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23rd Annual Scandinavian Atherosclerosis Conference

April 18-21, Krogerup Højskole, Humlebæk, Denmark

2017 Program

SCIENTIFIC COMMITTEE

Jacob Bentzon (Spain), Trine Ranheim (Norway)
Martin B. Mortensen (Denmark), Ilze Bot (The Netherlands)
Matti Jauhiainen (Finland), Katrine L. Rasmussen (Denmark)
Matteo Pedrelli (Sweden), Åsa Tivesten (Sweden)

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Organized by

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HOMEPAGE

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Tuesday, April 18, 2017

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 2017 NIKKILÄ MEMORIAL LECTURES	
19.35 – 19.40	Introduction of the 2017 Nikkilä Lecturer **Vesa Olkkonen** (*Finland*)
19.40 – 20.25	**2017 Nikkilä Lecture** Angiopoietin-like proteins 3, 4 and 8 – central organizers of fat storage and burning **Matti Jauhiainen** *(Finland)*
20.25 – 20.45	Discussion
20.45	Pub will be open

Wednesday, April 19, 2017

08.00 – 09.00	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Organized and chaired by Jacob Bentzon (*Spain*) and Trine Ranheim (*Norway*)
09.00 – 09.25	Effects of smooth muscle cell lineage and phenotype on vascular disease **Sanjay Sinha** *(United Kingdom)*
09.25 – 09.30	Discussion
09.30 – 09.45	Fibrous caps of murine atherosclerosis are derived from few medial SMCs undergoing coherent clonal expansion with a layered architecture **Kevin Jacobsen** *(Denmark)**
09.45 – 10.00	Lipid lowering by PCSK9 monoclonal antibodies reverses the pro-inflammatory profile of circulating monocytes **Annette Neele** *(The Netherlands)**
10.00 – 10.15	Targeting the ER stress response delays atherosclerosis and associated death in progeroid mice **Magda Hamczyk** (*Spain*)*
10.15 – 10.30	Loss of endothelial ADAM10 augments atherosclerosis development in mice **Kosta Theodorou** (*The Netherlands*)*
10.30 – 11.15	Coffee, posters and exhibitions
11.15 – 11.40	Gut microbiota and cardiovascular disease **Marius Trøseid** *(Norway)*
11.40 – 11.45	Discussion
11.45 – 12.00	Impaired interferon signalling may underlie high prevalence of cardiovascular disease in South Asians **Robin van Eenige** *(The Netherlands)**

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12.00 – 12.15	Lipocalin-2 contributes to experimental atherosclerosis in a stage-dependent manner **Jacob Amersfoort** (*The Netherlands*)
12.15 – 12.30	Testosterone is an endogenous regulator of BAFF and splenic B-cell number **Åsa Tivesten** *(Sweden)*
SESSION II	**CARDIOVASCULAR DISEASE** Organized and chaired by Martin B. Mortensen (*Denmark*) and Ilze Bot (*The Netherlands*)
12.30 – 12.55	Dissecting the impact of different B-cell functions on Atherosclerosis **Andrew B. Sage** *(United Kingdom)*
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 – 18.00	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
18.00 – 19.00	Dinner
19.00 – 19.25	Lipoprotein retention: a key step in atherogenesis **Jan Borén** *(Sweden)*
19.25 – 19.30	Discussion
19.30 – 19.45	Disruption of the biological clock aggravates atherosclerosis **Maaike Schilperoort** *(The Netherlands)****
19.45 – 20.00	Plasma stem cell factor levels are associated with risk of cardiovascular disease and death **Maria Wigren** *(Denmark)*
20.00 – 20.15	2.5-fold risk of ischemic stroke in individuals with clinical familial hypercholesterolemia: The Copenhagen General Population Study with 102,961 individuals **Sabina Beheshti** *(Denmark)**

