovascular disease(ASCVD), who are not definite statin eligible according to the 2016 European Society of Cardiology/European Atherosclerosis Society(ESC/EAS) guidelines, based on high concentrations of plasma triglycerides. **Methods and results:** From the Copenhagen General Population Study(2003-2015) 58,547 individuals aged 40-65 and free of ASCVD, diabetes, and statin use at baseline were included. Of these, 14% were definite statin eligible, 7% were not eligible and had triglycerides =3.0mmol/L(264mg/dL), and 79% were not statin eligible and had triglycerides <3.0mmol/L(264mg/dL). During 456,057 person-years of follow-up, 1,770 individuals experienced a major adverse cardiovascular event(MACE) and 734 experienced a myocardial infarction(MI). The cumulative incidences of MACE at age 70 were 8.1%(95% confidence interval(CI):7.3-8.9%)) and 14.6%(12.6-16.8%) in statin non-eligible individuals with triglycerides <3.0mmol/L(264mg/dL) and =3.0mmol/L(264mg/dL), and 16.5%(14.0-19.3%) in statin eligible individuals. Corresponding cumulative incidences of MI were 3.0%(2.7-3.3%), 7.8%(6.4-9.5%), and 7.1%(5.9-8.4%), respectively. The estimated 10-year risks of MACE were 2.8%(2.6-3.0%) and 5.7%(4.9-6.6%) in statin non-eligible individuals with triglycerides <3.0mmol/L(264mg/dL) and =3.0mmol/L(264mg/dL), and 7.6%(6.9-8.3%) in statin eligible individuals; the median age in these 3 groups were 51, 51, and 60 years, respectively. Corresponding risks of MI were 1.0%(0.9-1.1%), 3.0%(2.4-3.7%), and 3.3%(2.8-3.7%), respectively. **Conclusion:** Statin non-eligible individuals with triglycerides =3.0mmol/L(264mg/dL) had risk of ASCVD similar to statin eligible individuals, defined according to the 2016 ESC/EAS guidelines. This illustrates an unmet need for primary prevention, calling for expansion of guidelines on statin eligibility, and the potential for placebo-controlled randomized clinical trials in individuals with hypertriglyceridemia.

Barcoding in obese: who's at risk for the development of cardiovascular disease?

MAC Fontaine[1], T Aliyev[1,3], H Jin1, E Wijnands[1], MMJ van Greevenbroek[2], CJH van der Kallen[2], CG Schalkwijk[2], CDA Stehouwer[2], L Temmerman[1], JC Sluimer[1], EAL Biessen[1].

[1]Department of Pathology, School for Cardiovascular disease (CARIM), Maastricht University Medical Center, The Netherlands; [2]Department of Internal Medicine, School for Cardiovascular disease (CARIM), Maastricht University, The Netherlands; [3]CERN Open Lab, CERN, Switzerland.

Obesity is known to predispose to cardiovascular diseases (CVD); however not all obese subjects will develop CVD. So far, known biomarkers lack the discriminative power to predict CVD risk at an early stage. In search of predictive biomarkers to stratify the obese, we considered adipokines, which are secreted by adipose tissue and are able to regulate key processes of atherosclerosis. In this study we set out to profile adipokines in plasma from a prospective cohort (Cohort On Diabetes and Atherosclerosis Maastricht, CODAM) of obese subjects that suffered or did not suffer a CVD event during 7-year follow-up. To this end, we included baseline (1999) plasma samples of 92 individuals. The samples were divided into three groups: obese patients with CVD (n=41) or no CVD (n=41) after 7-year follow-up and healthy lean controls (n=10) and were matched for age, sex, smoking status, type 2 diabetes and waist size. Expression levels of 182 adipokines in plasma were determined using a semi-quantitative antibody array (RayBiotech, L182). A multi-biomarker risk signature, which included 10 adipokines, was generated by uni- and multivariate feature selection and subsequent classification using logistic regression. The signature based prediction model was evaluated using c-statistic (AUC:0.86, 95% CI: 0.8182;0.9018 and accuracy:85%). In our final model, 6 of 10 adipokines significantly and independently predicted the CVD outcome. Furthermore, in a direct comparison of our model to well-known risk factors of CVD, it appeared to perform significantly better in predicting CVD in obese population, than systolic blood pressure, total cholesterol and fasting plasma glucose combined (AUC:0.53, 95% CI: 0.4659;0.5940 and accuracy:46.3%). Taken together, these results show very promising predictive power of our newly developed 10 adikopines-multibiomarker. *For patent reason, adipokine names are not revealed.

Plasma transthyretin and risk of ischemic vascular disease in the general population: a prospective cohort study

Mette Christoffersen MSc (Pharm.) PhD[1], Louise S. Hornstrup MD PhD[1], Ruth Frikke-Schmidt MD DMSc[1,2], Anne Tybjærg-Hansen MD DMSc[1,2]

[1]Department of Clinical Biochemistry, Rigshospitalet; [2]The Copenhagen City Heart Study, Bispebjerg Hospital; [1,2]Copenhagen University Hospitals and Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

Aim: Wildtype transthyretin can cause cardiac amyloidosis attributable to destabilization of transthyretin tetramers in plasma. We have recently shown that heterozygote carriers of a stabilizing genetic variant in the gene encoding transthyretin (TTR), T119M, had increased levels of serum transthyretin and a reduced risk of vascular disease compared to non-carriers. In the present study, we tested the hypothesis that low levels of transthyretin are associated with increased risk of ischemic vascular disease (IVD) in the general population. **Methods:** Plasma levels of transthyretin were measured in 7,263 individuals without IVD from the Copenhagen City Heart Study. During a mean follow-up of 16 years, 1,545 developed ischemic heart disease (IHD), 653 suffered a myocardial infarction (MI), 788 developed ischemic cerebrovascular disease (ICVD), and 615 suffered an ischemic stroke (IS). **Results:** Transthyretin concentrations decreased with increasing age, and were higher in men than in women in all age-groups. Multifactorially adjusted hazard ratios for IVD increased stepwise with decreasing transthyretin levels up to 1.52 (95% confidence interval, 1.20-1.93) for IHD, 1.75 (1.20-2.56) for MI, 1.48 (1.06-1.72) for ICVD and 1.90 (1.31-2.75) for IS, in individuals with transthyretin levels below the 6th percentile (extreme low) versus individuals with the highest levels of transthyretin (>80th percentile). Results were slightly attenuated after adjustment for C-reactive protein and albumin. **Conclusion:** Low plasma transthyretin level is an independent predictor of risk of IHD, MI, ICVD, and IS in the general population, even after adjustment for markers of inflammation and nutritional status.

LDL cholesterol is more important for some forms of atherosclerotic disease than others. Using the Norwegian cohort of familial hypercholesterolemia as a model disease to generate new understanding of the role of LDL cholesterol

Liv J Mundal[1,2], Jannicke Igland[3], Kirsten Holven[2,4], Martin P Bogsrud[4,5], Trond P Leren[6], Kjetil Retterstøl[1,2]

[1]The Lipid Clinic, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P. Box 4950 Nydalen, 0424 Oslo, Norway; [2]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Box 1110, Blindern, 0317 Oslo, Norway; [3]Department of Global Public Health and Primary Care, University of Bergen, Kalfarveien 31, 5018 Bergen, Norway; [4]National Advisory Unit for familial hypercholesterolemia, Oslo University Hospital, Rikshospitalet, P.O Box 4950, Nydalen Norway. [5]Unit for Cardiac and Cardiovascular Genetics, Oslo University Hospital, Oslo University Hospital, P. Box 4950 Nydalen, 0424 Oslo, Norway Norway.

Objective: Elevated LDL cholesterol increase the risk of atherosclerotic disease and it is generally accepted that LDL cholesterol play a more important role for the development of coronary heart disease than e.g for ischemic stroke. The aim of this study was to explore the role of LDL cholesterol in different types of atherosclerotic disease. We used subjects with a pathogenic familial hypercholesterolemia (FH) mutation as a model to explore the isolated role of elevated LDL cholesterol. **Methods:** We calculated the incidence of hospitalization for different atherosclerotic disease in all patients with a known pathogenic FH mutation in Norway (n= 4273) from 2001-2009 and compared it with the incidence in the entire Norwegian population. We estimated standard incidence ratios (SIRs) with 95% confidence intervals (95% CIs) by indirect standardization with incidence rates for the total population stratified by sex, calendar year and one-year age groups as reference rates. We defined incident cases as first time hospitalization. **Results:** In adult FH patients (25 years and above) there were 671 cardiovascular events during the observation period, 271 events in women and 392 in men. SIRs increased in the following order for men: Stroke 1.1 (0.7-1.8), cerebrovascular disease 1.2 (0.8-1.7). atrial fibrillation 1.9 (1.4-2.5), heart failure 2.3 (1.6-3.2), myocardial infarction 2.3 (1.8-3.0) peripheral arterial disease 2.7 (1.8-4.1), aortic aneurism 2.9 (1.7-5.0), coronary heart disease 4.2 (3.6-5.0), aortic stenosis 7.4 (5.0-10.9). In women the pattern was slightly different so that excess risk for heart failure was lower than for atrial fibrillation and excess risk for aortic aneurism was lower than for peripheral artery disease. **Conclusion:** The excess risk associated with elevated LDL cholesterol from birth showed a clear gradient across the various types of disease and aortic stenosis had the highest SIR in both men 7.4 (5.0-10.9) and women 8.5 (5.8-12.4).

Using metabolic profiling and gene expression analyses to explore molecular effects of replacing saturated fat with polyunsaturated fat- a randomized controlled dietary intervention study

Stine M. Ulven[1], Jacob J. Christensen[1], Ottar Nygård[2], Asbjørn Svardal[2], Lena Leder[1,3], Inger Ottestad[1], Lene F. Andersen[1] and Kirsten B. Holven[1,4]

[1]Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, Oslo, Norway; [2]Department of clinical medicine, University of Bergen; [3]Mills SA, Oslo, Norway; [4]National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway

Background: Replacing saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) reduces the plasma LDL cholesterol (LDL-C) and thereby the risk of cardiovascular disease (CVD). However, other molecular links between dietary intake of fatty acids, metabolic dysregulation and CVD remain to be discovered. **Objective:** The aim of the present study was to gain further understanding of the molecular effects of improved dietary fat quality on human health. **Method:** Recently we conducted a double blind randomized controlled trial replacing SFA with PUFA among healthy subjects with moderate hypercholesterolemia (n=99). In the present sub-study, we performed a comprehensive metabolic profiling using multiple platforms (both NMR and LC-MS technology), and we analysed peripheral blood mononuclear cells (PBMC) gene expression by quantitative real-time polymerase chain reaction (qPCR). **Results:** A large number of lipid subclasses, myristoyl- and palmitoylcarnitines, tryptophan and kynurenine were reduced when SFA was replaced with PUFA in the diet. In contrast, bile acids, certain ketone bodies and certain amino acids were increased by the intervention. Branched-chain amino acids and gut flora metabolites were unaffected. The mRNA levels of LDLR, LXRA, FASN, ABCG1, CXCR2, CD8A, GATA3, TNFSF14, and IRAK1 were increased, while the mRNA expression levels of UCP2, IRF4 and TNFRSF1A were decreased in PBMCs after replacing SFA with PUFA. **Conclusion:** Replacement of SFA with PUFA in the diet affected genes and metabolites involved in key metabolic events. Applying metabolomics in randomized controlled dietary intervention trials hold the potential to extend our knowledge of biological and molecular effects of dietary fat quality.

Oral Presentations – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Quercetin lowers plasma triglycerides accompanied by white adipose tissue browning in diet-induced obese mice

Eline N. Kuipers[1,2], Andrea D. van Dam[1,2], Ntsiki M. Held[3], Isabel M. Mol[1,2], Riekelt H.L. Houtkooper[3], Patrick C.N. Rensen[1,2], Mariëtte R. Boon[1,2]

[1]Department of Medicine, Division of Endocrinology, LUMC, Leiden, the Netherlands; [2]Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, the Netherlands; [3]Laboratory of Genetic Metabolic Diseases, AMC, Amsterdam, the Netherlands.

Background and aim: Obesity and dyslipidaemia are major risk factors for developing cardiovascular disease (CVD). Quercetin, a natural flavonoid, lowers plasma triglycerides (TG) in human intervention studies and its intake is associated with lower CVD risk. The aim of this study was to elucidate the mechanism by which quercetin lowers plasma TG levels in diet-induced obesity. **Methods and results:** 9-week-old C57Bl/6J mice received a high-fat diet (45% of calories derived from fat) with or without quercetin (0.1% w/w) for 12 weeks. Quercetin decreased plasma TG levels from 9 weeks on, without affecting food intake, body composition or energy expenditure. Mechanistically, quercetin did not decrease intestinal fatty acid (FA) absorption. Rather, quercetin induced a slight reduction in liver ApoB expression, which suggests decreased VLDL-TG production. Interestingly, quercetin also markedly increased uptake of [3H]oleate derived from glycerol tri[3H]oleate-labeled lipoprotein-like particles by the subcutaneous white adipose tissue (sWAT) together with markedly increased mRNA expression of Ucp1 specifically in sWAT. Accordingly, only quercetin-treated animals showed UCP-1 protein-positive cells in sWAT, which is fully compatible with increased browning. **Conclusion:** Quercetin decreases plasma TG levels and induces browning of sWAT, accompanied by a markedly increased TG-derived FA uptake by sWAT.

The ApoM/S1P axis controls triglyceride metabolism and brown fat activity

Christina Christoffersen[1,2], Christine K. Federspiel[1], Anna Borup[1,2], Pernille M. Christensen[1], Andreas N. Madsen[2,3], Markus Heine[6], Carsten H. Nielsen[5], Andreas Kjaer[5], Birgitte Holst[2,3], Joerg Heeren[6], and Lars B. Nielsen[1,2,4,7]

[1]Department of Clinical Biochemistry, Rigshospitalet, 2100 Copenhagen, Denmark; [2]Department of Biomedical Sciences, University of Copenhagen, 2200 Copenhagen, Denmark; [3]Novo Nordisk Center for Basic Metabolic Research, University of Copenhagen, 2200 Copenhagen, Denmark; [4]Department of Clinical Medicine, University of Copenhagen, 2200 Copenhagen, Denmark; [5]Department of Clinical Physiology, Nuclear Medicine & PET and Cluster for Molecular Imaging, Rigshospitalet and University of Copenhagen, 2200 Copenhagen, Denmark; [6]Department of Biochemistry and Molecular Cell Biology, Universitätsklinikum Hamburg-Eppendorf, 20246 Hamburg, Germany; [7]Present address: Aarhus University, Aarhus, Denmark

The understanding of energy metabolism is of crucial importance, as obesity and its consequences are an increasing burden on society. Triglycerides can be stored in white adipose tissue, whereas brown adipose tissue (BAT) utilizes triglycerides to generate heat. Activation of BAT improves the metabolic status in both patients with type II diabetes and rodents by e.g. improving insulin sensitivity. Apolipoprotein M (apoM) carries sphingosine-1-phosphate (S1P), which interacts with five receptors controlling diverse biological processes e.g. maintaining the endothelial barrier. Thus, the aim was to explore the role of apoM and S1P in energy metabolism. ApoM-deficient mice (KO) had increased amount of BAT compared to WT at room temperature (RT). Furthermore, apoM-KO mice had accelerated turnover of postprandial plasma triglycerides compared to WT primarily due to an increased uptake of triglycerides in BAT. ApoM-KO mice were also protected against diet-induced obesity and showed an improved glucose tolerance. Exposing the animals to cold or ß3-adrenergic stimulation increased, as expected, the BAT mass in WT mice, whereas no changes was seen in apoM-KO. Hence, apoM-KO mice at RT had a phenotype similar to cold-exposed WT mice. To investigate the changes in BAT function in apoM-KO mice, S1P and its receptors were explored. The distribution of S1P receptors on BAT showed S1P1 mainly expressed on endothelial cells and S1P3 on adipocytes. Antagonists of the S1P1 receptor lead to increased BAT mass and reduced postprandial triglycerides in WT mice, whereas the reverse effect on BAT mass was seen in apoM-KO mice treated with S1P1 agonist. These data indicate that modification of S1P1 receptor signaling can regulate the BAT mass and activity. Thus, the data reveal a new link between the apoM/S1P axis and energy metabolism by controlling postprandial triglyceride metabolism and diet-induced obesity.

A diurnal rhythm in brown adipose tissue causes rapid clearance of plasma lipids at wakening

Sander Kooijman[1,2], Wietse In het Panhuis[*2,3], Rosa van den Berg[1,2], Mahesh Umapathysivam[4], Fredrik Karpe[4], Maaike Schilperoort[1,2], Sander Kersten[3], Patrick C.N. Rensen[1,2]

*[1]Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands; [2] Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands; [3]Nutrition, Metabolism and Genomics group, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; [4]Oxford Centre for Diabetes, Endocrinology and Metabolism, Radcliffe Department of Medicine, University of Oxford, Oxford OX3 7LE, United Kingdom. *presenting author*

Background: Brown adipose tissue (BAT) has emerged as a novel target for the treatment of cardiometabolic disorders due to its capacity to increase energy expenditure through thermogenesis. Recently, we showed that circadian disruption by prolonged light exposure reduces BAT activity, resulting in adiposity (PNAS 2015). Since this finding implies the presence of a circadian component in BAT, we aimed to investigate whether BAT activity is rhythmic in mice and humans and to delineate underlying mechanisms. **Methods & Results:** In wild-type C57Bl/6J mice we injected glycerol tri[3H]oleate-labeled triglyceride (TG)-rich lipoprotein-like particles at 6 time points during a 24 h period, and observed a high amplitude rhythm in [3H]oleate uptake by BAT that synchronized with the light/dark cycle. Highest uptake was found at the onset of the active period, which coincided with high LPL expression and low Angptl4 expression. In APOE*3-Leiden.CETP mice diurnal rhythmicity in BAT activity determined the rate at which lipids were cleared from the circulation, thereby imposing the daily rhythm in plasma lipid concentrations. In mice as well as humans, postprandial lipid excursions were nearly absent at waking. Interestingly, in humans we observed a much higher cold-induced thermogenesis and temperature of BAT as assessed by thermal imaging in the morning as compared to the evening, indicating that BAT activity is also rhythmic in humans. **Conclusion & future perspectives:** We found a strong diurnal rhythm in the TG-derived fatty acid uptake by BAT, as reflected by the diurnal rhythm in LPL activity. Since the rhythm in LPL activity could be mediated by the antiphase rhythm in Angptl4 expression, we currently evaluate diurnal BAT activity in Angptl4-/- mice. We anticipate that diurnal BAT activity is an important factor to consider when studying the therapeutic potential of promoting BAT activity in cardiometabolic disorders.

Inhibition of the endocannabinoid system by treatment with a CB1R antagonist improves dyslipidemia

Robin van Eenige, Lauren L. Tambyrajah, Yanan Wang, Patrick C.N. Rensen, Sander Kooijman

Dept. of Medicine, Div. of Endocrinology, Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands.