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20.15 – 20.45	Coffee, posters and exhibitions
20.45 – 21.00	Association of apolipoprotein M with cardiovascular risk factors and kidney injury in patients with chronic kidney disease **Ida Maria Hjelm Sørensen** *(Denmark)**
21.00 – 21.15	Genetic variants in CYP7A1 and risk of myocardial infarction and symptomatic gallstone disease **Faiza Qayyum** *(Denmark)**
21.15 – 21.30	Incidence of acute myocardial infarction and coronary heart disease in patients with genotyped familial hypercholesterolemia in Norway during 2001-2009 **Kjetil Retterstøl** (*Norway*)
21.30-21.45	Body Mass Index and Risk of Alzheimer Disease: A Mendelian Randomization Study of 399,536 Individuals **Ruth Frikke-Schmidt** (*Denmark*)
21.45 –	Pub will be open

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Thursday, April 20, 2017

08.00 – 09.00	Breakfast.
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Organized and chaired by Matti Jauhiainen (*Finland*) and Katrine L. Rasmussen (*Denmark*)
09.00 – 09.25	Diagnosis, treatment and prognosis of patients with familial hypercholesterolemia **Erik Stroes** *(The Netherlands)*
09.25 – 09.30	Discussion
09.30 – 09.45	Effect of weight reduction on Lipoprotein(a) levels in obese and Type 2 diabetes patients **Monique Mulder** (*The Netherlands*)
09.45 – 10.00	High lipoprotein(a) and low risk of major bleeding in the general population: a Mendelian randomization study **Børge G. Nordestgaard** (*Denmark*)
10.00 – 10.15	Complex functions of High Density Lipoproteins (HDL) - HDL increases inflammatory and bacterial clearance responses in macrophages **Emiel van der Vorst** *(Germany)**
10.15 – 10.30	Lysosomal oxidation of LDL and its implications for atherosclerosis **Feroz Ahmad** *(United Kingdom)**
10.30 – 11.15	Coffee, posters and exhibitions
11.15 – 11.40	New insight into regulation of chylomicron assembly and secretion **Gary F. Lewis** *(Canada)*
11.40 – 11.45	Discussion
11.45 – 12.00	Loss of Function of GALNT2 Lowers High-Density Lipoproteins in Humans, Nonhuman Primates, and Rodents **Katrine Schjoldager** *(Denmark)*
12.00 – 12.15	Protein destabilization as a regulating factor in intravascular lipolysis **Michael Ploug** *(Denmark)*
12.15 – 12.30	Cidea improves the metabolic profile through expansion of adipose tissue and modulation of lipid metabolism **Alexander W. Fischer** *(Germany)**

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12.30 – 12.45	Butyrate via the gut-brain circuit reduces appetite and activates brown adipose tissue **Patrick Rensen** *(The Netherlands)*
12.45 – 14.00	Lunch
SESSION IV	**OTHER TOPICS** Organized and chaired by Matteo Pedrelli (*Sweden*) and Åsa Tivesten (*Sweden*)
14.00 – 14.25	Shifting concepts in the description of the "vulnerable plaque" **Gerard Pasterkamp** *(The Netherlands)*
14.25 – 14.30	Discussion
14.30 – 14.45	Disruption of the cholesterol efflux transporters ABCA1 and ABCG1 alters megakaryocyte proplatelet production **Amber Ouweneel** *(The Netherlands)**
14.45 – 15.00	Biological clock strongly regulates brown adipose tissue activity: implications for postprandial triglyceride metabolism **Rosa Van den Berg** *(The Netherlands)**
15.00 – 15.15	Cold-triggered bile acid synthesis shapes the gut microbiome **Anna Worthmann** (*Germany*)*
15.15 – 15.30	Blood-brain barrier transcytosis genes and risk of dementia -a study of 74,754 individuals **Ida Juul Rasmussen** *(*Denmark) *
15.30 – 16.15	*Coffee, posters and exhibitions*
16.15 – 16.40	Lifestyle and cardiovascular prevention – from theory to clinical practice **Mai-Lis Hellenius** *(Sweden)*
16.40 – 16.45	Discussion
16.45 – 17.00	Extreme High High-Density Lipoprotein Cholesterol is Paradoxically Associated with High Mortality in Men and Women: two Prospective Cohort Studies **Christian Medom Madsen** (*Denmark)* *

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17.00 – 17.15	Protein components of intracellular membrane contact sites in endothelial cells: Roles of ORP2 and protrudin in angiogenesis **Vesa Olkkonen** *(Finland*)
17:15 – 17:30	Low LDL-cholesterol, genetic variation, and risk of Alzheimer's dementia and Parkinson's disease **Marianne Benn** *(Denmark*)
17.30 – 19.00	Time free
19.00 – 19.30	**Cocktail**
19.30 –	**Banquet and dancing**

Friday, April 21, 2017

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

23rd Annual Scandinavian Atherosclerosis Conference

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2017 Posters

Wednesday, April 19, 2017

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. Each poster can be mounted on any of the boards – (first come - first served basis). Pins will be provided. ***Posters should be no more than 80 cm wide and 120 cm high.***

SESSION I - INFLAMMATION AND VASCULAR BIOLOGY

Organized and chaired by Jacob Bentzon (*Spain*) and Trine Ranheim (*Norway*)

The effect of adipose tissue products on monocyte adhesivity to the endothelium
Soňa *Čejková (Czech Republic)**

Altered leukocyte distribution under hypercholesterolemia: a cross-sectional study in children with familial hypercholesterolemia
*Jacob J. Christensen (Norway)**

Detection of pyruvate kinase isoform M2 (PKM2) in arterial wall cells, intact arteries and human atherosclerotic lesions
Michael Davies (Denmark)

Vascular stiffening: mechanisms and therapeutic options in a mouse model of Hutchinson Gilford Progeria Syndrome
*Lara del Campo (Spain)**

Targeting B cells with an agonistic BTLA antibody in atherosclerosis
*Hidde Douna (The Netherlands)**

Role of macrophages in the production of extracellular traps during chronic inflammation – a pathway to lesion development in atherosclerosis?
Clare Hawkins (Denmark)

Effects of Imiquimod-induced psoriasis-like lesions and digoxin in low density lipoprotein receptor-deficient mice
Marie Madsen (Denmark) – presented by Tanja X. Pedersen

Quantitative analysis of Monocyte subpopulations and CD3+ iNKT cells in patients with atherosclerotic plaque and dyslipidemia
*Meisam Naeimi Kararoudi (Italy)**

Uremia does not affect neointima formation in mice
Tanja X. Pedersen (Denmark)

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Apolipoprotein E Deficiency Increases Remnant Lipoproteins and Accelerates Progressive Atherosclerosis in Yucatan Minipigs
*Jeong Shim (Denmark)**

The role of NRF2 in bone marrow derived macrophages in atherosclerosis
Virve Sihvola (Finland)

Deficiency of the hemoglobin scavenger receptor CD163 in macrophages exacerbates development of atherosclerosis in apolipoprotein E deficient mice
Pia Svendsen (Denmark)

Hematopoietic CARD9 deficiency reduces macrophage number in atherosclerotic plaques within a hyperglycemic atherosclerotic mouse model
*Kathrin Thiem (The Netherlands)**

Mitochondrial dysfunction prevents repolarization of inflammatory macrophages
*Jan Van den Bossche (The Netherlands)**

HDL deficiency abrogates lipid lowering-induced plaque stabilization in apolipoprotein E knockout mice
Ronald van der Sluis (The Netherlands)

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SESSION II - CARDIOVASCULAR DISEASE

Organized and chaired by Martin B. Mortensen (*Denmark*) and Ilze Bot (*The Netherlands*)

Relationship between microfibrillar-associated protein 4 (MFAP4) and vascular patency in lower extremity peripheral artery disease (PAD)
*Line Ea Hemstra (Denmark)**

Elevated Lp(a) levels strongly increase the risk for cardiovascular disease in patients with familial hypercholesterolemia
Kirsten Holven (Norway)

Characterization of the antibody immune response in atherosclerosis
*Cristina Lorenzo (Spain)**

Treat to target Familial Hypercholesterolemia: a prospective study on effects from aggressive lipid lowering treatment in an outpatient setting in patients with Familial hypercholesterolemia
Irene Mork (Norway)

Maternal cholesterol levels may predict LDL cholesterol levels in children with familial hypercholesterolemia
*Ingunn Narverud (Norway) **

Inhibition of immunoproteasomal subunit LMP7 attenuates atherosclerosis
*Frank Schaftenaar (The Netherlands)**

A microenvironment-specific protective role for CD8+ T cells in advanced atherosclerosis
*Janine van Duijn (The Netherlands)**