Aim: The endocannabinoid system (ECS) is involved in several metabolic functions, such as energy storage and nutrient transport. We showed that cannabinoid receptor type 1 (CB1R) antagonism reverses obesity through activating brown fat. As activation of brown fat promotes the lipolytic conversion and subsequent hepatic clearance of triglyceride-rich lipoproteins, we assessed the effects of the CB1R antagonist rimonabant on VLDL metabolism in APOE*3-Leiden.CETP transgenic mice, a well-established mouse model for human-like lipoprotein metabolism. **Methods:** Female mice were fed a Western-type diet (containing 16% fat and 0.1% cholesterol) with or without supplementation of 0.017% w/w rimonabant. Body weight and food intake were monitored throughout the study. After 4 weeks, body composition was determined by echo-MRI and plasma triglycerides (TG) and cholesterol were measured in 4h fasted plasma samples. In addition, we measured energy expenditure by means of indirect calorimetry and investigated clearance of VLDL-TG and remnants. **Results:** Rimonabant transiently reduced food intake, which normalized after 4 days of treatment, and reduced fat mass after 4 weeks of treatment (-48%, $p<0.05$). Rimonabant lowered plasma cholesterol and TG levels (-31%, $p<0.05$ and -49%, $p<0.01$, respectively) and reduced the respiratory quotient during the light period (0.80 vs. 0.82, $p<0.01$) indicative of increased fatty acid oxidation. Rimonabant did not affect VLDL-TG-derived FA uptake by brown fat or hepatic clearance of remnants. **Conclusions:** In conclusion, rimonabant treatment of cholesterol-fed APOE*3-Leiden.CETP mice alleviates dyslipidemia independent of brown fat activation. Current experiments are focused on investigating underlying mechanisms and studying the potential benefit of CB1R antagonism for atherosclerosis development.

Susceptibility of LDL particles to aggregate is reduced by PCSK9 inhibitor and by healthy diet

Ruuth Maija K[1,2], Nguyen Su Duy[1], Vihervaara Terhi[3], Hilvo Mika[3], Uusitupa Matti[4], Schwab Ursula[4,5], Savolainen Markku J[6], Jauhiainen Matti[7,8], Käkelä Reijo[9], Baruch Amos[10], Laaksonen Reijo[3], Kovanen Petri T[1] & Öörni Katariina[1,2]

[1]Wihuri Research Institute, Helsinki, Finland; [2]Faculty of Medicine, Research Program Unit, University of Helsinki, Helsinki, Finland; [3]Zora Biosciences, Espoo, Finland; [4]School of Medicine, Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland; [5]Institute of Clinical Medicine, Internal Medicine, Kuopio University Hospital, Kuopio, Finland; [6]University of Oulu, Oulu, Finland, and Medical Research Center, Oulu University Hospital, Oulu, Finland; [7]Minerva Foundation, Institute for Medical Research, Helsinki, Finland; [8]Genomics and Biomarkers Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland; [9]Helsinki University Lipidomics Unit, Department of Biosciences, University of Helsinki, Helsinki, Finland; [10]Genentech Research and Early Development, South San Francisco, CA, USA

Aims: We have previously shown that inter-individual differences in the intrinsic propensity of LDL particles to aggregate upon modification associate with coronary death and that aggregation-prone LDL particles are enriched in sphingolipids. Here we examined whether in humans PCSK9 inhibition or dietary changes, both of which can influence LDL lipidome, would alter the susceptibility of LDL to aggregate. **Methods:** Plasma samples were derived from EQUATOR study, a randomized placebo-controlled phase II trial with a fully human monoclonal anti-PCSK9 antibody RG7652. LDL was isolated before and after treatment with RG7652 (n=25) or placebo (n=15) for 29 days. LDL was isolated also from healthy subjects participating in the SYSDIET study, in which they were randomly assigned to a Healthy Nordic diet (n=33) or a Control diet (n=24) for 18-24 weeks. Aggregation of LDL was triggered by lipolysis and followed by dynamic light scattering. LDL lipid composition was analysed by mass spectrometry. **Results:** Lipidomic analysis of LDL particles revealed that PCSK9 inhibitor decreased the proportion of sphingolipids and increased the proportion of phosphatidylcholines in LDL particles and rendered them less aggregation-susceptible. Increased consumption of vitamin E and decreased consumption of sucrose in the Healthy Nordic diet were associated with similar changes in LDL lipidome and the aggregation susceptibility of LDL. Placebo-treatment or control diet had only minor effects on LDL lipid composition and they did not alter the aggregation-susceptibility of LDL particles. **Conclusions:** PCSK9 inhibition and healthy diet reduce LDL-sphingomyelin and LDL aggregation susceptibility, which may partially explain the anti-atherosclerotic effects of these interventions.

Low LDL-cholesterol by PCSK9 variation and mortality in 109,800 individuals from the general population

Associate professor, chief physician Marianne Benn[1,3,5], Professor, chief physician Anne Tybjærg-Hansen[1,3,4,5], and Professor, chief physician Børge G. Nordestgaard[2,3,4,5]

[1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital, Denmark; [2]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark; [3]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark; [4]The Copenhagen City Heart Study, Frederiksberg Hospital, Copenhagen University Hospital, Denmark; and [5]Faculty of Health and Medical Sciences, University of Copenhagen, Denmark.

Introduction: Low levels of low-density-lipoprotein (LDL)-cholesterol due to inhibition of proprotein convertase subtilisin kexin 9 (PCSK9) may be associated with low risk of cardiovascular and all-cause mortality. **Hypothesis:** We tested whether low LDL-cholesterol due to genetic variation in PCSK9 (R46L (rs11591147), R237W (rs148195424), and I474V (rs562556)), is associated with cardiovascular mortality, cancer mortality, mortality from other causes than cardiovascular disease and cancer, and all-cause mortality in the general population. We tested this hypothesis in 109,800 individuals from two Danish general population studies, the Copenhagen General Population Study and the Copenhagen City Heart Study. **Results:** A weighted allele score of PCSK9 genetic variants was associated with an up to 0.47mmol/L (13.7%; p<0.001) lower LDL-cholesterol and 9.4g/L (8.3%; p<0.001) lower apolipoprotein B, but not with concentrations of lipoprotein(a), triglycerides, or high-density-lipoprotein-cholesterol. An increasing number of weighted PCSK9 alleles were associated with stepwise lower LDL-cholesterol and lower cardiovascular mortality (p-trend across alleles=0.007), with a hazard ratio of 0.56 (95%confidence interval: 0.35-0.90; p=0.016) in individuals with the highest number of weighted PCSK9 alleles compared to individuals with the lowest number. Median age at cardiovascular death was 84.4 (inter-quartile range: 74.0-88.8) versus 81.8 (74.2-87.5) years in individuals with genetically lowest versus highest LDL cholesterol. In genetic, causal analyses a 1 mmol/L lower LDL-cholesterol had risk ratios of 0.44 (0.20-0.98; p=0.045) for cardiovascular mortality, 0.99 (0.60-1.63; p=0.97) for cancer mortality, 1.18 (0.94-1.47; p=0.15) for mortality from other causes, and 0.98 (0.80-1.19; p=0.81) for all-cause mortality. **Conclusion:** Low LDL-cholesterol levels due to PCSK9 variants caused a lower risk of cardiovascular mortality, but had no causal effect on cancer mortality, mortality from other causes than cardiovascular disease and cancer, or all-cause mortality.

Metabolomic consequences of genetic inhibition of PCSK9 compared with statin treatment

Peter Würtz

University of Helsinki & Nightingale Health Ltd, Finland

Background: Both statins and PCSK9 inhibitors lower LDL cholesterol levels to reduce cardiovascular risk. To enhance the molecular understanding of these two therapies, we assessed the detailed metabolic effects of genetic variants in HMGCR and PCSK9 to mimic naturally occurring trials of the drug targets. **Methods:** 228 circulating metabolic measures, including lipoprotein subclasses, fatty acids, and amino acids, were quantified by NMR metabolomics for ~70,000 individuals. The metabolite associations with genetic inhibition of PCSK9 were compared to the corresponding associations for genetic variants in HMGCR (target of statins). The results were further compared with the detailed metabolic changes due to statins in two randomized trials. **Results:** For the same lowering in LDL cholesterol, the comprehensive metabolic effects of statin therapy were nearly identical to those caused by HMGCR gene variation (R2=0.95). However, both genetic and therapeutic inhibition of HMGCR was more efficacious at lowering cholesterol in VLDL particles than the predicted effect of PCSK9 inhibition. Association patterns were similar for alterations in lipoprotein lipid composition and fatty acid balance, and null associations across non-lipid metabolites. **Conclusions:** PCSK9 inhibition results in similar effects as statin therapy across the detailed metabolic profile. However, for the same lowering of LDL cholesterol, PCSK9 inhibition is predicted to be slightly less efficacious at lowering VLDL cholesterol, which could potentially translate into differences in cardiovascular risk reduction.

Low HDL cholesterol as a "HbA1c" for long-term average elevated triglyceride-rich remnants: three Copenhagen studies including 120,828 individuals and patients

Anne Langsted, Anne Marie Reimer-Jensen, Anette Varbo, Børge G. Nordestgaard.

Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Denmark

Aim: HDL cholesterol is inversely associated with plasma triglycerides. Elevated triglyceride-rich remnants represent a causal factor for ischemic cardiovascular disease, marked by low HDL cholesterol. We tested the hypothesis that low HDL cholesterol is a marker of average elevated triglycerides. **Methods:** We studied cross-sectionally 109,000 individuals from the Copenhagen General Population Study, dynamically 1000 patients from Copenhagen University Hospital with lipid measurement at 10 repeated hospital visits, short-term 300 individuals during an oral fat load, and long-term 10,000 individuals from the follow-up examination of the Copenhagen General Population Study with two lipid measurements 10 years apart. **Results:** Cross-sectionally in the general population, levels of HDL cholesterol were inversely associated with levels of triglycerides with an R2=0.26. Dynamically for individuals with 10 or more measurements of both HDL cholesterol and triglycerides, the maximum absolute difference for individuals with triglycerides =5mmol/L was 1.1mmol/L for triglycerides and 0.2mmol/L for HDL cholesterol. Short-term after an oral fat load in individuals with fasting triglycerides <2mmol/L, median triglycerides increased 61% while HDL cholesterol decreased 1%; corresponding values were +75% and -4% at triglycerides of 2-4.99 mmol/L and +96% and -1% at triglycerides =5mmol/L. Long-term for individuals with two measurements of both triglycerides and HDL cholesterol ten years apart, the absolute change in triglycerides for the highest quintile was -0.82mmol/L and for the lowest quintile +0.36mmol/L, while for HDL cholesterol the corresponding numbers were -0.23mmol/L and -0.10mmol/L. **Conclusion:** Low HDL cholesterol is a stable marker of average elevated triglycerides. This suggests that low HDL cholesterol can be used to monitor long-term average elevated triglyceride-rich remnants, just like high HbA1c is a long-term monitor of average elevated glucose levels.

Oral Presentations – Abstracts –

Other Topics

SESSION IV

Intake of added sugars and all-cause and cardiovascular mortality

Stina Ramne, Joana Alves Dias, Emily Sonestedt

Nutritional epidemiology, Department of Clinical Sciences Malmö, Lund University

Background: Sugar consumption has been associated with risk factors for cardio-metabolic disease. However, most of the evidence is found for sugar-sweetened beverages. Due to inconsistent evidence for the effects of total intake of added sugars, and hence to difficulties in making unanimous sugar recommendations, the aim of this study was to examine associations between added sugar and mortality. **Method:** In the prospective Malmö Diet and Cancer cohort (n = 24272), dietary data was collected through a modified diet history method. The Swedish death registry was used to assess mortality. The association between added sugar intake, divided into six categories of energy percentages from <5E% to >20E%, and mortality risk was examined using adjusted cox proportional hazards regression. **Results:** Higher added sugar intake was associated with higher age, lower socioeconomic status and other unfavourable lifestyle factors. A U-shaped association between added sugar intake and mortality was observed, with lowest risk in the intake category of 7.5-10E%, which therefore was used as reference. Intakes of both >20E% and <5E% of added sugar was significantly associated with a higher risk for total mortality, HR 1.30 (95% CI 1.12-1.51) and HR 1.23 (95% CI 1.11-1.35), respectively, as well as for cardiovascular mortality, HR 1.40 (95% CI 1.09-1.82) and HR 1.22 (95% CI 1.02-1.47), respectively. A linear positive trend for ApoB/ApoA1 ratio and a linear negative trend for HDL was seen with increasing added sugar intake. When examining different sugar-rich products, a linear positive association was observed between sugar-sweetened beverages and mortality, but an inverse association for treats. **Conclusion:** The risk was almost equally increased for low-consumers as for high-consumers of added sugar, despite them having a more favourable lifestyle in general. Though, the associations seem to be dependent on the type of sugar-rich products consumed.

Increased glucose and fatty acid metabolism and metabolic flexibility in myotubes from trained subjects

Nils Gunnar Løvsletten[1], Jenny Lund[1], Jørgen Jensen[2], G. Hege Thoresen[1,3], Arild C. Rustan[1]

[1]Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Norway; [2]Department of Physical Performance, Norwegian School of Sport Sciences, Norway; [3]Department of Pharmacology, Institute ofClinical Medicine, Univesity of Oslo, Norway

Aims: Physical activity is known to have an important role in both prophylaxis and treatment of a number of metabolic diseases. To explore exercise-related metabolic effects in skeletal muscle, we compared glucose and fatty acid metabolism in skeletal muscle cells (myotubes) established from trained (athletes) and sedentary untrained young male subjects. Untrained was defined as VO2max < 46 ml/kg/min whereas trained was defined as VO2max > 60 ml/kg/min. **Methods:** Myoblasts were cultured and differentiated to myotubes from satellite cells isolated from biopsies of musculus vastus lateralis. Glucose and fatty acid metabolism was studied in myotubes using D-[14C(U)]glucose, [1-14C]deoxy-D-glucose and [1-14C]oleic acid, respectively. Accumulation of labelled substrates was assessed by scintillation proximity assay. Lipid distribution was measured by thin layer chromatography. Gene and protein expressions were also studied. **Results:** Myotubes established from trained subjects showed higher glucose accumulation and oxidation. These cells were also more sensitive to the suppressive action of acutely added oleic acid. There was no difference in basal and insulin-stimulated glycogen synthesis between the two donor groups. Myotubes from trained subjects also showed higher fatty acid oxidation, both complete oxidation (CO2 formation) and ß-oxidation, compared to myotubes from untrained subjects. However, myotubes from trained individuals showed lower fatty acid accumulation and lower incorporation of fatty acids into total cellular lipids, triacylglycerol, diacylglycerol and cholesteryl ester. **Conclusions:** Myotubes from trained subjects were more flexible to exploit the fuel source available, demonstrated by the suppressive activity of fatty acids on glucose oxidation. Furthermore, myotubes from trained individuals had higher glucose and fatty acid metabolism compared to myotubes from untrained subjects. Whether these properties in the satellite cells are inherent from birth or acquired through lifestyle remains unknown.

OSBP-related protein 2 (ORP2) – a novel regulator of Akt signaling and cellular energy metabolism

Henriikka Kentala[1], Annika Koponen[1], Helena Vihinen[2], Juho Pirhonen[1,3], Gerhard Liebisch[4], Zoltan Pataj[4], Annukka Kivelä[1], Shiqian Li[1,3], Leena Karhinen[3], Eeva Jääskeläinen[1], Robert Andrews[5], Leena Meriläinen[2], Silke Matysik[4], Elina Ikonen[1,3], You Zhou[5,6], Eija Jokitalo[2], and Vesa M. Olkkonen[1,3]

[1]Minerva Foundation Institute for Medical Research, FI-00290 Helsinki, Finland; [2]Electron Microscopy Unit, Institute of Biotechnology, FI-00014 University of Helsinki, Helsinki, Finland; [3]Department of Anatomy, Faculty of Medicine, FI-00014 University of Helsinki, Finland; [4]Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, D-93053 Regensburg, Germany; [5]Systems Immunity Research Institute, Cardiff University, Cardiff, CF14 4XN, UK; [6]Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, CF14 4XN, UK

OSBP-related protein 2 (ORP2) is a ubiquitously expressed oxysterol-binding protein homolog previously implicated in endoplasmic reticulum (ER) - lipid droplet (LD) contacts, triacylglycerol (TG) metabolism, cholesterol transport, adrenocortical steroidogenesis, and actin-dependent cell dynamics. Here, we characterize the role of ORP2 in carbohydrate and lipid metabolism by employing ORP2-knock-out (KO) hepatoma cells (HuH7) generated by CRISPR-Cas9-mediated gene editing. The ORP2-KO and control HuH7 cells were subjected to RNA sequencing, analyses of Akt signaling, carbohydrate and TG metabolism, the extracellular acidification rate (ECAR), and the lipidome, as well as to transmission electron microscopy (TEM). The loss of ORP2 resulted in a marked reduction of active phosphorylated Akt(Ser473) and its target Glycogen synthase kinase 3ß(Ser9), consistent with defective Akt signaling, a conclusion supported by the RNA sequencing data. ORP2 was found to form a physical complex with the key controllers of Akt activity, Cdc37 and Hsp90, and to co-localize with Cdc37 and active Akt(Ser473) at lamellipodial regions of the plasma membrane. The loss of ORP2 reduced glucose uptake, glycogen synthesis, glycolysis, mRNAs encoding glycolytic enzymes, and SREBP-1 target gene expression, and led to defective TG synthesis and storage. According to quantitative TEM analysis, ORP2-KO did not reduce but rather increased ER–LD contacts under basal culture conditions but disturbed their expansion upon fatty acid loading, suggesting that ORP2 is not necessary for ER-LD tethering but may play a role in the dynamic regulation of such organelle contacts. ORP2-KO did not significantly affect the composition of cellular membrane phospholipids, cholesterol, nor oxysterols. However, the RNA sequencing revealed altered expression of >10 mRNAs of cholesterol metabolism, indicating that the KO cells may have adapted to a disturbance of cholesterol homeostasis via gene regulatory changes. Together with our recently published investigation (Kentala et al., FASEB J 2017, Nov 1. PMID: 29092904), the present study identifies ORP2 as a new regulatory nexus of Akt signaling, cellular energy metabolism, actin cytoskeletal function, cell migration and proliferation.

GPR120 agonism promotes metabolic health by stimulating calcium-dependent mitochondrial respiration in brown fat

Maaike Schilperoort[1,2], Andrea D. van Dam[1,2], Geerte Hoeke[1,2], Irina G. Shabalina[4], Trond Ulven[5], Sander Kooijman[1,2], Mark Christian[3], Patrick C.N. Rensen[1,2]

[1]Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, the Netherlands; [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands; [3]Department of Metabolic and Vascular Health, the University of Warwick, Coventry, the United Kingdom; [4]Department of Molecular Biosciences, The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, Stockholm, Sweden; [5]Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark

Background: We have shown that activation of brown adipose tissue (BAT) protects from atherosclerosis development by reducing hypercholesterolemia (Nat Commun 2015) and enhancing reverse cholesterol transport (Nat Commun 2017). A potential novel target to activate BAT is the G protein-coupled receptor 120 (GPR120), which expression is strongly increased by cold exposure in both white and brown fat, suggesting a role for GPR120 in thermogenesis. Therefore, we aimed to elucidate the importance of GPR120 for BAT activity. **Methods & Results:** Activation of GPR120 by the selective agonist TUG-891 (intraperitoneal injection of 35 mg/kg) increases fat oxidation acutely (within 1 h), while prolonged daily treatment (= 1.5 weeks) reduces body weight in fat mass in wild-type mice, but not in GPR120-/- mice. These effects coincided with decreased brown adipocyte lipid content and increased nutrient uptake from plasma by BAT, confirming increased BAT activity. Consistent with these observations, GPR120-deficiency reduced expression of genes involved in nutrient handling in BAT. Mechanistically, GPR120 signaling in brown adipocytes in vitro promoted differentiation and acutely induced O2 consumption. This was strongly dependent on intracellular calcium, a downstream target of G-protein signaling. In fact, we showed that the GPR120-induced rise in intracellular calcium led to mitochondrial depolarization and subsequently fragmentation, thereby stimulating mitochondrial respiration. In addition, the GPR120 agonist TUG-891 activated the mitochondrial uncoupling protein 1 (UCP1), which may act synergistically with mitochondrial fragmentation to increase respiration. **Conclusion:** In conclusion, GPR120 signaling stimulates the metabolic activity of brown adipocytes in vitro and in vivo. These result indicate that GPR120 agonism is a promising novel therapeutic strategy to increase lipid combustion and reduce cardiovascular disease.