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SESSION III - LIPOPROTEINS AND LIPID TRANSPORT

Organized and chaired by Matti Jauhiainen (*Finland*) and Katrine L. Rasmussen (*Denmark*)

Activation of lipoprotein lipase and endothelial lipase in thermogenic adipocytes promotes HDL turnover and reverse cholesterol transport
*Nicola Schaltenberg (Germany)**

Novel ApoA-I derived peptides with anti-diabetic and anti-CVD effect
Shelley Edmunds (Sweden)

Real-time imaging of vascular brown adipose tissue lipoprotein lipase activity
Markus Heine *(Germany)*

Protein destabilization controls LPL activity and regulation
Kristian Kølby Kristensen (Denmark)

Depletion of TM6SF2 disturbs membrane lipid composition and dynamics in HuH7 hepatoma cells
*Hanna Ruhanen (Finland)**

High quality fish oil has a more favourable effect than oxidized fish oil on intermediate and low density lipoprotein subclasses
*Amanda Rundblad (Norway)**

Familial longevity is characterized by preserved circadian rhythmicity of serum cholesterol levels in healthy middle-aged individuals
*Lauren Tambyrajah (The Netherlands)**

PNPLA2 selectively mediates the secretion of triglyceride-rich lipoproteins by human hepatoma cells
*Apostolos Taxiarchis (Sweden)**

The anti-inflammatory function of HDL is impaired in type 2 diabetes
Uwe Tietge (The Netherlands)

GPR120 as a novel target to reduce obesity in mice
*Andrea van Dam (The Netherlands)**

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A role for the LXR target IDOL in the regulation of intestinal LDLR expression
Nienke van Loon (The Netherlands)

Plasma apolipoprotein M is increased in postmenopausal women
*Adelina Yafasova (Denmark)**

Subjects with familial hypercholesterolemia have larger postprandial increase of small VLDL after intake of saturated fat compared to polyunsaturated fat – a randomized controlled trial
Linn Kristin Lie Øyri (Norway)

***Participate in the young investigator's award.**

SESSION IV - OTHER TOPICS

Organized and chaired by Matteo Pedrelli (*Sweden*) and Åsa Tivesten (*Sweden*)

Impaired fatty acid synthesis affects immune cells activation: Focus on sterol regulatory element binding factor-1c on T lymphocytes
*Fabrizia Bonacina (Italy)**

Milk cholesterol content is tightly regulated and remains stable regardless of strong dietary or genetic manipulations in hypo- and hypercholesterolemic mice
*Lidiya Dimova (The Netherlands)**

T-cell activation is associated with artery stiffness in patients with breast cancer
Anastasiya Filatova (Rusia) – presented by Aleksandra Shchinova

Familiar hypercholesterolemia – treatment and quality of life
*Oda Haug Larsen (Norway)**

Common genetic variation in ABCA7 and risk of dementia, ischemic heart and cerebrovascular disease
*Emilie Westerlin Kjeldsen (Denmark)**

Investigating the underlying mechanisms in high risk CAD by studying patient specific iPSCs derived hepatocyte models in vitro
Mostafa Kiamehr (Finland)

Gut-derived bacterial LPS links western diet with adipocyte dysfunction through mitochondrial damage
*Lucia Martinez de la Escalera (United Kingdom)**

Regulation of ANGPTL8 by microRNA 221 and inflammation in adipocytes
Raghavendra Mysore (Finland)

Glucose handling and white adipocyte phenotype are altered in proteoglycan 4 deficient mice
*Joya Nahon (The Netherlands)**

Carbohydrate response element binding protein regulates de novo lipogenesis in brown adipose tissue
*Christian Schlein (Germany)**

NLRP3 inflammasome promote myocardial remodeling during diet-induced obesity
Marina Sokolova (Norway)

Evaluation of Aortic Valve Morphology in Hyperlipidemic Mice by Magnetic Resonance Imaging (MRI)
*Jonna Weisell (Finland)**

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Oral presentations – Abstracts

Inflammation and Vascular Biology

SESSION I

Fibrous caps of murine atherosclerosis are derived from few medial SMCs undergoing coherent clonal expansion with a layered architecture

Jacobsen K[1,2], Lund MB[2], Shim J[2], Gunnersen S[2], Füchtbauer EM[4], Kjolby M[3], Carramolino L[1], Bentzon JF[1,2]*

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; [2]Deparment of Clinical Medicine, Aarhus University, Aarhus, Denmark; [3]Department of Biomedicine, Aarhus University, Aarhus, Denmark; [4]Department of Molecular Biology, Aarhus University, Aarhus, Denmark

Aim: Fibrous cap SMCs are essential in atherosclerosis protecting lesions from rupturing and causing thrombosis. To gain insight into recruitment of fibrous cap SMCs, we mapped the clonal architecture of SMCs in murine plaques using aggregation chimeras and random recombination of a fluorescence reporter transgene.

Methods: Chimeras, created by aggregation of eGFP+Apoe-/- and Apoe-/- mouse embryos, were fed chow until 10 months of age. Myh11-CreERT2 mice, expressing tamoxifen-inducible Cre recombinase in SMCs, were crossed with R26R-Confetti mice that harbor a multi-cassette reporter transgene, which can be randomly recombined by Cre recombinase to express one of four fluorescent proteins. Confetti mice were injected with tamoxifen and atherosclerosis was subsequently induced by liver-specific adeno-associated virus-mediated gene transfer of murine D377Y-mPCSK9 followed by 12, 24 or 36 weeks of high fat diet.

Results: Fluorescence microscopy of the aorta in balanced eGFP+Apoe-/- <-> Apoe-/- aggregation chimeras (n=4) showed a mixed population of eGFP+ and non-fluorescent medial SMCs with a small patch size. Fibrous caps in plaques had much larger groups of cells of the same genotype organized in layers aligned with the underlying endothelium. In Confetti mice, the arterial media showed mosaic expression of the four fluorescence proteins in 57±7% (mean±SD) of SMCs as determined in un-diseased segments of the aortic root. Fibrous caps identified after 24 and 36 weeks of plaque development (n=10 aortic roots analyzed in each group) consisted of large endothelium-aligned layers of SMαA+ SMCs of the same color, confirming results in the aggregation chimeras. The bulk of the SMαA- SMC population in the basal part of murine plaques from Confetti mice also belonged to clonal populations of varying size and exhibited heterogeneous phenotypes including chondroid metaplastic cells and cells with intracellular crystalline material.

Conclusion: During plaque development single medial SMCs undergo significant coherent clonal expansion.

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Lipid lowering by PCSK9 monoclonal antibodies reverses the pro-inflammatory profile of circulating monocytes

A.E. Neele[1]*, S.J. Bernelot Moens[2]*, J. Kroon[3], F.M. van der Valk[2], J. Van den Bossche[1], M.A. Hoeksema[1], R.M. Hoogeveen[2], J.G. Schnitzler[3], M.T. Baccara-Dinet[4], G. Manvelian[5], E.S.G. Stroes[2]#, M.P.J. de Winther[1,6] #

[1]Experimental Vascular Biology, Medical Biochemistry, [2]Vascular Medicine, [3]Experimental Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands; [4]Sanofi, Clinical Development, R&D, Montpellier, France; [5]Regeneron Pharmaceuticals., Tarrytown, NY, USA; [6]Institute for Cardiovascular Prevention (IPEK), Munich, Germany

*Contributed equally, #Contributed equally

Aims: Migration of monocytes into the arterial wall contributes to arterial inflammation and atherosclerosis progression. Since elevated LDL levels have been associated with activation of monocytes, intensive LDL lowering may reverse these pro-inflammatory changes. Subjects with elevated LDL levels are currently treated with statins, which are also described to have pleiotropic anti-inflammatory effects. Using proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies which selectively reduce LDL we studied the impact of LDL lowering on monocyte phenotype and function in patients with familial hypercholesterolemia (FH).

Methods and Results: We assessed monocyte phenotype and function using flow cytometry, and a trans-endothelial migration assay in FH patients(n=22) and healthy controls (n=18). Monocyte chemokine receptor (CCR) 2 expression was approximately 3-fold higher in FH patients compared with controls. CCR2 expression correlated significantly with plasma LDL levels and was positively associated with intracellular lipid accumulation. Monocytes from FH patients also displayed enhanced migratory capacity ex vivo. After 24 weeks of PCSK9 monoclonal antibody treatment (n=17), plasma LDL was reduced by 49%, which coincided with reduced intracellular lipid accumulation and reduced CCR2 expression. Functional relevance was substantiated by the reversal of enhanced migratory capacity of monocytes following PCSK9 monoclonal antibody therapy. Finally, PCSK9 inhibition reduced TNF and enhanced IL-10 production upon stimulation. All changes were comparable in subjects who were treated with statins indicating that the anti-inflammatory effects were mediated through LDL lowering.