Very high plasma levels of HDL cholesterol and risk of dementia in the general population

Emilie Westerlin Kjeldsen[1], Anne Tybjærg-Hansen[1-3], Børge G. Nordestgaard[2-4], Ruth Frikke-Schmidt[1,2]

[1]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; [2]The Copenhagen General Population Study; [3]The Copenhagen City Heart Study; [4]The Department of Clinical Biochemistry, Herlev Hospital, Herlev, Denmark; [1-4] Copenhagen University Hospitals and Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

No easily accessible biomarker for dementia is currently available. The associations between plasma levels of lipids and lipoproteins and risk of dementia have not been clarified. Especially reports on high-density lipoprotein (HDL) cholesterol are conflicting. Our aim was to examine whether HDL cholesterol on a continuous scale covering extreme levels are associated with dementia and its subtypes in the general population. A total of 49,765 men and 62,215 women were included from two prospective population-based studies, the Copenhagen General Population Study and the Copenhagen City Heart Study. Analyses were conducted separately for men and women and multifactorially adjusted for risk factors and apolipoprotein E (APOE) genotype. Multifactorially adjusted restricted cubic spline models with HDL cholesterol on a continuous scale showed that men and women with extreme HDL cholesterol concentrations had significantly increased risk of any dementia and Alzheimer's disease. When further adjusting for triglycerides and APOE genotype results remained. Hazard ratios from Cox proportional hazards regression showed similar results as the restricted cubic spline analyses. For any dementia, men in the 96th-99th and 100th percentile had multifactorially adjusted hazard ratios of 1.44(95% confidence interval 1.11-1.88) and 2.02(1.35-3.03) when compared with men in the reference group (41st-60th percentile). For Alzheimer's disease, women in the 100th percentile had a multifactorially adjusted hazard ratio of 1.60(1.02-2.49) when compared with women in the reference group (81st-95th percentile). Men and women in the general population with very high plasma levels of HDL cholesterol have increased risk of any dementia and Alzheimer's disease independent of triglycerides and APOE genotype.

Vascular disease, related risk factors and development of dementia – a nationwide study of the Danish population

Jesper Qvist Thomassen[1], Janne Schurmann Tolstrup[2], Ruth Frikke-Schmidt[1,3]

[1]Department of Clinical Biochemistry, Rigshospitalet; [2]National Institute of Public Health, University of Southern Denmark; [3]Faculty of Health and Medical Sciences, University of Copenhagen

Aim: The prevalence of dementia is increasing globally and vascular disease and related risk factors are major contributors to this neurodegenerative disease. The specific impact of vascular disease and vascular risk factors for development of dementia at the individual and population level is unknown. **Methods:** We tested if ICD10 diagnoses of cerebrovascular and ischemic heart disease, diabetes, and hyperlipidemia predicted future risk of dementia and its subtypes. We defined an exposure period from 1994-2004 where comorbidities, risk factors and socio-economic factors were harvested from Statistics Denmark. The follow-up period was from 2004 to 2014 identifying all incident dementia subtype diagnoses. We included individuals above 65 years of age at 2004, totaling 789,531 individuals with 61,177 dementia diagnoses. **Results:** Multifactorially adjusted hazard ratios were 1.51(95% confidence interval 1.47-1.55) for all dementia, 1.47(1.43-1.51) for unspecified dementia, 0.95(0.90-1.00) for Alzheimer's disease, and 3.09(2.93-3.26) for vascular dementia for cerebrovascular disease versus no cerebrovascular disease. Corresponding estimates were 1.08(1.06-1.11), 1.09(1.06-1.12), 0.98(0.94-1.02), and 1.33(1.26-1.41), for ischemic heart disease versus no ischemic heart disease, 1.46(1.42-1.51), 1.51(1.46-1.57), 1.13(1.06-1.20), and 1.96(1.82-2.12) for diabetes versus no diabetes. Finally, the population attributable fractions of cerebrovascular disease and diabetes were 21% and 5% for vascular dementia and 4% and 3% for all dementia. **Conclusion:** The present study shows that vascular diseases, diabetes, and hyperlipidemia are important risk factors for dementia both at the individual and at the population level. Since dementia at present remains effectively untreatable, reducing the risk becomes increasingly important. These data underscores the importance of early prevention, diagnosis and treatment of vascular disease for neurocognitive health.

Absolute 10 year risk of dementia by age, gender and APOE genotype for targeted prevention in 104,537 individuals from the general population

Katrine L. Rasmussen MD PhD[1,2,3], Anne Tybjærg-Hansen MD DMSc[1,2,3], Børge G. Nordestgaard MD DMSc[2,3,4], Ruth Frikke-Schmidt MD DMSc[1,2]

[1]Department of Clinical Biochemistry, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark; [2]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark; [3]Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark; [4]The Copenhagen City Heart Study, Frederiksberg Hospital, Nordre Fasanvej 57, DK-2000 Frederiksberg, Denmark; [1-4]Copenhagen University Hospital and Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen, Denmark.

Background: Dementia is a major cause of disability in the elderly, and risk factor reduction may have the potential to delay or prevent the disease. For targeted prevention estimation of age, gender, and apolipoprotein E (APOE) genotype specific absolute risk to identify high risk individuals is needed. The aim was to determine the absolute 10 year risk of Alzheimer disease and all dementia, by age, gender, and APOE genotype. **Methods:** 104,537 individuals from the general population were followed until occurrence of event, death, emigration or November 10th 2014, whichever came first. **Results:** In e44 carriers, the absolute 10 year risks of Alzheimer disease in women and men were 7% and 6% at age 60-69, 16% and 12% at age 70-79, and 24% and 19% at age 80+. Corresponding values for all dementia were 10% and 8%, 22% and 19%, and 38% and 33 %, respectively. Multifactorially adjusted hazard ratios (HRs) for all dementia increased from e22 to e32 to e33 to e42 to e43 to e44 (p for trend=1x10-100). Multifactorially adjusted HRs for e44 versus e33 were 8.74 (95% confidence interval: 7.08-10.79) for Alzheimer disease, 2.87 (1.54-5.33) for vascular dementia, 4.68 (3.74-5.85) for unspecified dementia, and 5.77 (4.89-6.81) for all dementia. **Conclusions:** The present age, gender, and APOE genotype specific 10 year absolute risk estimates for Alzheimer disease and all dementia generated from a large prospective study of the general population with more than 3,000 e44 carriers robustly identify high risk groups for potential targeted preventive interventions.

Dietary Sargassum fusiforme improves memory and reduces amyloid plaque load in an Alzheimer's disease mouse model

Jeroen Bogie[1]*, Cindy Hoeks[1]*, Melissa Schepers[1], Ann Cuypers[2], Frank Leijten[3], Yupyn Chintapakorn[4], Surachai Pornpakakul[5], Anja Kerksiek[10], Hong-Bing Liu[6], Niels Hellings[1], Pilar Martinez-Martinez[8], Eric Sijbrands[3], Jochen Walter[7], Jerome Hendriks[1], Albert Groen[8], Bart Staels[9], Dieter Lütjohann[10], Tim Vanmierlo[1#], Monique Mulder[3#]

[1]Department of Biochemistry and Neuroimmunology, Biomedical research institute, Hasselt University, Martelarenlaan 42, 3500 Hasselt Belgium; [2]Centre for Environmental Sciences, Hasselt University, Martelarenlaan 42, 3500 Hasselt, Belgium; [3]Department of Internal Medicine, Laboratory of Vascular Medicine, Erasmus University Medical Center, Wytemaweg 80, 3015 CN, Rotterdam, the Netherlands; [4]Center of Excellence in Environment and Plant Physiology, Department of Botany, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; [5]Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; [,#]equal contribution*

Accumulating evidence suggests a key role for disturbed brain sterol homeostasis in Alzheimer's disease (AD). Liver X receptors (LXRs) are master regulators of sterol transport and synthetic LXR agonists improve memory performance in AD mice. However, these LXR agonists induce hypertriglyceridemia in plasma and in liver, which has hampered their use in the clinic. Phytosterols are naturally occurring LXR agonists that do not increase plasma triglycerides. Therefore, we hypothesized that phytosterols can act as LXR-dependent cognition enhancers in AD. The capacity of phytosterols and extracts to activate LXRs in vitro was defined using a luciferase-based nuclear receptor reporter assay. We show that phytosterols commonly present in a Western type diet hardly ligate LXRs. However, a lipid extract of the seaweed Sargassum fusiforme, containing the phytosterol 24(S)-Saringosterol, activated LXRs in vitro and in vivo. Dietary supplementation with lipid extracts of Sargassum fusiforme significantly improved short-term memory in APPswePS1dE9 mice and reduced hippocampal amyloid-ß plaque load by 81%. None of the side effects typically induced by synthetic LXR agonists were observed. These findings point towards Sargassum fusiforme being an attractive option for translational add-on treatments in the emerging field of nutritional neuroscience.

Posters – Abstracts –

Inflammation and Vascular Biology

SESSION I

Pathways of endothelial dysfunction during inflammation in atherosclerosis

Benjamin Rayner[1,2], Adrian Abdo[1,2], David van Reyk[3] and Clare Hawkins[1,2,4].

[1]Heart Research Institute, Sydney, Australia; [2]Sydney Medical School, University of Sydney, Australia; [3]School of Life Sciences, University of Technology Sydney, Australia; [4]Department of Biomedical Sciences, University of Copenhagen, Denmark.

The oxidant hypochlorous acid (HOCl) produced by myeloperoxidase (MPO) is strongly implicated in the acceleration of lesion development in atherosclerosis by multiple pathways, including the induction of endothelial dysfunction and modification of low-density lipoprotein (LDL). MPO also utilises thiocyanate (SCN-) to produce the oxidant hypothiocyanous acid (HOSCN), which has a different pattern of reactivity compared to HOCl. The role of HOSCN in lesion development is not clear, as elevated plasma SCN- has been linked with both the propagation and prevention of atherosclerosis. In this study, we examined the ability of HOSCN, and LDL modified by this oxidant, to alter endothelial function. In vitro experiments with human coronary artery endothelial cells (HCAEC) show that by targeting intracellular thiols (R-SH), HOSCN can induce enzyme inactivation, alter the cellular redox environment and perturb cytosolic Ca2+ levels. This culminates in mitochondrial dysfunction and the activation of different stress-related signaling cascades that can promote cell death by apoptotic pathways. Exposure of HCAEC to HOSCN-modified LDL decreases the production of nitric oxide (NO•) and induces the loss of endothelial nitric oxide synthase (eNOS) activity. This occurs to a similar extent to that seen with HOCl-modified LDL. In each case, these effects are related to eNOS uncoupling, rather than altered expression, phosphorylation or cellular localisation. We also demonstrate that HOSCN and LDL-modified by HOSCN, inhibit endothelium-mediated vasorelaxation ex vivo in rat aortic ring segments. Together, these data provide new insights into role of MPO and LDL modification in the induction of endothelial dysfunction, which has implications for both the therapeutic use of SCN- within the setting of atherosclerosis and for smokers, who have elevated plasma levels of SCN-, and are more at risk of developing cardiovascular disease.

Does hypochlorous acid (HOCl) induced modification of extracellular matrix (ECM) of the arterial wall contribute to atherosclerosis?

Huan Cai, Christine Y. Chuang, and Michael J. Davies

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

Objective: This study examines the effects of the strong inflammatory oxidant HOCl on ECM synthesised by human coronary artery smooth muscle cells (HCASMCs), and whether modified ECM proteins modulate HCASMC behaviour. **Methods:** HCASMC-derived ECM was exposed to increasing concentrations of HOCl (0 - 200 µM) with damage investigated using antibodies against specific ECM proteins via ELISAs and Western blots. Effects on adhesion to, and proliferation on, HOCl-modified ECM was examined using calcein-AM and MTS. Effects of HOCl-modified ECM on mRNA expression were determined by real-time PCR. **Results:** A loss of antibody reactivity against the cell-binding fragment (CBF) of fibronectin, laminins, type IV collagen and versican were detected upon exposure to increasing concentrations of HOCl via ELISA. Western blots showed that treatment with 200 µM HOCl resulted in a reduction of epitope recognition against fibronectin CBF, laminins and type IV collagen. Interestingly, preliminary data showed HOCl-modified HCASMC-ECM resulted in a concentration-dependent reduction of HCASMC cell adhesion and proliferation from as little as 10 µM of HOCl treatment. In addition, mRNA expression of multiple types of genes in HCASMCs associated with the inflammatory response (IL-6, COX-2), matrix proteins (FN1) as well as matrix metalloproteinases (MMP1, MMP11, MMP13), was up-regulated by ECM pre-treated with 1 µM or higher HOCl for 2 h. **Conclusion:** HOCl induces structural damage to ECM proteins, including fibronectin, versican, laminins, and type IV collagen, which subsequently can modulate HCASMC cell adhesion and proliferation. Our results reveal a novel pathway through which oxidation by HOCl modifies ECM components, and contributes to behavioural switching of HCASMCs, which is a key process during the progression of atherosclerosis.

Hypercholesterolemia affects macrophage metabolism and function

Jeroen Baardman[1], Michel van Weeghel[2], Saskia van der Velden[1], Sanne Verberk[3], Koen H.M. Prange[1], Dave Speijer[1], Menno P.J. de Winther[1] & Jan Van den Bossche[1,3]

[1]Experimental Vascular Biology, Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [2]Laboratory Genetic Metabolic Diseases, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [3]Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, the Netherlands

Foam cells are lipid-loaded macrophages and participate in all stages of atherosclerosis, Metabolic reprogramming arose as a key controller of macrophage activation and we hypothesized that metabolic changes in foam cells could underlie their phenotype.
To investigate the effect of foam cell formation on macrophage metabolism, low-density lipoprotein receptor (Lldr)-/- mice were fed a high-fat diet (HFD) or a chow normal-fat diet (NFD) as control. Thioglycollate-elicited peritoneal macrophages from the HFD group were lipid-laden foam cells as demonstrated by Oil Red O staining. Compared to the macrophages from the NFD group, those foam cells displayed attenuated lipopolysaccharide (LPS)-induced expression of inflammatory genes, secretion of pro-inflammatory cytokines and nitric oxide (NO) production. Extracellular flux analyses and mass spectrometry demonstrated that this reduced inflammatory phenotype in foam cells is not accompanied with major changes in glycolysis or a reconfigured TCA cycle. Interestingly, LPS-elicited induction of pentose phosphate pathway (PPP) intermediates ribose-5P/ribulose-5P and sedoheptulose-7P was reduced upon foam cell formation. Moreover, we discovered that 6-phosphogluconate dehydrogenase (Pgd) gene expression was blunted in foam cells, possibly explaining the reduced levels of these downstream PPP metabolites. To validate whether a suppression of the PPP in foam ells could support their attenuated LPS-induced inflammatory responses, we pharmacologically inhibited the PPP. Our observation that suppression of the PPP diminishes LPS-induced cytokine secretion and NO production supports the notion that this pathway is a key feature of inflammatory macrophage responses. Overall, this study reveals that foam cell formation and cellular metabolism are directly intertwined and together regulate macrophage function.

Characterisation of extracellular matrix materials in human atherosclerotic lesions

Christine Y. Chuang, Lasse Lorentzen, Per Hägglund and Michael J. Davies

Panum Institute, BMI, University of Copenhagen, Denmark

The vascular basement membrane (BM) is composed of specialized extracellular matrix (ECM) proteins that underlie the endothelial cells. The ECM is critical to the functional and mechanical properties of arteries by interacting with each other and growth factors to regulate cell activities. The BM is rich in laminin, a trimeric protein consisting of α-, β- and γ chains. The C-terminus of the α chain interacts with specific integrin on cells and plays a critical role in cell adhesion and signalling, while binding sites for perlecan, collagens, fibronectin and nidogen are present on specific domains. However, these protein-protein interactions are believed to be perturbed in atherosclerosis, and may contribute to endothelial cell (EC) dysfunction, uncontrollable smooth muscle cell (SMC) infiltration and proliferation, which subsequently alters the overall ECM composition. Hence, we hypothesise that specific laminin isoforms synthesized by EC and SMC are important in maintaining an intact and functional ECM environment in healthy arteries. Human coronary artery EC and SMC-derived native ECM has been characterised by ELISA. High reactivity for type IV collagen and heparan sulfate was detected in EC-ECM, whereas higher reactivity for laminins and chondroitin sulfate was detected in SMC-ECM. Immunocytochemistry and Western blotting experiments have confirmed that the ECM synthesised by EC and SMC show different distributions of laminin isoforms and chains. Advanced human atherosclerotic lesions also show different isoforms and the presence of laminin fragments consistent with ECM modification. Proteomics is being used to confirm the identity of the specific laminin isoforms. These data suggest that different laminin isoforms produced by EC and SMC have specific roles in maintaining a fully functional and intact ECM environment in healthy arteries, and that this balance is perturbed in atherosclerosis.

ATP citrate lyase as a negative regulator of inflammatory macrophage responses

Jeroen Baardman[1], Saskia van der Velden[1], Menno P.J. de Winther[1] & Jan Van den Bossche[1,2]*

*[1]Experimental Vascular Biology, Department of Medical Biochemistry, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [2]Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, the Netherlands; *supported by Netherlands Heart Foundation Junior (2013T003) and Senior (2017T048) Postdoc grant and NWO VENI*

Macrophages participate in all stages of atherosclerosis, one of the leading causes of death in the Western world. Both metabolic and epigenetic mechanisms now arise as key controllers of macrophage activity. Epigenetic enzymes require metabolites to support their chromatin-modifying activity. For example, histone acetylation transferases use acetyl-coenzyme A (Acetyl-CoA) as substrate for histone acetylation. Several enzymes are capable to synthesize acetyl-CoA in mammalian cells, including ATP citrate lyase (Acly), and are possibly involved in regulating macrophage activation. We hypothesized that modulating acetyl-CoA availability via targeting Acly will affect the epigenetic machinery and phenotype of macrophages. Here we found that inhibition of glycolysis impairs the LPS-induced secretion of inflammatory cytokines and expression of inflammatory genes in bone marrow-derived macrophages (BMDMs). These effects were accompanied with reduced acetylation of H3K27 at promoter and enhancer regions of inflammatory genes, emphasizing the tight relationship between metabolism and epigenetics in activated macrophages. Interestingly, deletion of Acly increased the LPS-induced expression of inflammatory genes, secretion of inflammatory cytokines and production of nitric oxide in BMDMs. This enhanced inflammatory activation was accompanied with increased production of reactive oxygen species. Moreover, while loss of Acly augments inflammatory activation, expression of canonical IL-4 induced genes were reduced after Acly deletion in BMDMs. Overall, these results pointing towards a negative role of Acly in regulating LPS-induced inflammation in macrophages. Given the importance of macrophage-induced inflammation in atherosclerosis, we are currently investigating the effects of myeloid-specific deletion of Acly on atherogenesis.