Conclusions: Elevated LDL levels in FH patients induce pro-inflammatory changes in monocytes, which is dampened by LDL lowering by PCSK9 monoclonal antibody therapy. LDL lowering was paralleled by reduced intracellular lipid accumulation, suggesting that LDL lowering itself is associated with anti-inflammatory effects on circulating monocytes.

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Targeting the ER stress response delays atherosclerosis and associated death in progeroid mice

Magda R. Hamczyk[1], Ricardo Villa-Bellosta[1], Víctor Quesada[2], Pilar Gonzalo[1], María J. Andrés-Manzano[1], Carlos López-Otín[2], Vicente Andrés[1]

[1]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), CIBER de Enfermedades Cardiovasculares, Madrid, Spain; [2]Departamento de Bioquímica y Biología Molecular, Instituto Universitario de Oncología (IUOPA), Universidad de Oviedo, Oviedo, Spain.

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disease caused by progerin, a variant of nuclear lamin A. Patients exhibit accelerated atherosclerosis and premature aging and die mainly from myocardial infarction or stroke in their early teens. The mechanisms underlying progerin-induced atherosclerosis remain largely unexplored. Here, we generated an HGPS-like mouse model of ubiquitous progerin expression that reproduces the main features of human HGPS, including adventitial thickening, vascular smooth muscle cell (VSMC) loss, accelerated atherosclerosis, and shortened lifespan. Notably, these alterations were also seen when progerin was specifically expressed in VSMCs. Transcriptomic studies of progeroid aorta identified the endoplasmic reticulum stress and unfolded protein responses as possible drivers of progerin-induced atherosclerosis and VSMC death. Targeting this stress pathway with a chemical chaperone inhibited atherosclerosis in both progeria models and extended lifespan in the VSMC-specific model. Our results identify a mechanism underlying HGPS cardiovascular disease that could be targeted in patients.

Acnowledegements: Work supported by grants from the Spanish Ministry of Economy, Industry and Competitiveness (MINECO) (SAF2013-46663-R, SAF2016-79490-R) and the Instituto de Salud Carlos III (RD12/0042/0028) with co-funding from the Fondo Europeo de Desarrollo Regional (FEDER). The Instituto Universitario de Oncología is supported by Obra Social Cajastur. The CNIC is supported by the MINECO and the Pro-CNIC Foundation, and is a Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505).

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Loss of endothelial ADAM10 augments atherosclerosis development in mice

Kosta Theodorou[1]*, Emiel P.C. van der Vorst[1]*, Timo Rademakers[2], Jaap van Buul[2], Carl Blobel[3], Michael Lehrke[4], Corinna Lebherz[4], Daniela Dreymueller[5], Andreas Ludwig[5], Jacob F. Bentzon[6,7], Erik A.L. Biessen[1], Marjo M.P.C. Donners[1]

[1]Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht University, The Netherlands; [2]Department of Molecular Cell Biology, Sanquin Research and Landsteiner Laboratory, Amsterdam, The Netherlands; [3]Hospital for Special Surgery, Weill Cornell Medicine, Rockefeller University, New York, USA; [4]Department of Internal Medicine and [5]Institute for Pharmacology and Toxicology, RWTH Aachen University, Germany; [6]Centro Nacional de Investigaciones Cardiovasculares Carlos III, Spain, and [7]Dept. of Clinical Medicine, Aarhus University, Denmark