White Blood Cell Differential Count and Screening-Detected Abdominal Aortic Aneurysms in Men

Marcus Langenskiöld[1], Kristian Smidfelt[1], Joakim Nordanstig[1], Marta Lantero Rodriguez[2], Göran Bergström[2], Åsa Tivesten[2]

[1]Department of Vascular Surgery, Sahlgrenska University Hospital; [2]The Wallenberg Laboratory for Cardiovascular and Metabolic Research; Institute of Medicine, The Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden.

Background: An abdominal aortic aneurysm (AAA) is a pathological widening of the abdominal aorta, which may result in a life-threatening rupture of the aortic wall. In Sweden, all men are invited to ultrasound screening for AAA at 65 years of age. The trigger of AAA formation is unknown, but vascular extracellular matrix degradation and inflammation likely are central to the pathogenesis. In accordance, CRP and white blood cell (WBC) count are increased in AAA patients. However, associations with subclasses of circulating leukocytes have not been addressed previously. **Aim:** To study the association between WBC differential count and presence of AAA at ultrasound screening in 65-year old men. **Methods:** In region Västra Götaland (1.5 million inhabitants), men with AAA (diameter >30 mm) at screening (cases) and AAA-free screened men (controls; matched for site in a 1:10 consecutive randomization order) were invited to participate in the study "Gothia 3A". During 2013-2017, WBC differential count was analyzed in 151 cases and 224 controls at the Gothenburg screening site. **Results:** In accordance with previous studies, AAA cases had higher frequency of smoking, hypertension, and hypercholesterolemia. In univariate analyses, blood levels of neutrophils, lymphocytes, monocytes and basophils were higher among AAA cases, while blood levels of eosinophils were similar in cases and controls. Neutrophil, lymphocyte and monocyte counts remained associated with AAA presence in multivariate models; odds ratio was 1.8 (95% CI 1.4-2.3) and 1.4 (1.1-1.9) per standard deviation increase in neutrophil and lymphocyte count, respectively. **Conclusion:** Several subclasses of circulating leucocytes are associated with AAA presence, independently of other previously known risk factors such as smoking. Understanding the inflammatory process involved in AAA pathogenesis will be an important task for future research.

YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

Association between plasma omega 6 fatty acids and expression of inflammation-related genes in peripheral blood mononuclear cells: a cross-sectional study in healthy children

Jacob J. Christensen[1,6], Siril S. Bakke[11], Stine M. Ulven[1], Kjetil Retterstøl[1,6], Tore Henriksen[2,8], Jens Bollerslev[2,7], Terje Espevik[11], Liv Ryan[11], Pål Aukrust[3,4,5,9], Bente Halvorsen[3,4,5], Kirsten B. Holven[1,10]

[1]Department of Nutrition, [2]Faculty of Medicine, [3]Research Institute of Internal Medicine, University of Oslo, P.O box 1046 Blindern, 0317 Oslo, Norway; [4]Institute of Clinical Medicine, [5]K.G. Jebsen Inflammatory Research Center, Oslo, Norway; [6]The Lipid Clinic, [7]Section of Specialized Endocrinology, [8]Department of Obstetrics, [9]Section of Clinical Immunology and Infectious Diseases, [10]Norwegian National Advisory Unit on Familial Hypercholesterolemia, Oslo University Hospital, Rikshospitalet, Oslo, Norway; [11]Department of Cancer Research and Molecular Medicine, Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway.

Background and aim: Atherosclerosis is mainly driven by close interaction between lipids and the immune system. Some animal studies suggest that a high dietary intake of omega 6 is pro-inflammatory, but whether omega 6 fatty acids (FA) actually cause inflammation in humans is debated. The aim of the present study was to investigate how expression of inflammation-related genes in peripheral blood mononuclear cells (PBMCs) and circulating plasma cytokines associate with plasma omega 6 status in healthy children. **Methods:** We recruited 58 healthy children and measured expression of 460 inflammation-related genes in PBMCs using Nanostring technology. Also, we measured seven circulating plasma cytokines. We applied various statistical and bioinformatics analyses to associate these measurements with omega 6 status (upper versus lower tertile). **Results:** After adjusting for multiple testing, the expression of HLA-DPB1 (MHC class II DP beta 1), TICAM1 (TLR adaptor molecule 1) and PDCD2 (CD279, programmed cell death 1) was higher with high omega 6 FA status, and the expression of IL11RA (IL11 receptor alpha) and ZAP70 (TCR zeta-chain associated protein kinase 70kDa) was higher with low omega 6 FA status. Interestingly, PTGS2 (COX2) was positively associated with omega 6 FA level. Moreover, gene set enrichment analysis, gene ontology, pathway analysis and Metacore enrichment analyses revealed a large number of significant gene sets, ontologies and pathways. Some interesting examples include signal transduction, response to abiotic and biotic stimuli, lipid binding, and other immune-related gene sets. Interestingly, most gene sets were higher expressed with higher omega 6 FA status. Plasma cytokines, on the other hand, did not associate with omega 6 FA status. **Conclusion:** We found an association between omega 6 status and expression of inflammation-related genes in PBMCs in healthy children. Our results suggest a role for omega 6 FAs in the interphase of lipids and inflammation, which warrants further examination in intervention trials.

IFNy-stimulated B cells effectively inhibit T follicular helper cells and reduce atherosclerosis by expression of high levels of PD-L1.

H. Douna, J. Amersfoort, F.S. Schaftenaar, M. Kroner, I. Bot, A. Foks, G.H.M. van Puijvelde, J. Kuiper

Division of Biotherapeutics, LACDR, Leiden University, The Netherlands

Antibodies are the culmination of a full B cell reponse to an antigen. In germinal centers, T follicular helper cells (Tfh cells) present antigen to B cells. B cells that recognize the antigen receive further stimulation from Tfh cells and will proliferate into plasma cells which secrete large amounts antibodies. It has recently been shown that blocking this Tfh-germinal center B cell axis by regulatory CD8 T cells reduces the lesion size in ApoE KO mice. Others have also shown that this axis can be blocked by B cells that express high levels of PD-L1. These B cells effectively reduced the onset of EAE in an experimental mouse model. We thus hypothesized that PD-L1hi B cells would inhibit the Tfh-germinal center axis in ApoE KO mice and hence decrease lesion size.We found that stimulation of B cells with IFNy effectively increased the expression of PD-L1 on B cells and that these cells were highly effective in blocking Tfh cells in vitro and in vivo. To determine whether these B cells could also reduce atherosclerosis, we used a collar model in ApoE KO mice that were given an Western type diet for 6 weeks and either received three injections of PBS, non-stimulated or IFNy-stimulated B cells. We found that total plaque volume was significantly reduced by 65% in mice receiving IFNy-stimulated B cells compared to mice receiving PBS (PBS: 7.0 ± 5.1x107 µm3 vs. IFNy: 2.4±1.4x105 µm3; $p<0.05$) and the same trend of a 52% reduction was observed when compared to mice receiving non-stimulated B cells (Non-stimulated: 5.0 ± 2.5x107 µm3 vs. IFNy: 2.4±1.4x105 µm3; $p=0.15$). In short, IFNy is an effective inducer of PD-L1 on B cells which results in potent inhibition of Tfh cells. In addition, adoptive transfer of IFNy-stimulated B cells reduces total plaque volume in a collar model in ApoE-/- mice. To further confirm our hypothesis that this reduction is indeed due to the inhibition of the Tfh-germinal center axis, we are currently determining the level of Tfh cells and of total and antigen-specific antibodies in these mice.

Mcl-1 deficient macrophages lead to a pro-atherogenic, giant-cells enriched plaque phenotype.

Fontaine MAC[1], Westra MM[2], Bot I[2], Franssen A[1], Bot M[2], de Jager SCA[2], Dzhagalov I[3], He Y-W[3], van Vlijmen BJM[4], Gijbels MJJ[1], Reutelingsperger CP[5], van Berkel TJC[2], Sluimer JC[1], Temmerman L[1], Biessen EAL[1]

[1]Experimental Vascular Pathology Group, Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands; [2]Division of Biopharmaceutics, Leiden Amsterdam Centre for Drug Research, Leiden University, Leiden, The Netherlands; [3]Department of Immunology, Duke University Medical Center, Durham, NC, USA; [4]Hemostasis and Thrombosis Research Center, Dept. of Hematology, Leiden University Medical Center, Leiden, The Netherlands; [5]Deptartment of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands.

The Bcl-1 family member myeloid cell leukemia 1 (Mcl-1) plays an important role in survival and differentiation of leukocyte subsets. Here, we investigated the impact of Mcl-1 deletion (Mcl-1-/-) in neutrophils and macrophages on atherosclerotic plaque development and stability in high-fat diet (HFD) fed LDL receptor-deficient (LDLr-/-) mice by a bone marrow transplantation strategy. Mcl-1 myeloid depletion resulted in markedly lower circulating neutrophils both before and under HFD conditions (-82% to -91%, $p<0.001$). Neutrophil content in Mcl-1-/- atherosclerotic lesions was decreased by 72% compared to wild-type (WT) lesions ($p=0.046$). Surprisingly, despite this atheroprotective effect provided by the reduced neutrophil presence, Mcl-1-/- deletion had no effect on plaque and necrotic core size or macrophage content in the lesion. Mcl-1-/- deficient peritoneal macrophages and bone marrow-derived macrophages (BMDMs) had an increased sensitivity to oxLDL-induced cell death (63% and 155%, respectively. $p<0.01$), compatible with the increased apoptosis in atherosclerotic plaques of Mcl-1-/- chimeras (77%, $p=0.002$). OxLDL and vLDL accumulation by peritoneal Mcl-1-/- macrophages and BMDMs was enhanced both in vitro and in vivo. Interestingly, Mcl1-/- peritoneal foam cells formed up to 45% more multinucleated giant cells (MGCs) in vitro compared to WT ($p<0.05$), which concurred with the 118% increased MGC presence in atherosclerotic lesions of Mcl1-/- mice ($p<0.05$). After 13 days in culture, Mcl-1-/- BMDMs were still enriched in MGCs. However, they lost their enhanced lipid uptake capacity and pro-apoptotic phenotype compared to control cells, despite maintained Mcl-1 knockdown. Taken together, these findings suggest that Mcl-1-/- deletion in macrophages leads to a pro-atherogenic, MGC-enriched phenotype, which is completely counteracted by the neutropenia in circulation and plaque.

Vaccination with ApoB100 derived HLA-A2 restricted CD8 T cell epitopes did not reduce atherosclerosis in male LDLrKO hApoB100tg HLA-A2tg mice.

F.H. Schaftenaar, H. Douna, J. Amersfoort, B. Slutter, G.H.M van Puijvelde, J. Kuiper.

Leiden University, LACDR, Biotherapeutics

CD8 T cells make up a large amount of lymphocytes in atherosclerotic plaques, however their role in atherosclerosis has not been thoroughly studied. Since ApoB100 is a key antigen in atherosclerosis we were interested in the role of ApoB100 specific CD8 T cells in atherosclerosis. HLA-A2 restricted CD8 T cell epitopes derived from human ApoB100 were in silico predicted using The “Proteasomal cleavage/TAP transport/MHC class I combined predictor”of the immune epitope database. Best 6 epitopes were selected to be synthesized based on a consensus HLA-A2 binding value of 1%, processing score of at least 1.5, and a final score which is a combination of MHC binding and processing scores. Binding of peptides to HLA-A2 was confirmed for all peptides with a T2 cell peptide HLA-A2 stabilization assay. To determine whether these putative CD8 T cell epitopes were capable of inducing a peptide specific CD8 T cell response in vivo, HLA-A2tg mice were vaccinated with single peptide pulsed HLA-A2tg DCs. After 7 days splenocytes from the vaccinated mice were exposed to the peptides and recall responses were measured by flow cytometric determination of IFN-ã producing CD8 T cells. 5 of 6 peptides were found capable of inducing a peptide specific recall response. To study whether vaccination with ApoB100 derived CD8 T cell epitopes could influence atherosclerosis, male hApoB100tg HLA-A2tg LDLrKO mice were vaccinated with peptide pulsed bone marrow derived DCs and boosted with a peptide vaccination adjuvanted with áCD40 and Poly(I:C). After 11 weeks of WTD animals were sacrificed. At this timepoint peptide specific CD8 T cells could still be detected in the spleen. Although vaccination with ApoB100 derived CD8 T cell epitopes successfully induced peptide specific CD8 T cells, vaccination did not affect aortic root plaque size.

Increment of Chondrocyte-like Cells in Aortic Valves in Hypercholesterolaemic Mice

Jonna Weisell[1], Anna-Kaisa Ruotsalainen[2], Jaana Rysä[1]

[1]School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [2]A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland

Aims: In aortic stenosis (AS), valvular calcification makes valves thicker causing impairment of the valve function. This is caused by accumulation of oxidized LDL and inflammatory cells into the valve, followed by extra cellular matrix remodelling. As disease progresses, chondrocytes, cartilage, and endochondral calcification may also be present in calcified valves. Here, we wanted to study if chondrocytes are involved in pathobiological process of valvular calcification in hypercholesterolaemic mouse model of AS. **Methods:** LDLr-/-ApoB100/100 mice were fed a western diet (WD) (42 % kcal from fat) or a normal chow diet (control) for 5 months. Histological stainings of aortic valves were conducted with hematoxylin-eosin, Mac-3 for the macrophages, Alizarin red for calcification and S100 for the chondrocyte-like cells. Mice hearts were imaged using echocardiography and the ejection fraction (EF) was determined. **Results:** EF declined in LDLr-/-ApoB100/100 mice fed with WD (27%, $p<0.001$) compared to control mice. Histological analysis revealed more severe atheromatous changes in WD fed mice compared to controls; the aortic root was dilated (+61 %, $p<0.001$), plaque area in the atheroma (+110 %, $p<0.001$) and the cusp area (+94 %, $p<0.001$) were increased. Macrophage infiltration increased into the plaque (+49 %, $p<0.01$) and the cusp (+434 %, $p<0.001$) after WD when compared to control mice. Interestingly, number of chondrocyte-like cells increased significantly after WD (+460 % $p<0.05$). Calcification was not detected in either group. **Conclusion:** This study is first to identify increment of chondrocyte-like cells in mouse aortic valves in hypercholesterolaemic mice fed with WD

Effects of a novel selenosugar on primary human vascular cells, mouse aortic rings and apoE-/- mice

Triantafyllos Zacharias[1], Konstantina Flouda[2], Thomas A. Jepps[1], Christina Christoffersen[1,3,4], Tanja X. Pedersen[1], Bente Gammelgaard[2], and Michael J. Davies[1]

[1]Department of Biomedical Sciences, and [2]Department of Pharmacy, University of Copenhagen, Denmark. Department of Clinical Biochemistry, [3]Rigshospitalet and [4]Bispebjerg Hospital, Denmark.

Background: Atherosclerosis is characterized by chronic low-grade inflammation, large numbers of activated leukocytes, and the deposition of cholesterol and lipids in the artery wall. Activated leukocytes produce oxidants that can convert low-density lipoproteins (LDL) into pro-atherogenic particles that are internalized by macrophages to form lipid-laden cells, and also modify the structure of the extracellular matrix (ECM). This project is investigating a novel seleno-sugar (SeTal) as a potential regulator of oxidative stress, inflammation and atherosclerosis. **Methods:** The effects of SeTal have been examined on human coronary artery endothelial and smooth muscle cells, on aortic rings from mice, and on apoE-/- mice fed a high fat or standard diet. **Results:** SeTal did not decrease cell viability at up to 2 mM. Pretreatment of cells with SeTal before exposure to H2O2 and HOCl protected against toxicity. The levels and activity of the selenoproteins glutathione peroxidase 1 and thioredoxin reductase 1 were unaffected by SeTal. Studies on murine aortic rings showed that high concentrations of SeTal induced aortic relaxation after 3 h of treatment, and preserved endothelial cell-dependent relaxation after exposure to 50 µ. HOCl. Dosing, via drinking water, of SeTal in apoE-/- mice did not affect weight gain, water consumption, or animal welfare. SeTal was detected in the plasma, the liver and kidneys of the animals as an intact species. Total cholesterol, total triglycerides and the inflammatory markers MCP-1, IL-6 and SAA were unaffected. The effects of SeTal on arterial plaque deposition and composition are currently being determined, and will be presented. **Conclusions:** These data indicate that this synthetic seleno-sugar is well tolerated by cells, organs and mice even at high concentrations, and that this species protects against oxidative stress and stimulates relaxation of aortic rings, consistent with the enhanced rate of wound healing seen in previous studies.

Posters – Abstracts –

Cardiovascular Disease

SESSION II

Intake of SFA compared to PUFA induce lower postprandial LDL receptor gene expression in PBMC in subjects with and without FH

Linn K. L. Øyri[1*], Ingunn Narverud[1,2*], Martin P. Bogsrud[2], Patrik Hansson[1], Lena Leder[3], Marte G. Byfuglien[3], Marit B. Veierød[4], Stine M. Ulven[1] and Kirsten B. Holven[1,2]

[1]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway; [2]Norwegian National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Norway; [3]Mills AS, Oslo, Norway; [4]Oslo Centre for Biostatistics and Epidemiology, Department of Biostatistics, Institute of Basic Medical Sciences, University of Oslo, Norway. *Contributed equally

Aim: The long-term cholesterol lowering effect of replacing intake of saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) is well established, however not fully explained mechanistically. We examined the postprandial response of meals with different fat quality on expression of lipid genes in peripheral blood mononuclear cells (PBMC) in subjects with and without familial hypercholesterolemia (FH). **Methods:** Thirteen subjects with FH (without current lipid-lowering treatment) and 14 normolipidemic controls were included in a randomized controlled double-blind crossover study with two meals, each with 60 grams (~70 E%) of fat, either mainly SFA (~40 E%) or PUFA (~40 E%). Expression of 34 lipid genes in PBMCs were analysed by qPCR from fasting and 6 h postprandial blood samples. A linear mixed model for repeated measures was used. **Results:** 1. Intake of SFA compared to PUFA induced lower gene expression of LDL receptor (p=0.01) and higher of ABCG1 (p=0.002), with no interaction with group. 2. FH compared to control subjects had significantly lower gene expression of FDPS, SREBP2, ACC, CPT1A/2, MSR1, NR1H3 and higher of MYLIP (0.007=p=0.05), with no interaction with meal (except for NR1H3) or time point (except for SREBP2, ACC and CPT1A). 3. In controls only, the postprandial gene expression was significantly reduced for DHCR24, LSS, SREBP1/2, FADS1/2, HMGCS1 and ACC (0.001=p=0.03), with no interaction with meal. 4. In FH subjects only, the postprandial gene expression was increased for SORL1 (p=0.03), with no interaction with meal. **Conclusions:** Intake of SFA compared to PUFA induced lower postprandial gene expression of LDL receptor and higher of ABCG1 in PBMCs. This may potentially result in increased plasma cholesterol level.

Statin use is associated with carotid plaque composition: The Rotterdam Study

Blerim Mujaj, MD, MSc[1], Daniel Bos, MD,PhD[1,2,5], Mariana Selwaness, MD, PhD[2], Maarten J.G. Leening, MD, PhD[1,3,5], Maryam Kavousi, MD, PhD[1], Jolanda Wentzel, PhD[4], Aad van der Lugt, MD, PhD[2], Albert Hofman, MD, PhD[5], Bruno H. Stricker, MMed, PhD[1], Meike W. Vernooij, MD, PhD[1,2], Oscar H. Franco, MD, PhD[1]

Departments of [1]Epidemiology, [2]Radiology and Nuclear Medicine, [3]Cardiology, [4]Biomechanics, Erasmus MC, Rotterdam, the Netherlands. Department of [5]Clinical Epidemiology, Harvard TH Chan School of Public Health, Boston, USA

Background: Statins represent a key treatment for cardiovascular disease. Nevertheless, the direct effects of statin treatment on the composition of atherosclerotic plaques remain elusive. **Objectives:** We aimed to investigate the association of statin treatment with the presence of different plaque components located in the carotid arteries within a population-based setting. **Methods:** From the population-based Rotterdam Study, 1740 participants with carotid atherosclerosis (mean age 72.9 years, 46% women) underwent MRI of the carotid arteries to determine the presence of calcification, lipid core, and intraplaque hemorrhage. Information for the duration and dosage of statin use was obtained from pharmacy records for all participants. We used logistic regression models to study the association of statin use with the presence of plaque components. **Results:** Statin treatment was associated with a higher presence of calcification (OR: 1.73 [95%CI:1.22-2.44]). Longer duration of use strengthened this association (OR: 1.82 [95%CI:1.00-3.33] for 10 to 48 months, and OR 1.74 [95%CI: 1.09-2.77] for > 48 months, compared to OR: 1.65 [95%CI:0.94-2.89] for ≤ 10 months). Current statin treatment was also associated with a lower presence of lipid core (OR: 0.66 [95%CI:0.42-1.04]), but only when using statins for 10 months or less. Any dosage of statins was associated with a higher presence of calcification, whilst only high dosages (DDD > 1.33) were associated with a lower presence of lipid core. **Conclusions:** Active, high-dosage statin use seems to beneficially influence the composition of carotid atherosclerosis by shifting the composition from vulnerable plaque with a lipid core to more stable calcified plaque.

Risk of nocturnal dipping status for coronary heart disease is driven by nighttime blood pressure level

Wen-Yi Yang, MD[1,2], Jan A. Staessen, MD, PhD[1,3]; on behalf of the International Database on Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) Investigators

[1]Studies Coordinating Centre, Research Unit Hypertension and Cardiovascular Epi-demiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium; [2]Department of Cardiology, Shanghai General hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China; [3]Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, the Netherlands.

Background: Nocturnal dipping patterns have been associated with increased cardio-vascular risk. Whether coronary risk of dipping pattern is driven by nighttime systolic blood pressure (BP) level remains unknown. **Methods**: Among 11,135 participants (49.3% women; mean age, 53.4 years) enrolled in 13 population studies from three continents of the International Database on Ambulatory Blood Pressure in Relation to Cardiovascular Outcomes (IDACO), we assessed continuous dipping ratio (nighttime /daytime systolic BP) and categorical dipping patterns and compared the dipping status with nighttime systolic BP in relation to incident coronary risk after multi-variable adjustment. **Results:** This study included 2018 extreme dippers (ratio≤0.80), 5617 dippers (0.80<ratio≤0.90), 2908 non-dippers (0.90<ratio≤1.0) and 691 reverse dippers (ratio>1.0). The respective nighttime systolic BPs were 103.3, 109.9, 119.2 and 135.0 mm Hg in these four dipping groups (P<0.0001 for trend). Over a follow-up of 13.8 median years, incident coronary heart disease, myocardial infarction, coronary revascularisation, coronary and sud-den death in 922, 616, 180, 388 and 83 participants. The multivariable-adjusted hazard rati-os of all coronary disease were 1.14 (P<0.0001) in relation to 10 mm Hg-increase of nighttime systolic BP, 1.06 (P<0.0001) in relation to 0.05-increment of dipping ratio and 0.82 (P=0.033) for extreme dippers, 1.01 (P=0.95) for non-dippers, and 1.31 (P=0.020) for re-verse dippers. In additional models including nighttime BP and either continuous (P=0.30) or categorical (P=0.88) dipping status, only nighttime systolic BP (P<0.0001) remained signifi-cant. In subgroups of the dippers and non-dippers, nighttime BP was still a significant risk predictor (P≤0.00070), while dipping ratio (P≥0.062) or pattern (P≥0.16) was not associated with incident risk in nighttime BP subgroups. **Conclusions**: Our results shows that the prognostic value of nocturnal dipping status for coronary disease is driven by nighttime BP level, and relaxes the clinicians from unmanage-able dipping status to focus on handy ambulatory BP level for clinical practice.

YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION II

Lifestyle related cancer deaths in patients with familial hypercholesterolemia

Henriette W. Krogh[1], Liv J. Mundal[1,2], Jannicke Igland[3], Kirsten B. Holven[1,4], Karianne Svendsen[1], Martin P. Bogsrud[4,5], Trond P. Leren[5], Kjetil Retterstøl[1,2]

[1]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; [2]The Lipid Clinic, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway; [3]Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway;[4] National Advisory Unit for familial hypercholesterolemia, Oslo University Hospital, Oslo, Norway; [5] Unit for Cardiac and Cardiovascular Genetics, Oslo University Hospital, Oslo, Norway.

Aims: Diet and lifestyle is a cornerstone in treatment of familial hypercholesterolemia (FH) in addition to almost life-long treatment with lipid-lowering medication. The majority of FH patients in Norway receive dietary counselling from clinical dietitians as part of their follow-up regimen. Earlier we have shown that FH patients have healthier food choices compared to persons without FH. The very long exposure to lipid-lowering medication combined with dietary and lifestyle advice suggest that FH is a relevant model disease in studying the impact of such treatment on the occurrence of lifestyle related cancers. The aim of this study was to investigate whether lifestyle related cancer deaths in patients with a registered FH mutation is different from that of the general population. **Methods and results:** We compared deaths among patients with a registered FH mutation with the Norwegian population (~5 million people) from 1992-2013 and calculated standardized mortality ratios (SMRs) with 95% confidence intervals (95% CIs). Data from 5518 patients (2831 women and 2687 men) were available. Mortality ratios for the total population stratified by sex, calendar year and one-year age groups served as reference rates. The definitions of lifestyle related cancers were in accordance with American Cancer Society's definitions. In total, there were 189 deaths among the FH patients of which 56 deaths were caused by cancer. Overall cancer mortality and deaths due to smoking-related cancers (n=32) tended to be lower in the FH patients compared with the general population, but the difference was not statistically significant (SMR=0.77, (0.60-1.01) and SMR=0.72, (0.51-1.02), respectively). Deaths due to alcohol-related cancers were similar to the general population (n=17, SMR=0.89, (0.56-1.44)). **Conclusion:** There were no differences in overall cancer mortality, smoking-related cancer mortality or alcohol-related cancer mortality between FH patients and the Norwegian population although the FH patients receive diet and lifestyle advice, and comprehensive lipid-lowering medication.

Sex Specific Analysis On Efficacy And Safety Of The ''Big Five'' Cardiovascular Drugs: Systematic Reviews And Quantitative Meta-Analyses

M. Schreuder, H. Boersma, J.E. Roeters van Lennep

Introduction: Cardiovascular disease (CVD) is the major cause of mortality in Europe for men and women. The most commonly prescribed drugs for these patients are referred to as the "Big Five": aspirin, P2Y12 inhibitors, statins, beta-blockers and ACE-inhibitors. With this project we will address the question whether sex differences exist in the efficacy and safety (adverse drug reactions) of the "Big Five" CVD drugs. **Patients, materials and methods:** We will perform five systematic reviews and quantitative meta-analyses applying sex-specific analyses on the efficacy and safety of the "Big Five". A specific literature search will be carried out per drug in Ovid Medline, Embase and Cochrane Central in order to identify relevant RCT's. The indication for treatment will be restricted to secondary prevention for coronary artery disease. Inclusion criteria are RCT's with a sample size bigger than 100, age ≥18 years old and a minimum follow-up time of 3 months. **Results:** The first literature search was performed on statins. Full text screening resulted in a final inclusion of 45 different RCT's. However, sex-specific data on efficacy and safety was only available in 5 trials. In these 5 trials, we found a significant sex difference between the risk ratios of men and women in all-cause mortality (ACM) in the statin vs. placebo trials ($p = 0.03$). Among safety, no significant differences between sexes were objected in discontinuation of therapy as a result of ADR. **Conclusions:** This meta-analysis suggests that men might have more benefit of statins on ACM. However, it should be taken into account that this analysis was pooled with a small amount of RCTs with in-between heterogeneity and only 18% of the cohorts were women. We are in contact with the authors of the remaining 40 trials for their sex-specific data in order to get more valid results.

High lipoprotein (a) levels do not exacerbate aortic valve velocity in a group of FH subjects

Ingunn Narverud[1,2], Martin Prøven Bogsrud[2], Anders Hovland[3,4], Inger Aagnes[3], Gisle Langslet[2,5], Kjetil Retterstøl[1,5], Cecilie Wium[5], Arne Svilaas[5], Kjell Erik Arnesen[5], Linn KL Øyri[1,2], Pål Aukrust[6,7,8,9], Bente Halvorsen[6,7,8], Arne Yndestad[6,7,8], Stine Ulven[1], Kirsten B Holven[1,2]

[1]Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, Norway; [2]Norwegian National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Norway; [3]Division of Internal Medicine, Nordland Hospital, Norway; [4]Department of Clinical Medicine, University of Tromsø, Norway; [5]Lipid Clinic, Oslo University Hospital, Norway; [6]Research Institute for Internal Medicine, Oslo University Hospital, Norway; [7]Institute of Clinical Medicine, University of Oslo, Norway; [8]K.G. Jebsen Inflammatory Research Center, Norway; [9]Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Norway

Aim: Subjects with familial hypercholesterolemia (FH) are characterized by increased LDL cholesterol levels, and premature atherosclerosis and cardiovascular disease. Aortic valve peak velocity (AV Vmax) may be considered as a subclinical marker of atherosclerosis in the aortic valve. Lipoprotein (a) (Lp[a]) is proposed as an independent risk factor for CVD, and may mediate atherosclerosis, at least partly, by its anti-fibrinolytic and pro-inflammatory properties. Furthermore, Lp(a) has been associated with aortic stenosis. However, less is known about the effect of Lp(a) on atherosclerotic markers in patients with primarily genetic verified FH. We aimed to investigate atherosclerotic markers in FH subjects with and without high Lp(a) levels and healthy controls. **Methods:** FH subjects with (n=68) and without (n=77) high Lp(a) defined as Lp(a) > 75 nmol/L, and healthy controls (n=14) were recruited. Circulating atherosclerotic markers were analyzed in non-fasting blood samples. AV Vmax was estimated by continuous wave Doppler. **Results:** Our main results were: i) FH subjects have higher AV Vmax and D-dimer independent of Lp(a) levels compared to controls; ii) FH subjects with high Lp(a) have higher fibrinogen levels compared to controls; iii) FH subjects without high Lp(a) have higher a1-antitrypsin compared to controls; iv) Lp(a) levels were significantly correlated with fibrinogen, and AV Vmax was significantly correlated with D-dimer in the total population. **Conclusions:** FH subjects have higher AV Vmax compared to controls, possibly due to atherosclerosis affecting the aortic valve. Some differences in hemostatic markers between FH and controls were also found independent of Lp(a) levels.

Predilection of Low Protein C-induced Spontaneous Atherothrombosis for the Right Coronary Sinus in Apolipoprotein E-deficient mice

Amber B. Ouweneel[1], Marco Heestermans[2,3], Jasmin Hassan[1], Meander Kloosterman[2,3], Marion J.J. Gijbels[4,5], Bart J.M. van Vlijmen[2,3], Miranda Van Eck[1]

[1]Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands; [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Pathology and department of Molecular Genetics, Cardiovascular Research Institute Maastricht, Maastricht, The Netherlands; [5]Department of Medical Biochemistry, Amsterdam Medical Center, Amsterdam, The Netherlands

Previously we have shown that silencing of anticoagulant protein C using RNA interference (siProc) evokes low incident but spontaneous atherothrombosis in the aortic root of apolipoprotein E–deficient (Apoe-/-) mice. The aims of the current study were 1) to determine if plaque characteristics or circulating factors correlate with atherothrombosis susceptibility in this model, 2) to increase the incidence of atherothrombosis by transiently increasing blood pressure using phenylephrine treatment, and 3) to add an additional predefined vascular site by applying a semi-constrictive collar around the carotid artery.
SiProc-driven spontaneous atherothrombosis in the aortic root reproduced and occurred at an incidence of 23% (9 out of 39 mice), while the incidence of atherothrombosis in the carotid artery was 2.6% (1 out of 39 mice). Phenylephrine treatment did not increase atherothrombosis at either site. Aortic root plaques with an associated thrombus were lower in collagen (-THR: 13.4% (2.1-29.6), +THR: 8.3% (6.4-15.3), P=0.031) and macrophages (-THR: 9.1% (0.8-34.9), +THR: 6.7% (4.5-13.7), P=0.028), and mice with atherothrombosis had higher platelet counts (-THR: 1280x109/L (728-1632), +THR: 1512x109/L (1004-1696), P<0.001). Remarkably, our data revealed that thrombus formation preferably occurred on plaques in the right coronary sinus of the aortic root (7 out of 9, P=0.018). In conclusion, atherothrombosis was found to be associated with an increase in platelets and plaques lower in collagen and macrophage content. Our study highlights that there is a predilection of low protein C-induced spontaneous atherothrombosis in Apoe-/- mice for the right coronary sinus.

Plasma methionine and risk of myocardial infarction: effect modification by established risk factors

Indu Dhar[1,2] , Vegard Lysne[1], Reinhard Seifert[1], Gard FT Svingen[3] , Per M Ueland[1,4], Ottar K Nygård[1,2,3]

[1]Department of Clinical Science, University of Bergen, 5021 Bergen, Norway; [2]KG Jebsen Centre for Diabetes Research, University of Bergen, 5009 Bergen, Norway; [3]Department of Heart Disease, Haukeland University Hospital, 5021 Bergen, Norway; [4]Bevital AS, 5021 Bergen, Norway

Aim: Methionine is a sulfur-containing essential amino acid involved in epigenetic regulation and lipid metabolism. Methionine deficiency is associated with fatty liver, which in turn is related to atherosclerosis. The prospective relation between circulating methionine and incident acute myocardial infarction (AMI) is however unknown. **Methods:** We studied the associations of plasma methionine with incident AMI in 4156 patients (77% men; median age 62 years) undergoing coronary angiography for stable angina pectoris, and among whom 80% were treated with statins. Risk associations were estimated using Cox-regression analyses. **Results:** Plasma methionine was positively related to apolipoprotein (apo) A1, whereas a significant inverse association was observed with age, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and apo B at baseline (all P<0.05). During a median follow-up of 7.5 years, 534 (12.8%) patients experienced an AMI. There was no overall association between plasma methionine and incident AMI; however, methionine was inversely associated with risk among patients with high (> median) as compared to low (= median) levels of serum LDL-C or apoB (multivariate adjusted HRs per SD [95% CI] 0.84 [0.73-0.96] and 0.83[0.73-0.95], respectively; P-interaction= 0.02). A similar but not statistically different trend towards an inverse risk relationship for methionine was also observed with high serum high-density lipoprotein cholesterol or apoA levels. We also observed an inverse risk associations in patients aged 65 years or below (younger), and those without diabetes or hypertension (P-interaction= 0.03, all). **Conclusions:** High plasma methionine was associated with decreased risk of AMI in subjects with high serum atherogenic lipid levels, but also in subgroups with presumably lower cardiovascular risk. The influence of methionine status on residual cardiovascular risk in patients with coronary heart disease should be further investigated.

Clinical familial hypercholesterolaemia and risk of peripheral arterial disease and chronic kidney disease: The Copenhagen General Population Study

Frida Emanuelsson[1, 2], Børge G Nordestgaard[2, 3, 4], Marianne Benn[1, 2, 4]

[1]Department of Clinical Biochemistry, Rigshospitalet Copenhagen University Hospital, Blegdamsvej 3, 2100 Copenhagen, Denmark; [2]Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, 2200 Copenhagen, Denmark; [3]Department of Clinical Biochemistry, and [4]The Copenhagen General Population Study, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev Ringvej 75, 2730 Herlev, Denmark.

Aims: Individuals with familial hypercholesterolaemia (FH) have high risk of coronary artery disease, but whether they also have high risk of peripheral atherosclerotic disease is unclear. In individuals with clinical FH, we tested the hypotheses 1) that the risks of peripheral arterial disease (PAD) and chronic kidney disease (CKD) are elevated, and 2) that ankle-brachial index (ABI) and estimated glomerular filtration rate (eGFR), as markers of PAD and CKD, are associated with high risk of myocardial infarction. **Methods:** We used Dutch Lipid Clinic Network (DLCN) criteria to diagnose clinical FH in 106,172 individuals from the Copenhagen General Population Study. **Results:** Compared with individuals with unlikely FH, multivariable adjusted odds ratios of PAD were 1.84 (95% confidence interval 1.70-2.00) in those with possible FH and 1.35 (1.00-1.83) in individuals with probable/definite FH. For CKD, the corresponding odds ratios were 1.93 (1.79-2.09) and 2.49 (1.91-3.25). The risk of myocardial infarction increased from unlikely FH and ABI>0.9 or eGFR=60mL/min/1.73m2 to possible/probable/definite FH and ABI=0.9 or eGFR<60mL/min/1.73 with multivariable adjusted hazard ratios of 4.86 (2.50-9.47) and 2.20 (1.71-2.83), respectively. **Conclusion:** In the general population, clinical FH based on the DLCN criteria is associated with an increased risk of PAD and CKD. Furthermore, reduced ABI and eGFR are associated with high risk of myocardial infarction in clinical FH. This suggests that individuals with FH should be routinely screened for PAD and CKD, and that ABI and eGFR may be used as prognostic tools in the management and treatment of FH.

Increased levels of the cysteine protease legumain in plasma and atherosclerotic plaques from patients with carotid stenosis

Ngoc Nguyen Lunde[1], Sverre Holm[2], Tuva B. Dahl[2,3], Inass Elyouncha[1], Bjørnar Sporsheim[4], Ida Gregersen[2,3], Azhar Abbas[5], Mona Skjelland[5], Terje Espevik[4], Rigmor Solberg[1], Harald Thidemann Johansen[1] and Bente Halvorsen[2,3]

[1]Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway; [2]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet (OUH), Oslo, Norway; [3]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway; [4]Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway; [5]Department of Neurology, OUH, Oslo, Norway

The cysteine protease legumain has previously been shown to be up-regulated in unstable atherosclerotic plaques [1-2]. Legumain in atherosclerosis was further elucidated by examining plasma and carotid plaques from patients with carotid stenosis. Also, legumain secretion from monocyte-derived macrophages treated with atherogenic lipids during macrophage polarization was studied. Levels of legumain in plasma from patients with carotid stenosis (n = 254), healthy controls (n = 91), and secreted from monocyte-derived macrophages were assessed by enzyme-linked-immunosorbent assay. Quantitative PCR and immunoblotting of legumain were performed on isolated plaques and legumain localization was visualized by immunohistochemistry and fluorescence microscopy. Monocyte-derived macrophages polarized to M1 or M2 macrophages were treated with VLDL, oxLDL or cholesterol crystals (CC) and the level of legumain analyzed. Patients with carotid stenosis had significantly higher levels of plasma legumain compared with healthy controls, although there was no correlation between the level of legumain and the degree of stenosis and legumain was not an independent factor to identify patients with carotid plaques. Moreover, patients with symptoms the last 2 months had higher expressions of legumain, its inhibitors cystatin C and E/M, and the macrophage markers CD80 (M1) and CD163 (M2). Legumain co-localized with both M1 and M2 macrophages within plaques, and legumain mRNA expression was significantly higher in plaques compared to non-atherosclerotic control arteries. Furthermore, in vitro studies showed significantly increased secretion of legumain from pro-inflammatory M1 compared to pro-resolving M2 macrophages, and particularly in M1 treated with CC. In carotid plaques, legumain was localized to structures resembling foam cells. In conclusion, legumain was increased in both plasma and plaques of patients with carotid stenosis and might be a new biomarker of atherosclerosis.

Reference:
1. Mattock, K.L., et al., Legumain and cathepsin-L expression in human unstable carotid plaque. Atherosclerosis, 2010. 208(1): p. 83-9.
2. Clerin, V., et al., Expression of the cysteine protease legumain in vascular lesions and functional implications in atherogenesis. Atherosclerosis, 2008. 201(1): p. 53-66.

Lipoprotein(a) are associated with stenosis in the carotid artery in patients with a mild-to-moderate stenosis

Dianne H.K. van Dam-Nolen[1,2], Anouk C. van Dijk[1], M. Eline Kooi[3], Jeroen Hendrikse[4], Paul J. Nederkoorn[5], Peter J. Koudstaal[2], Monique T. Mulder[6], Aad van der Lugt[1]

[1]Department of Radiology and Nuclear Medicine, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands; [2]Department of Neurology, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands; [3]Department of Radiology and Nuclear Medicine, CARIM School for Cardiovascular Diseases, Maastricht University Medical Center, Maastricht, The Netherlands; [4]Department of Radiology, University Medical Center Utrecht, Utrecht, The Netherlands; [5]Department of Neurology, Academic Medical Center, Amsterdam, The Netherlands; [6]Department of Internal Medicine, Erasmus University Medical Center Rotterdam, Rotterdam, The Netherlands

Background and purpose: An elevated plasma lipoprotein(a) [Lp(a)] level is an independent risk factor for cardiovascular disease and recurrent ischemic stroke. Lp(a) concentrations are largely determined by the number of KIV repeats in the LPA gene encoding apo(a). Previous studies have shown an association between high Lp(a) levels and stenosis in the coronary arteries. However, the association between Lp(a) levels and atherosclerosis in the carotid artery is yet unknown. The aim of this study was to analyze the association between Lp(a) levels and imaging biomarkers of the atherosclerotic plaque in the carotid artery. **Methods:** We selected 182 patients with determined Lp(a) concentrations of the Plaque At RISK-study (PARISK) in which patients with ischemic symptoms and an ipsilateral carotid artery stenosis of 30-69% were included. Imaging biomarkers of carotid atherosclerosis (severity and composition) were determined by MDCTA (n=162) and MRI (n=173). We defined elevated Lp(a) as >50 mg/dL. Univariable and multivariable linear and logistic regression analyses were used to investigate the association between Lp(a) levels and markers of atherosclerosis: degree of stenosis, plaque ulceration, presence of calcification, lipid-rich necrotic core (LRNC) and intraplaque hemorrhage, volume of calcifications and LRNC. The smallest apo(a) isoform, as determined by immunoblotting, was used as a continuous variable. **Results:** Elevated Lp(a) levels were significantly associated with degree of ECST stenosis (beta=6.71, 95% CI: 1.35-12.07). After adjusting for age, gender, hypertension, hypercholesterolemia, diabetes mellitus and current smoking, the association remained significant (beta=6.66, 95% CI: 1.28-12.04). There was no effect of the apo(a) isoform. Elevated Lp(a) levels were not associated with the other imaging biomarkers. **Conclusions:** Lp(a) is significantly associated with degree of stenosis in the carotid artery in patients with a history of ischemic symptoms and a mild-to-moderate carotid artery stenosis.

Posters – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Treat-To-Target Familial Hypercholesterolemia (TTT-FH): A 8-10 years prospective study in adult patients with familial hypercholesterolemia (FH) treated in a specialized Lipid Clinic.

Arnesen, Kjell-Erik; senior consultant, MD; Randsborg, Karoline; clinical nutritionist; Mork, Irene; clinical nutritionist; Thorvall, Marlene; clinical nutritionist;. Ose, Leiv; professor emeritus, Dr. Med; Svilaas, Arne; senior consultant, Dr. Med; Langslet, Gisle; senior consultant, MD; Wium, Cecilie; senior consultant, Dr. Med; Retterstøl, Kjetil; professor, Dr. Med.

Avdeling for Endokrinologi, Sykelig Overvekt og Forebyggende medisin (EFSPA), Lipidklinikken. Oslo Universitetssykehus HF. Rikshospitalet. Norge.

Aim: To study the effect of maximal tolerable lipid lowering treatment (LLT) from 2006-2017. **Methods:** 216 patients with FH were followed from 2006 and the next 8-10 years prospectively. **Results:** First lipid measurement was at mean age (95%CI) 26.8 (25.0, 28.5) years with a total cholesterol of 10.0 (9.7, 10.3) and LDL 7.6 (7.1, 8.1). 92% had genetically FH, diagnosed at mean age of 33.9 years. Men started LLT earlier than women at age 31.9 years (29.8, 34.0) versus 37.8 (35.6, 39.9). High intensity statins were used by 77.8%. Double LLT was used by 51.4%, triple by 26.4 % and quadruple by 3.7%. Side effects were reported by 33.5 %, mainly muscular complaints. At V3 the achieved mean TC was 5.0 (4.8, 5.2), LDL 3.0 (2.9, 3.2), non-HDL 3.6 (3.4, 3.8). LDL treatment target was reached in 30.3% of the patients in primary prevention and only 8.5% in secondary prevention. Weight, waist and HbA1c% increased significantly during the study period. There were 46 CVD events during the study period. Of these events 26 were recurrent and 22 first time CVD events. **Conclusions:** Despite up to quadruple LLT no more than 8.5% and 30.3% of the patients reached the lipid targets. Women started LLT 6 years later than men.

Interrelation level of leptin, postprandial hypertriglyceridemia, endothelial dysfunction and body mass index in patients with coronary heart disease combined with non-alcoholic fatty liver disease.

M.Grechanyk.

Ukraine, the Dnieper, Vernadsky 9. Department of Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine

Objective: to estimate the frequency of leptin levels, postprandial hypertriglyceridemia (PPG), endothelial dysfunction, body mass index (BMI) in patients with coronary heart disease (CHD) combined with non-alcoholic fatty liver disease (NAFLD). **Methods:** We studied 48 patients with CHD in combination with NAFLD mean age 57,6±2,04 (group A) and 24 patients with CHD without NAFLD mean age 55,8±4,2 (group B). Patients with diabetes were excluded. The study group was divided into 3 subgroups according to BMI (subgroup 1 (35%) - patients who are overweight, 2 (40%) - obesity 1 degree, 3 (25%) - obesity grade 2). Studied endothelial function, PPG, leptin levels. **Results:** According to the reactive hyperemia test data, endothelial dysfunction was found in 45 patients (91%) in a group A and in 12 patients (50%) in a group B, vasospastic response observed in 2 patients (9%) in a group A. Reactive hyperemia index (RHI) was lower (4,87±3,3%,) in the group A than in group B (8,8±3,4%) ($p<0,05$). In patients with grade 2 obesity identified the highest leptin levels (43,6 ± 20,2, $p <0,05$), which is 44% higher than in the subgroup with obesity 1 degree (24,4 ± 14,6, $p <0.05$) and 63% greater than in the subgroup with overweight. The greatest increase in triglycerides after fat loading test with fixed subgroup 1 (70%) and in the group B (116%). There was correlation between the endothelial dysfunction and level of leptin ($r=0,76$, $p=0,03$) in a subgroup 2 however in a subgroup 1 and 3 such connection was not observed. **Conclusion:** In patients with NAFLD in combination with CHD endothelial dysfunction were more expressed than in those who had no NAFLD. There was correlation between the endothelial dysfunction and level of leptin in patients who are obesity 1 degree however in patients who are overweight and obesity 2 degree such connection was not observed. "

Characterization of MDA-LDL-specific Antibody Response in Atherosclerosis

Martos-Folgado I[1], Lorenzo C.[1], Delgado P.[1], Busse C.E.[2], Wardemann H.[2], Ramiro AR.[1]

[1]B Cell Biology Lab, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [2]B Cell Immunology Lab, German Cancer Research Center, Heidelberg, Germany.

Introduction: Atherosclerosis is a chronic inflammatory disorder of the arteries that causes thrombosis and myocardial infarction or stroke. Adaptive immunity is involved in atherosclerosis, but the precise role of the immune response in disease initiation and progression is still ill-defined. It is generally believed that neoantigens generated in the context of atherosclerosis progression are responsible for triggering an adaptive immune response. Among those neoantigens, the most studied are the oxidized forms of low density lipoprotein (e.g. MDA-LDL). Intriguingly, immunization experiments with MDA-LDL in proatherogenic mice have been reported to be protective. However, the immune response to MDA-LDL and the mechanisms responsible for this protective role have not been yet addressed. **Objectives:** We aim at characterizing the humoral immune response and the antibody repertoire generated upon MDA-LDL immunization in a non-atherogenic mouse model. **Results:** We have performed kinetics analyses of C57BL/6 or AID Cre+/ki / R26tdTom+/ki reporter mice immunized with MDA-LDL plus adjuvant or adjuvant alone, and have found that MDA-LDL triggers a germinal center response and that mice progressively accumulate memory B cells, plasma cells and high titers of specific IgM and IgG antibodies. In addition, the BCR repertoire of immunized mice is being analysed by single cell sorting, sequencing and cloning. Finally, experiments are in progress to characterise the contribution of the antibody response in the atheroprotective role of MDA-LDL. We expect our results will help to understand atherosclerosis-associated neoantigens and their role in the progression of the disease.

Antioxidant capacity of HDL is strongly influenced by size and cholesterol level of this particle and cholesterol ester transfer protein activity

Maria Camila Pruper de Freitas, Flávia de Conti Cartolano, Alexandre Orsi Campos Ventura, Mariana Baldini Prudencio, Nágila Raquel Teixeira

Damasceno - Department of Nutrition, Faculty of Public Health, University of Sao Paulo, Brazil.

Objective: To identify components of high-density lipoprotein (HDL) able to modulate the antioxidant capacity of HDL. **Methods:** Cross-sectional study (n = 343) including individuals of both sexes, age from 30 to 74 years old. Plasma and HDL lipids and proteins were evaluated by standard. HDL subfractions (n=10) were evaluated by the Lipoprint® method. The antioxidant capacity of HDL was evaluated by the Lag time method, where area under the curve performed in the production of conjugated dienes was calculated. From these data, univariate and multiple linear regression models were tested to identify de influence of plasma and HDL components in the antioxidant capacity of HDL. Variables with p <0.20 in the univariate models were selected for the final multiple model. All models were adjusted by previous chronic diseases and drug use. Statistical significance adopted was p <0.05. **Results:** The antioxidant capacity of HDL was associated with the cholesterol content and size of this lipoprotein and also the activity of the CETP, regardless of the presence of chronic diseases and the use of drugs. The final multiple model explained 11% of the variability in antioxidant capacity of HDL. Increasing in cholesterol content (1.0 mg/dL), HDL size (1%) and reduction in CETP activity (1.0 pmol/min) were associated with 0.12, 0.18 and 0.19 units in the antioxidant capacity of HDL, respectively. **Conclusion:** Therefore, our results highlighted that negative impact of CETP in cholesterol content and size of HDL influence directly the antioxidant capacity of this particle and probable its functionality.

Oxidized LDL stimulates production of inflammatory extracellular vesciles by platelets

Maarit Neuvonen and Pia Siljander

EV-Group, Faculty of Bio- and Environmental Sciences and Faculty of Pharmacy, University of Helsinki, Finland

The role of platelets in generation of thrombosis in late stage atheroma is well-established. However, it is becoming evident that platelets are activated in hyperlipidemic conditions already before plaque formation can be detected. Platelets can be activated and produce extracellular vesicles (EVs) by different pathways, including interaction with oxidized LDL, which is generated during atherosclerosis. Platelet-derived EVs transport e.g. growth factors and cytokines, which can modulate cells of the developing atheroma. We analyzed EV production from platelets, isolated from platelet concentrates, after different activating stimuli including LDL, oxidized LDL, HDL and compared these to thrombin + collagen co-stimulus, a strong EV-inducing signal. Platelet activation was monitored by flow cytometry of P-selectin exposure. EVs were isolated by serial ultracentrifugation to separate them from lipoproteins and soluble proteins, and analyzed by EV-dedicated high-resolution flow cytometry. We found that the lipoproteins induced platelet EV production in a concentration- and time-dependent manner at concentration levels relevant to physiological conditions, and further, detected inhibitory effects of the different lipoprotein stimuli on each other. These EVs were able to induce TNF-alpha expression when fed to recipient cells. In conclusion, hyperlipidemia endorsed changes in lipoprotein profiles can manifest in altered platelet EV generation capable of increasing proinflammatory cytokines.

YIA Poster Walk – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Genetic variants in APOM revealed no association with diabetes mellitus and ischemic heart disease in the general population

Stefan Hajny[1,2], Mette Christoffersen[1], Lars Bo Nielsen[4], Anne Tybjærg Hansen[1,2], Christina Christoffersen[1,2,3]

[1]Department of Clinical Biochemistry, Rigshospitalet, University Hospital of Copenhagen, Denmark; [2] Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [3]Department of Clinical Biochemistry, Bispebjerg and Frederiksberg Hospital; [4]Aarhus University Hospital, The Faculty of Health Sciences , Aarhus University, Aarhus, Denmark

Apolipoprotein M (apoM) is mainly carried by HDL particles and previous studies suggested a protective role of apoM in various pathophysiological mechanisms including diabetes mellitus (DM) and ischemic heart disease (IHD). Plasma apoM correlates with fasting insulin, BMI, total cholesterol and HDL as well as LDL in humans. Five single nucleotide polymorphisms (SNP) in the apoM promoter region and two SNP's in the corresponding coding sequence have been extensively analysed to detect associations with DM and IHD. Results are however inconclusive so far. We used a mendelian randomization strategy to analyse novel genetic variants in APOM to identify correlations with DM and IHD in the European general population. Participants of the Copenhagen City Heart study (n=9,087) and Copenhagen General Population study (n=96,715) have been analysed and available data from GWAS consortia have been incorporated. Depending on the genotyping approach available SNP's were investigated in respective subsets (>19,750 individuals) or the entire cohort. We explored 7 novel SNP's in the human apoM gene in addition to previously reported genetic variants, yielding 7 promoter, 3 exonic and 3 intronic SNP's in total. Associations between the genetic variants and parameters on diseases related biomarkers such as plasma lipids, glucose and BMI have been studied in addition to the endpoints DM and IHD. The study confirmed an association between the apoM plasma concentration and DM (OR=0.3, 95% CI=0.2-0.5) as well as IHD (OR=0.7, 95% CI=0.5-0.9). One promoter variant decreased the apoM plasma levels by 11% (p=5*10-5), without altering disease susceptibility (DM:OR=1.0, 95% CI=0.7-1.5; IHD: OR=0.9, 95% CI=0.7-1.2). Finally, neither of the remaining variants was associated with an increased risk for DM and IHD nor plasma biomarkers. In conclusion, we identified a single SNP in the human apoM promoter region which altered the corresponding apoM plasma concentration by 11%, an association with DM or IHD could however not be identified for any of the investigated SNPs.

USF1 silencing promotes cholesterol efflux, prevents cholesterol uptake and alleviates inflammation in macrophages

Maija Ruuth[1], Jarkko Soronen[2], Essi Kaiharju[3], Krista Merikanto[3], Julia Perttilä[2], Jari Metso[2], Miriam Lee-Rueckert[1], Marja-Riitta Taskinen[4], Petri T. Kovanen[1], Katariina Öörni[1], Vesa M. Olkkonen[2], Pirkka-Pekka Laurila[3,5], Matti Jauhiainen[2,3]

[1]Wihuri Research Institute, Helsinki, Finland; [2]Minerva Foundation Institute for Medical Research, Helsinki, Finland; [3]National Institute for Health and Welfare, Helsinki, Finland; [4]Diabetes and Obesity Research Program, University of Helsinki, Helsinki, Finland; [5]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

Aim - The upstream stimulatory factor 1 (USF1) was originally associated with familial combined hyperlipidemia. We recently showed that inactivation of USF1 in mice protects against cardiometabolic derangements and leads to high plasma HDL-C and low triglyceride levels. Here the aim was to study the impact of USF1 silencing on HDL functionality in macrophages, focusing on cholesterol efflux, cholesterol uptake and inflammation. **Methods** - USF1 was silenced using shRNA in THP-1 cells. Cholesterol efflux and acetyl-LDL uptake were measured in THP-1 macrophages. Cytokines were analyzed with ELISA methods. Gene expression microarray was performed in peritoneal macrophages from Usf1-/- mouse. **Results** - USF1 deficiency improved the function of HDL. HDL particles derived from Usf1-/- mice removed cholesterol more efficiently from macrophages, which was attributed to a higher amount of phospholipids per HDL particle. Silencing of USF1 in THP-1 macrophages enhanced the cholesterol efflux capacity of these cells associated with increased ABCA1 mRNA levels. Secretion of pro-inflammatory cytokines MCP-1 and IL-1ß was reduced demonstrating attenuated inflammatory burden in USF1 deficient macrophages. Lack of USF1 protected against LPS-induced macrophage cholesterol uptake and was associated with a reduced SR-A1 mRNA level. Usf1 deficiency in mouse induced upregulation of macrophage genes involved in fatty acid ß-oxidation and mitochondrial oxidative phosphorylation thus resembling the transcriptome of the brown adipose tissue of Usf1-/-mice. **Conclusions** - Our findings identify USF1 as a novel factor regulating HDL functionality, demonstrating that USF1 deficiency improves the functional quality of HDL particles, enhances cholesterol efflux, attenuates cholesterol accumulation, and reduces macrophage inflammation. Improved cholesterol flux through macrophages and alleviated inflammation are associated with the anti-atherogenic function of USF1 deficiency.

Treat-To-Target Familial Hypercholesterolemia (TTT-FH): A prospective study in adult patients with FH. Pregnant women have many years off lipid lowering medication.

Randsborg, Karoline; clinical nutritionist. Arnesen, Kjell-Erik; senior consultant, MD. Mork, Irene; clinical nutritionist. Thorvall, Marlene; clinical nutritionist. Ose, Leiv; professor emeritus, Dr. Med. Svilaas, Arne; senior consultant, Dr. Med. Langslet, Gisle; senior consultant, MD. Wium, Cecilie; senior consultant, Dr. Med. Retterstøl, Kjetil; professor, Dr. Med.

Avdeling for Endokrinologi, Sykelig Overvekt og Forebyggende medisin (EFSPA), Lipidklinikken. Oslo Universitetssykehus HF. Rikshospitalet. Norge.

Aim: To study consequences for lipid lowering therapy (LLT) in females with familial hypercholesterolemia (FH) due to pregnancy and lactation. **Methods:** In 2006 FH patients (n=357) were evaluated at visit 1, at visit 2 one year later (n=332), and visit 3 during 2014-17 (n=216). We present data of LLT during pregnancy and lactation. **Results:** At visit 3 100 patients (47.3%), were female, mean age 52.5 years. Age at start of LLT among women was mean 37.8 years, which is 5.9 years later than in men. Only 22 of the women used LLT before pregnancy, with an average LLT of 7.9 years. 44 women initiated LLT first after the childbirth. During the study 22 women discontinued LLT due to pregnancy, and later restarted LLT. Total time off LLT during the conception-pregnancy-lactation period was mean 4.2 years. Most women had one child during the study. **Conclusions:** Women started LLT 5.9 years later than men, and 44% initiated LLT first after childbirth. Time off LLT for FH women was approximately 4 years per childbirth. Time off LLT is far longer than the pregnancy-lactation period. This might have impact on females' CVD risk.

Testosterone reduces brown fat activity in male mice

Marta Lantero Rodriguez[1], Maaike Schilperoort[2], Jan Kroon[2], Elin Svedlund Eriksson[1], Inger Johansson[1], Malin C. Levin[1], Patrick C.N. Rensen[2], Åsa Tivesten[1]

[1]Wallenberg Laboratory for Cardiovascular and Metabolic Research, Institute of Medicine, Sahlgrenska Academy, Göteborg, Sweden; [2]Department of Medicine, Division of Endocrinology and Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

Background and aim: Brown adipose tissue (BAT) burns large amounts of triglyceride (TG)-derived fatty acids (FA) for thermogenesis and has been suggested to protect against hyperlipidemia. The volume of BAT as measured by [18F]FDG-PET-CT increases during adolescence when androgen levels rise, an effect that is greater in boys than in girls. However, little is known if and how androgens regulate BAT mass and activity. Therefore, we aimed to study the effect of the male androgen testosterone on BAT activity. **Methods and results:** In line with the findings in humans, BAT was enlarged in C57Bl/6J male mice paralleling increasing testosterone levels across adolescence. To confirm a role of testosterone, we performed short-term castrations (2-3w) in 10-11w old male mice, which renders mice completely testosterone deficient. Testosterone deficiency reduced BAT weight (-30%; $P<0.01$), indicative of an increased BAT activity, and reduced serum TG levels (-50%; $P<0.05$). We further determined tissue-specific uptake of FA derived from radiolabeled lipoprotein-like particles in sham-operated and castrated mice treated with vehicle or testosterone. In line with the above results, castration markedly enhanced FA uptake by BAT (+350%; $P<0.0001$) while testosterone supplementation to castrated mice increased BAT weight and decreased FA uptake by BAT. Mechanistically, castration increased expression of genes involved in mitochondrial biogenesis (Pgc1a) and FA uptake/lipolysis (Lpl) in BAT. **Conclusions:** Testosterone deficiency increases BAT activity, resulting in reduced BAT mass and serum TG levels in male mice. These results suggest that testosterone is an important negative regulator of BAT activity, potentially saving energy for its anabolic functions.

HDL Cholesterol Efflux Predicts New Onset Diabetes After Transplantation (NODAT) in Renal Transplant Recipients Independent of HDL Cholesterol Levels

Tamas Szili-Torok[1], Wijtske Annema[1], Stephan J.L. Bakker[2], and Uwe J.F. Tietge[1]

[1]Department of Paediatrics, [2]Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

In renal transplant recipients (RTR) new onset diabetes after transplantation (NODAT) is a frequent and serious complication limiting survival of both graft and patient. However, the underlying pathophysiology remains incompletely understood. In vitro and in preclinical models, HDL can preserve beta cell integrity, largely due to its cholesterol efflux function. Currently, no data are available evaluating this concept in humans. Therefore, the aim of this study was to investigate whether baseline cholesterol efflux in RTR is prospectively associated with incident NODAT during follow-up. In this prospective longitudinal study 405 diabetes-free RTR with a functioning graft for >1 year were included. During a median follow-up of 9.6 [6.6-10.2] years 57 patients (14.1%) developed NODAT. Cholesterol efflux capacity at baseline was quantified using incubation of human macrophage foam cells with apolipoprotein B-depleted plasma. Baseline efflux was significantly lower in patients developing NODAT during follow-up compared with the NODAT free group (6.84 [5.84-7.50] vs. 7.44 [6.46-8.60], resp., p=0.001). Kaplan-Meier analysis showed a lower risk for incident NODAT with increasing gender-stratified tertiles of HDL efflux capacity (p<0.001). Receiver operator characteristic (ROC) curves confirmed that cholesterol efflux capacity predicts NODAT (p=0.001). Cox regression analysis demonstrated that cholesterol efflux was significantly associated with NODAT (HR, 0.53; 95% CI, 0.38-0.76; p<0.001), even after adjustment for HDL cholesterol levels (p=0.015), constituents of the metabolic syndrome (p=0.018), immunosuppressive medication (p=0.004) and kidney function (p=0.01). In conclusion, this study establishes HDL cholesterol efflux capacity as a strong predictor of NODAT in RTR independent of a number of other recognized risk factors.

Effects of fish and krill oil on gene expression in peripheral blood mononuclear cells and circulating markers of inflammation: a randomised controlled trial.

Amanda Rundblad[1], Kirsten B. Holven[2,3], Inge Bruheim[4], Mari C. Myhrstad[1] and Stine M. Ulven[2]

[1]Department of Nursing and Health Promotion, Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, P.O. Box 4 St. Olavs plass, 0130 Oslo, Norway; [2]Department of Nutrition, Institute for Basic Medical Sciences, University of Oslo, P.O. Box 1046 Blindern, 0317 Oslo, Norway; [3]National Advisory Unit on Familial Hypercholesterolemia, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, P.O. Box 4950 Nydalen, 0424 Oslo; [4]Rimfrost AS, N-6099 Fosnavaag, Norway

Marine omega-3 fatty acids reduce CVD risk, mainly by effects on lipid metabolism and inflammation. Although the underlying mechanisms of these effects are not fully understood, they include alteration of gene expression by regulation of the activity of transcription factors. Krill oil is a source of marine omega-3 fatty acids that has been shown to modulate gene expression in animal studies, however, the effect in humans is not known. Hence, we aimed to compare the effect of intake of krill oil, lean and fatty fish with a similar content of omega-3 fatty acids, and high-oleic sunflower oil added astaxanthin (HOSO) on expression of genes in glucose and lipid metabolism and inflammation in peripheral blood mononuclear cells (PBMC) as well as circulating inflammatory markers. In an 8-week trial, healthy men and women aged 18-70 years with fasting TAG of 1.3-4.0 mmol/L were randomised to receive krill oil capsules (n 12), HOSO capsules (n 12) or lean and fatty fish (n 12). The weekly intakes of marine omega-3 fatty acids from the interventions were 4654 mg, 0 mg and 4103 mg, respectively. The mRNA expression of four genes, PPARGC1A, SCD, ABCA1 and CD40, were differently altered by the interventions. Furthermore, within-group analyses revealed that krill oil downregulated the mRNA expression 13 genes, including genes involved in glucose and cholesterol metabolism and ß-oxidation. Fish altered the mRNA expression of four genes and HOSO downregulated 16 genes, including several inflammation-related genes. There were no differences between the groups in circulating inflammatory markers after the intervention. In conclusion, we show that intake of krill oil and HOSO added astaxanthin alter the PBMC mRNA expression of more genes than intake of fish.

Added sugar intake and micronutrient dilution on a Swedish population.

Esther González-Padilla[1], Joana A. Dias[2], Emily Sonestedt[3]

[1]Nutritional Epidemiology. Department of Clinical Sciences in Malmö, Lund University. [2]Nutritional Epidemiology. Department of Clinical Sciences in Malmö, Lund University. [3]Nutritional Epidemiology. Department of Clinical Sciences in Malmö, Lund University.

Aim: Recently, the focus on the detrimental effects that a high intake of sugar may have on CVD as well as on whether certain micronutrients could affect the development of CVD has increased. There is little reliable data to support the association between the intake of energy-dense products, low in micronutrients and high in Added Sugars (AS), and the decrease in the intake of nutritious food. The aim of this study is to investigate the association between AS and micronutrient intake and to add to the connection between diet and CVD. **Methods:** Data was obtained from the Malmö Diet and Cancer Study, which included extensive dietary information from Malmö (Sweden) residents. AS was estimated by subtracting the sum of sugar from fruits, vegetables and juices from the sugar intake. The levels of daily intake of 9 micronutrients were analysed across 6 strata of AS intake, expressed as percentage of Total Energy Intake (TEI), for men and women separately. The model was adjusted for age, BMI and TEI. The percentage of individuals with micronutrient intakes below the Average Requirements (AR) and the Recommended Intake (RI) for the 6 groups were also analysed. **Results:** The sample population included 12.238 individuals (45% males), aged 45 to 68. Inverse associations could be observed between the levels of TEI derived from AS and micronutrient intake. The percentage of individuals with intake levels below AR and RI were most remarkable for folate, selenium and vitamin D. We found significant difference between the lowest and highest AS groups ranging from 10% to 40% difference in males and around 25% in females for all three micronutrients. Moreover, half or more of the females with the highest level of AS did not meet the daily AR of these 3 micronutrients. **Conclusion:** Our study shows a consistent association between high levels of AS and micronutrient dilution and helps to further understand the effect that diet has on CVD.

Posters – Abstracts –

Other Topics

SESSION IV

A preliminary report of lipid levels and selected biomarkers of vascular changes in children with idiopathic headaches

Kopyta I[1], Sarecka-Hujar B[2], Sordyl J[3], Francuz T[4], Matusik P[3], Małecka-Tendera E[3]

[1]Medical University of Silesia in Katowice, School of Medicine in Katowice, Department of Pediatric Neurology; [2]Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Pharmaceutical Technology; [3]Medical University of Silesia in Katowice, School of Medicine in Katowice, Department of Paediatrics and Paediatric Endocrinology; [4] Medical University of Silesia in Katowice, School of Medicine in Katowice, Department of Biochemistry

Background: Recent data suggest new potential risk factors for atherosclerosis, that may be associated with headaches however the data studies in children are scarce. The aim of the study was to analyse the blood levels of lipids and selected biomarkers of atherosclerosis in children with idiopathic headaches. **Methods:** The study population comprised 65 children (39 children with idiopathic headaches and 26 healthy individuals). Total cholesterol (TChol), HDL-, LDL- cholesterol and triacylglycerols (TG) levels were measured in every patient. Additionally brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) soluble CD40 ligand (sCD40L) and endothelial plasminogen activator inhibitor (serpin E1/PAI I) blood levels measurements were performed in 34 children. **Results:** We observed that TChol level was lower in the studied group compared to controls (121.04mg/dl vs 146.87mg/dl, p=0.019). In contrast, no differences in concentrations of TG, HDL- as well as LDL-cholesterol were found. There was significant difference in TChol level between girls with headaches and healthy girls (115.44mg/dl vs 144.58mg/dl, p=0.014). BDNF was significantly higher in the studied group (171.57pg/ml vs 64.04pg/ml, p=0.012). VEGF was significantly higher in boys with headaches than in girls (368.27pg/ml vs 142.86pg/ml, p=0.011). There were no statistical differences in levels of VEGF, sCD40L and PAI-1 between groups. **Conclusions:** Children with headaches have lower TChol and higher BDNF levels than controls. No significant differences in levels of TG, HDL-, LDL-cholesterol, VEGF, sCD40L and PAI-1 were found between children with headaches and healthy children.

Novel biomarker discovery in Non-Alcoholic steatihepatitis (NASH) – translation of complex data into clear results

Tanja Xenia Pedersen[1], Kristoffer Rigbolt[1], Sanne S. Veidal[1], Niels Vrang[1], Jacob Jelsing[1]

[1]*Gubra ApS, Hørsholm, Denmark*

Objectives: Non-Alcoholic Fatty Liver Disease (NAFLD) is a complex spectrum of liver diseases ranging from benign hepatic steatosis to the more aggressive necroinflammatory manifestation, Non-Alcoholic SteatoHepatitis (NASH). Today, diagnosis of NASH is done by an invasive liver biopsy procedure with a relatively high risk of complications followed by histopathological assessment of disease characteristics. Hence, there is an immediate need for validated and robust clinical biomarker assays that can identify disease stages and provide the basis for future standard of care. **Methods:** A meta-analysis of hepatic RNASeq datasets obtained from diet-induced obese (DIO) NASH mice was performed. Disease progression from 0-50 weeks with free access to a high trans-fat, fructose, and cholesterol diet, was compared to disease resolution following diet change and benchmark compound treatment. **Results:** This study demonstrates that a multi-dimensional analysis strategy is needed to elucidate complicated gene regulatory networks underlying the NASH pathology spectrum. DIO-animals had pronounced changes in lipid metabolic pathways but did not show the inflammatory and fibrotic components strongly manifested in DIO-NASH animals. With this understanding of the underlying biological processes, gene lists were funneled through a selection pipeline resulting in a manageable list of translatable biomarker candidates. We identified several known biomarker candidates (including vimentin and cytokeratin-18) showing methodological proof of concept, and created a panel of new biomarker candidates. **Conclusion:** We have identified a panel of novel NASH biomarker candidates by combining various RNASeq datasets in preclinical mouse models of NASH. Biomarker panels enabling patient stratification in terms of prognosis, diverse medical needs, and treatment follow-up will be of paramount importance in future NASH treatment and clinical trials.

YIA Poster Walk – Abstracts –

Other Topics

SESSION IV

Development of a novel rat model of diabetic cardiomyopathy

Louise Thisted[1], Keld Fosgerau[1], Thomas Secher[1], Morten Bækgaard Thomsen[2], Thomas Jespersen[2], Ross T. Lindsay[3], Niels Vrang[1], Lisbeth N. Fink[1], Tanja X. Pedersen[1], Nora E. Zois[1].

[1]Gubra ApS, Hørsholm, Denmark; [2]Department of Biomedical Sciences, University of Copenhagen, Denmark; [3]Department of Physiology, Development and Neuroscience, University of Cambridge, UK.

Cardiovascular complications are the leading cause of diabetes-related morbidity and mortality. Patients with diabetes experience specific structural and functional abnormalities of the myocardium, collectively known as diabetic cardiomyopathy (DC). No effective therapies targeting DC exist. This has, in part, been attributed to the failure of the available pre-clinical models to recapitulate essential features of the clinical condition. We hypothesized that cardiomyopathy can be superimposed on pre-existing diabetes by administration of the sympathomimetic agent isoprenaline (Iso) to provide a novel rat model of DC. Partial (90%) pancreatectomy (Px) was used as a model of Type 1 diabetes, which induced hyperglycemia within 2 weeks (blood glucose concentrations of 23.3 ± 3.7 mM). Five weeks post Px- or sham surgery, vehicle or Iso (1 mg/kg, SC) was administered once daily for 10 days. The study was terminated ten weeks after surgery and the hearts were isolated for either detailed histological and molecular characterization or functional ex vivo analyses in a Langendorff setup. The relative weight of the left ventricle (LV) was significantly increased in Px-Iso rats compared to sham- vehicle controls ($p<0.05$). Furthermore, Iso induced a significant increase in LV cardiac fibrosis as determined by quantitative histology ($p<0.001$). Detailed analyses of echocardiographic measurements and selected plasma marker levels will reveal whether the Px and/or Iso procedures result in LV remodeling and changed cardiac function. Moreover, transcriptome analyses and in vivo electrocardiographical changes monitored by cardiac telemetry will support the model characterization. In conclusion, the combination of the Px procedure and Iso treatment in the rat may induce key structural and functional changes of clinical DC and, thus, be a useful approach for the establishment of a novel animal model of this condition.

Proteoglycan 4 deficiency improves obesity phenotype in mice

Joya E. Nahon[1], Menno Hoekstra[1], Sander Kooijman[2], Patrick C.N. Rensen[2], Miranda Van Eck[1].

[1]Division of BioTherapeutics, LACDR, Leiden University, The Netherlands; [2]Department of Medicine, Division of Endocrinology, Leiden University Medical Center, The Netherlands

Proteoglycan 4 (Prg4) is a proposed peroxisome proliferator receptor gamma (PPAR.) target gene and highly expressed in metabolic tissues. However, the function of Prg4 in metabolism is unknown. Since the nuclear fatty acid receptor PPAR. is a key regulator of lipid and glucose metabolism, male Prg4 knock-out (KO) mice and wild-type (WT) littermates were challenged with a high fat diet. Liver triglyceride levels were 2.3-fold lower in Prg4 KO mice as compared to WT littermates ($p<0.001$). This was confirmed with an Oil Red O staining for neutral lipids. The decreased liver steatosis is most likely driven by decreased de novo lipogenesis, as judged by the significantly reduced expression of lipogenic genes ACC and SCD1 in Prg4 KO mice as compared to WT littermates (1.3- and 1.6-fold decrease; $p<0.05$). Radiolabeled flux studies showed less uptake of triglyceride-derived fatty acids by the white adipose tissue of Prg4 KO mice (1.8-fold decrease; $p<0.05$), which translated in a trend towards lower body weight. Additionally, the expression of inflammatory markers CD68 and MCP1 was lower in Prg4 KO adipocytes as compared to WT littermates (2.9- and 5-1-fold decrease; $p<0.01$). The favorable white adipose tissue phenotype in Prg4 KO mice as compared to WT mice coincided with improved glucose handeling during an oral glucose challenge (AUC: 1.4-fold decrease; $p<0.05$). In conclusion, Prg4 KO mice are protected from high fat diet-induced metabolic disruptions.

Adipokines Affect PCSK9 Expression: In Vitro And In Vivo Evidence

M.Botta[1], C.Macchi[1], D. Dall'Orto[1], P.Magni[1], A.Corsini[1], M.Ruscica[1], P.Dongiovanni[2], L.Valenti[2], J.Liu[3], N.Ferri[4], A.Cicero[5]

[1]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; [2]Pathophysiology and Transplantation, Università degli Studi Milano, UO Medicina Interna 1B, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20100 Milan, Italy; [3]Department of Veterans Affairs, Palo Alto Health Care System, 94304 Palo Alto, California; [4]Department of Pharmaceutical and Pharmacological Sciences, Università degli Studi di Padova, Padua, Italy; [5]Medical and Surgical Sciences Department, University of Bologna, Italy

Background: Adipose tissue is an endocrine organ secreting active molecules, the adipokines (eg, leptin and resistin). In a condition of dysfunctional visceral fat depots, adipokines are detrimental for cardiovascular system. **Aim:** To evaluate some of the molecular mechanisms linking the biology of adipokines and PCSK9. **Methods:** hepatic cell culture, qPCR, ELISA assay, siRNA, luciferase activity, ob/ob and high-fat diet mice. **Results**: In HepG2 cells leptin and resistin induced PCSK9 gene expression by +195% and +250%, respectively. Leptin and resistin enhanced PCSK9 promoter luciferase activity by +26% and +20%, respectively, through the involvement of Sterol Regulatory Element (SRE) motif and not that of HNF-1. Leptin raised the secretion of PCSK9 in HepG2 medium by +15%. The relationship among leptin, resistin and PCSK9 expression requires the mandatory involvement of STAT3, an important modulator in the inflammatory response. STAT3 knock-down abolished leptin- and resistin-induced hepatic expression of PCSK9 mRNA. Apolipoprotein B and microsomal triglycerides transfer protein mRNA were increased by leptin (+57% and +60%, respectively) and resistin (+ 50% for both). These effects were dependent on PCSK9; indeed, upon the use of siRNA anti-PCSK9 their activation was abolished. In a clinical setting (80 males, 56±4 y), a positive association between circulating leptin and PCSK9 levels (p=0.005) was found only in those with BMI<25 Kg/m^2. Ob/ob male mice, lacking leptin, showed significantly lower hepatic PCSK9 gene expression vs wildtype mice. In mice fed high-fat diet, a positive correlation between leptin, resistin and PCSK9 hepatic mRNA expression was found. **Conclusions:** There is a direct relationship between adipokines and PCSK9. The molecular basis beyond these findings requires the involvement of STAT3 pathway and the activation of SRE motif.

Reduced Wnt signaling in South Asian compared with white Caucasian pre-diabetic men

Laura GM Janssen[1,2], Mark JW Hanssen[3], Andrea D van Dam[1,2], Sander Kooijman[1,2], Kimberly Nahon[1,2], Hanneke Reinders[1,2], Ingrid Jazet[1], Wouter D van Marken Lichtenbelt[3], Patrick CN Rensen[1,2], Natasha M Appelman-Dijkstra[1,2,4], Mariëtte R Boon[1,2]

[1]Dept. Medicine, Div. Endocrinology, Leiden University Medical Center (LUMC), Leiden; [2]Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden; [3]Dept. Human Biology & Human Movement Sciences, NUTRIM School for Nutrition and Translational Research in Metabolism, Maastricht University Medical Center, Maastricht; [4]Center for Bone Quality, Div. Endocrinology, LUMC, Leiden, the Netherlands.

Aim: South Asians (SA) have an adverse metabolic phenotype compared to white Caucasians (WC), which includes abdominal obesity. It has been shown that the Wnt signaling coreceptor LRP5 is involved in body fat distribution, since mutations associated with reduced LRP5 function correlate with abdominal adiposity. Based on the hypothesis that decreased Wnt signaling may contribute to the adverse metabolic phenotype of SA, we aimed to unravel Wnt signaling in relation to insulin signaling in white adipose tissue and skeletal muscle. **Methods:** Ten pre-diabetic overweight Dutch SA men (age 47±7 years, BMI 30±3 kg/m2) and ten matched Dutch WC men were included. Fasting blood samples were drawn and assayed for sclerostin. Fasting sWAT and skeletal muscle biopsies were taken and expression of genes involved in Wnt and insulin signaling were assessed by RT-PCR. **Results:** Circulating levels of the Wnt inhibitor sclerostin were markedly higher in SA compared to WC (+64%, p<0.01). In sWAT, but not skeletal muscle, we observed lower gene expression of Wnt signaling coreceptors LRP5 (-43%, p=0.02) and LRP6 (-29%, p=0.02), as well as of other genes involved in Wnt signaling (FZD1; -33%, p=0.03, GSK3B; -32%, p=0.04, TCF7L2; -42%, p=0.01). These data were accompanied by lower expression of genes involved in insulin signaling (i.e. INSR, TBC1D4, SLC2A4, GYS1). Interestingly, in sWAT we observed strong correlations between the expression of genes in Wnt and insulin signaling, for example between LRP5 and INSR (R2=0.77, ß=0.29, p<0.001) and between LRP6 and INSR (R2=0.48, ß=1.13, p=0.001). **Conclusion:** Plasma levels of the Wnt inhibitor sclerostin are higher, and expression of Wnt and insulin signaling genes in sWAT are lower in pre-diabetic SA versus WC. These findings suggest that Wnt inhibition and reduced insulin signaling contribute to the adverse metabolic phenotype of SA males. Our data support a strong relation between Wnt and insulin signaling, which will be further unraveled in vitro.
Trial number: NCT02291458

Anti-oxidative function of follicular fluid high density lipoproteins predicts outcomes of modified natural cycle IVF

R.A. Nagy[1,2], A.P.A. van Montfoort[2,3], H. Groen[4], I. Homminga[2], D. Andrei[1,2], R.H. Mistry[1], J.L.C. Anderson[1], A. Hoek[2], U.J.F. Tietge[1]

[1]Department of Pediatrics, Center for Liver, Digestive, and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands; [2]Department of Obstetrics and Gynaecology, Section Reproductive Medicine, University of Groningen University, Medical Center Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands; [3]Department of Obstetrics & Gynaecology, GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, Maastricht, The Netherlands; [4]University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, The Netherlands

Aim: High density lipoproteins (HDL) are the main cholesterol carriers in follicular fluid (FF). Thus far, FF-HDL have been mainly studied in the context of steroid hormone synthesis. The aim of the current study is to investigate whether the anti-oxidative function of FF HDL relates to embryo quality in modified natural cycle in vitro fertilization (MNC-IVF). **Methods:** For the present cross sectional study, FF and plasma samples were collected between August 2013 and April 2017 during 491 consecutive MNC-IVF cycles of 244 patients in a single academic infertility treatment center. The anti-oxidative function of HDL as the capacity to prevent native LDL oxidation in vitro was determined in (i) FF from 375 MNC-IVF cycles, where one oocyte was retrieved and minimum FF blood contamination was present, and (ii) 19 matched plasma and FF samples. The association between HDL anti-oxidative function and oocyte and embryo quality as well as occurrence of an ongoing pregnancy was assessed. **Results:** The anti-oxidative function of FF-HDL was significantly higher than that of matching plasma-HDL (P <0.001); more than 80% of the total FF anti-oxidative capacity was attributable to the presence of HDL. A higher FF-HDL anti-oxidative function was related to a lower percentage of embryo fragmentation and the development of top quality embryos, associations that persisted after adjustment for BMI and smoking (P = 0.012 and P = 0.011, respectively). However, the anti-oxidative function of FF-HDL was not associated with the occurrence of an ongoing pregnancy. **Conclusion:** The present study demonstrates that FF has substantial anti-oxidative capacities that are mainly attributable to HDL. Moreover, increased FF anti-oxidant function predicts improved embryo quality. Definition of lifestyle interventions to increase the anti-oxidative capacity of FF-HDL thus holds potential to increase the success rates of IVF procedures and of natural reproduction.

Cardiovascular risk of children and adolescents with epilepsy is reduced by modified ketogenic diet

Prudencio, M.B.[1], Ricciarelli ,G.F.[1], Freitas, M.C.P.[1], Takakura, C.Y.[2], Sampaio, L.P.B.[2], Damasceno, N.R.T.[1]

[1]Public Health School of University of São Paulo, Brazil; [2]Children Institute, Hospital of Clinics of Medicine School of University of São Paulo, Brazil

Objective: Ketogenic diet (KD) has been proposed as an adjuvant treatment for obesity, cancer and neurology diseases, however, the impact of this diet in cardiovascular risk factors is controversy. Here, our goal was to investigate the impact of KD in cardiovascular risk of patients with refractory epilepsy. **Methods:** patients with epilepsy, both sex and ages between 1 to 18 years old were enrolled. After complete clinical evaluation blood samples were collected at baseline time (before start the KD treatment (T0) and after 6 months on KD treatment (T1). Lipid profile (total cholesterol, LDL-c, HDL-c and triglycerides), thiobarbituric reactive substances (TBRAS), lipoprotein particle size (HDL and LDL), oxidized LDL and cytokines were monitored. The carotid thickness was also measure using carotid ultrasound (USG). **Results:** Thirteen patients (77% boys) with mean age of 4 years old were selected. After 6 months, the efficacy of KD was confirmed (T0= 21 seizures/d and T1=4 seizures/d, $p<0.01$) and plasma ketones bodies (T0=1.0 mmolL; T1=3.1 mmolL; $p<0.01$) increased. Although the high content of fat in KD, after 6 months of treatment were not observed significant changes in total cholesterol (T0=173 mg/dL; T1=174 mg/dL; p=0.94), HDL-C (T0=53 mg/dL; T1=53 mg/dL; p=0.815) and LDL-C (T0=113 mg/dL, T1=108 mg/dL; p=0.686), however the triglycerides level increased (T0=76 mg/dL, T1=113 mg/dL; p=0.027). The TBARS level was similar (T0=22.2 mmolL; T1=20.4 mmolL; p=0.393). The LDL size (T0=268 nm; T1=266 nm; p=0.108) and oxidized LDL (T0=18.9 UL; T1: 21.6 UL; p=0.079) did not show significant differences during follow-up time. Inflammatory markers showed similar profile for IL1B (T0=2.0 pgmL; T1=1.8 pgmL, p=0.536), IL-6 (T0=3.0 pgmL; T1= 2.3 pgmL, p=0.632) and IL10 (T0=7.6 pgmL, T1=12.9 pgmL; p=0.116), while TNF-a level reduced (T0=10.2 pgmL; T1=6.6 pgmL; $p<0.01$). The right and left carotid thickness did not change after 6 months on KD treatment (T0=0.048 mm; T1=0.046 mm; p=0.7). **Conclusion:** Dyslipidemia, inflammation and oxidative stress – common side effects of classical KD, can be avoid by improvement in quality of fatty acids included in dietary protocol as used in our study, in which monounsaturated and polyunsaturated were the major fats.

Dietary Supplementation of Galactooligosaccharides reduces intestinal fat absorption leading to lower fat accumulation and improved insulin sensitivity

Rima H. Mistry*, Henkjan J. Verkade*, Uwe J.F. Tietge*

**Department of Pediatrics, Center for Liver, Digestive, and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

Background: Growing evidence suggests that the intestinal microbiota has considerable impact on obesity, cardiovascular diseases, and development of type 2 diabetes mellitus (T2DM) and thus holds potential for therapeutic intervention. Gut microbiota composition can be altered via dietary fibers which are utilized by bacteria for fermentation. Galactooligosaccharides (GOS) represent such a dietary fiber, recognized for its positive effect on gut microbiota. Little is known about the potential metabolic effects of GOS. **Aim:** Therefore, the aim of the present study was to investigate the effect of dietary GOS on lipid metabolism and the development of insulin resistance in mice. **Methods and Results:** C57BL/6 mice fed Western-type diet with GOS supplementation (7% w/w) for a period of 16 weeks showed significantly lower body weight gain ($p<0.01$) largely attributable to a reduction in perirenal (-30%, $p<0.01$) and epididymal fat (-10%, $p<0.01$). Liver triglyceride levels were also significantly decreased (-40%, $p<0.05$) in GOS fed mice. Liver cholesterol levels remained comparable in both groups. Insulin sensitivity was improved significantly in the GOS receiving group (20%, $p<0.05$). Respiratory exchange ratio (RER) and energy expenditure were similar in both groups over a circadian period suggesting no contribution to lower body weight gain in GOS fed mice. However, intestinal fat absorption in the GOS groups was significantly reduced (-35%, $p<0.01$) thus indicating a potential mechanism for the lower fat accumulation. **Conclusion:** In summary, this study demonstrates that Western-type diet supplemented with GOS reduces intestinal fat absorption resulting into lower body weight gain, adiposity, improved dyslipidemia and insulin sensitivity. These effects are expected to translate into metabolic health benefits, conceivably providing a dietary strategy against the increasing incidence of obesity, cardiovascular diseases and T2DM.

List of Participants 2018

Alitalo	Kari	kari.alitalo@helsinki.fi
Amersfoort	Jacob	j.amersfoort@lacdr.leidenuniv.nl
Anderson	Josephine	j.l.anderson@umcg.nl
Arnesen	Kjell-Erik	kearnese@online.no
Badimon	Lina	LBadimon@santpau.cat
Bartels	Emil D.	Emil.Daniel.Bartels@regionh.dk
Beata	Sarecka-Hujar	bsarecka-hujar@sum.edu.pl
Beheshti	Sabina	sabinabeheshti@gmail.com
Benn	Marianne	marianne.benn@regionh.dk
Bisgaard	Line Stattau	line.stattau.bisgaard@regionh.dk
Bonacina	Fabrizia	fabrizia.bonacina@unimi.it
Borup	Anna	anna.borup@regionh.dk
Botta	Margherita	margherita.botta@unimi.it
Baardman	Jeroen	j.baardman@amc.uva.nl
Cai	Huan	huan@sund.ku.dk
Carlsson	Annelie	annelie.carlsson@wlab.gu.se
Christensen	Jacob Juel	j.j.christensen@medisin.uio.no
Christoffersen	Christina	christina.christoffersen@regionh.dk
Christoffersen	Mette	mette.christoffersen.02@regionh.dk
Chuang	Christine	cchuang@sund.ku.dk
Davies	Michael	davies@sund.ku.dk
Dhar	Indu	Indu.Dhar@uib.no
Douna	Hidde	h.douna@lacdr.leidenuniv.nl
Emanuelsson	Frida	frida.karin.emanuelsson.lemvig@regionh.dk
Foged	Mads Møller	mmf@cancer.dk
Fontaine	Margaux	m.fontaine@maastrichtuniversity.nl
Frikke-Schmidt	Ruth	ruth.frikke-schmidt@regionh.dk
Gonzalez-Padilla	Esther	es7446go-s@student.lu.se
Grechanyk	Mariya	plomami@i.ua
Greve	Anders	anders.moeller.greve.01@regionh.dk
Hajny	Stefan	stefan.hajny.01@regionh.dk
Hansen	Anne Tybjærg	Anne.Tybjaerg.Hansen@regionh.dk
Hawkins	Clare	clare.hawkins@sund.ku.dk
Holven	Kirsten B	kirsten.holven@medisin.uio.no
In het Panhuis	Wietse	w.in_het_panhuis@lumc.nl
Janssen	Laura	l.g.m.janssen@lumc.nl

Jauhiainen	Matti	matti.jauhiainen@thi.fi
Johansson	Inger	inger.johansson@wlab.gu.se
Jones	Richard	RJones@akceatx.com
Ketelhuth	Daniel	Daniel.Ketelhuth@ki.se
Kjeldsen	Emilie	emilie.westerlin.kjeldsen@regionh.dk
Kopyta	Ilona	ilonakopyta@autograf.pl
Kovanen	Petri	petri.kovanen@wri.fi
Krogh	Henriette	h.w.krogh@medisin.uio.no
Kuipers	Eline	e.n.kuipers@lumc.nl
Langsted	Anne	anne.langsted.01@regionh.dk
Lantero Rodriguez	Marta	marta.lanterorodriguez@wlab.gu.se
Lorenzo Martín	Cristina	clorenzo@cnic.es
Lunde	Ngoc Nguyen	n.n.lunde@farmasi.uio.no
Løvsletten	Nils Gunnar	n.g.lovsletten@farmasi.uio.no
Laaksonen	Reijo	Reijo.Laaksonen@staff.uta.fi
Madsen	Christian Medom	christian.medom.madsen@regionh.dk
Mariana Baldini	Prudencio	mari_prud@hotmail.com
Martos Folgado	María Inmaculada	inmaculada.martos@cnic.es
Mistry	Rima	r.h.mistry@umcg.nl
Mujaj	Blerim	b.mujaj@erasmusmc.nl
Mulder	Monique	m.t.mulder@erasmusmc.nl
Murray	Andrew	ajm267@cam.ac.uk
Möller	Fredrik	fredrik.moller@nightingalehealth.com>
Nagy	Ruxandra	sandra.nagy@yahoo.com
Nahon	Joya	j.e.nahon@lacdr.leidenuniv.nl
Narverud	Ingunn	ingunn.narverud@medisin.uio.no
Neuvonen	Maarit	maarit.neuvonen@helsinki.fi
Nielsen	Sune	sune.fallgaard.nielsen@regionh.dk
Nordestgaard	Børge G.	boerge.nordestgaard@regionh.dk
Nordkvist	Sara	sara.nordkvist@med.lu.se
Olkkonen	Vesa	vesa.olkkonen@helsinki.fi
Ouweneel	Amber	a.b.ouweneel@lacdr.leidenuniv.nl
Pedersen	Tanja X.	txp@gubra.dk
Pruper de Freiras	Maria Camila	camilapruper@hotmail.com
Ramne	Stina	stina.ramne@med.lu.se
Randsborg	Karoline	karoline_randsborg@live.no

Ranheim	Trine	Trine.Ranheim@rr-research.no
Rasmussen	Katrine Laura	katrine.laura.rasmussen@regionh.dk
Rasmussen	Ida Juul	ida.juul.rasmussen@regionh.dk
Rensen	Patrick	p.c.n.rensen@lumc.nl
Retterstøl	Kjetil	kjetil.retterstol@medisin.uio.no
Romeo	Stefano	stefano.romeo@wlab.gu.se
Rundblad	Amanda	amanda.rundblad@hioa.no
Rustan	Arild	arild.rustan@farmasi.uio.no
Ruuth	Maija	maija.ruuth@gmail.com
Schaftenaar	Frank	f.h.schaftenaar@lacdr.leidenuniv.nl
Schilperoort	Maaike	m.schilperoort@lumc.nl
Schreuder	Michelle	m.m.schreuder@erasmusmc.nl
Stehr-Nielsen	Suzanna	stheut@akceatx.com
Stender	Stefan	Stefan.Stender@regionh.dk
Szili-Torok	Tamas	tamasszilitorok@me.com
Temmerman	Lieve	lieve.temmerman@mumc.nl
Thisted	Louise	lth@gubra.dk
Thomassen	Jesper Q.	jesper.qvist.thomassen@regionh.dk
Tietge	Uwe	u_tietge@yahoo.com
Tivesten	Åsa	asa.tivesten@medic.gu.se
van Dam-Nolen	Dianne H.K.	h.nolen@erasmusmc.nl
van Eenige	Robin	r.van_eenige@lumc.nl
van Puijvelde	Gijs	puijvelde@lacdr.leidenuniv.nl
Varbo	Anette	anette.varbo@regionh.dk
Verberk	Sanne	sanne.verberk@outlook.com
Ward	Liam	liam.ward@liu.se
Weisell	Jonna	jonna.weisell@uef.fi
Würtz	Peter	peter.wurtz@helsinki.fi
Yang	Wenyi	wenyi.yang@kuleuven.be
Zacharias	Triantafyllos	zacharias@sund.ku.dk
Øyri	Linn K. L.	l.k.l.oyri@medisin.uio.no
Öörnii	Katariina	kati.oorni@wri.fi