Through shedding of various membrane molecules, including adhesion molecules and chemokines, A Disintegrin And Metalloproteinase 10 (ADAM10) could regulate endothelial permeability and leukocyte recruitment, critical processes in inflammatory diseases like atherosclerosis. Indeed, proteomic analysis on mouse endothelial cell sheddome revealed ±300 differentially regulated proteins upon ADAM10 inhibition, of which 10% appeared involved in permeability and leukocyte transmigration. Accordingly, in vitro inhibition of endothelial ADAM10 decreased neutrophil adhesion and transmigration under flow.
To evaluate the causal role of endothelial ADAM10 in atherosclerosis development, we used wildtype or endothelial ADAM10-deficient (ADAM10fl/fl/Tie2-Cre; in brief ADAM10del) mice. Mice were rendered atherogenic by adeno-associated virus-mediated overexpression of PCSK9, resulting in persistent LDL receptor knockdown and hyperlipidemia after high cholesterol diet feeding (HCD).
Surprisingly, after 12 weeks of HCD diet feeding, ADAM10del mice showed significantly larger (±45%) and more advanced atherosclerotic lesions, with intraplaque hemorrhage in the brachiocephalic artery. Necrotic core area was increased (±87%) and macrophage content decreased (±49%). No differences were observed in granulocyte and collagen content.
In contrast to the in vitro findings, in vivo endothelial permeability, leukocyte adhesion and extravasation, as assessed by intravital multiphoton microscopy, were all increased.
In conclusion, this study reveals an unexpected protective effect of endothelial ADAM10 in atherosclerosis development. The underlying mechanisms remain to be determined.

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Impaired interferon signalling may underlie high prevalence of cardiovascular disease in South Asians

Robin van Eenige[1,2], Andrea D. van Dam[1.2], Mark J.W. Hanssen[3], Edwin Quinten[4], Hetty C. Sips[1.2], Cindy Hülsman[3], Ingrid M. Jazet[1], Wouter D. van Marken Lichtenbelt[3], Mariëlle C. Haks[4], Patrick C.N. Rensen[1,2], Mariëtte R. Boon[1,2,3]

[1]Dept. of Medicine, Div. of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden, The Netherlands;[3]Dept. of Human Biology & Human Movement Sciences, Maastricht University Medical Center, Maastricht, The Netherlands;[4]Dept. of Infectious Diseases, Leiden University Medical Center, Leiden, The Netherlands

Aim: South Asians have a 2-fold higher risk of developing CVD compared to white Caucasians, which cannot be explained by the classical risk factors including dyslipidemia. Since inflammation plays an important role in CVD development, we assessed inflammatory state in South Asians compared to white Caucasians.

Methods: We compared mRNA expression of 144 markers of immune function in skeletal muscle and white adipose tissue (WAT) of overweight pre-diabetic Dutch South Asian and matched white Caucasian men using a dual-color reverse transcriptase multiplex ligation-dependent probe amplification assay. Mitochondrial oxygen consumption in muscle biopsies was also assessed.

Results: In WAT, the expression of most macrophage markers and T cell subsets, pattern recognition receptors and inflammasome components were similar between South Asians and white Caucasians. Surprisingly, expression of especially interferon signalling genes was lower in South Asians, both in muscle (IFIT3, IFI44; -45-51%) and WAT (IFI35, IFI44, IFIT2, IFIT3, IFIT5, OAS1, STAT1; -13-40%). Interestingly, mitochondrial respiration in skeletal muscle correlated positively with IFIT2 ($R^2=0.28$, $p<0.05$), IFIT3 ($R^2=0.50$, $p<0.001$) and IFIT5 ($R^2=0.45$, $p<0.01$) expression in WAT. Ingenuity pathway analysis highlighted the anti-inflammatory IFNα/β signaling pathway to be lower in South Asians.

Conclusions: In conclusion, South Asians have impaired IFN signaling, which may contribute to their high CV risk by reducing energy metabolism. Indeed, South Asians have lower energy expenditure compared to white Caucasians and impaired IFNα/β signaling was recently shown to induce metabolic syndrome in mice. Whether IFN signaling in metabolic tissues directly influences mitochondrial function and metabolism in humans is an interesting field of future investigation.

Lipocalin-2 contributes to experimental atherosclerosis in a stage-dependent manner

J. Amersfoort[1], F.H. Schaftenaar[1], H. Douna1, M.J. Kroner[1], G.H.M. van Puijvelde[1], M.R. de Vries[2], P.H.A. Quax[2,3], J.Kuiper[1], I.Bot[1].

[1]Division of Biopharmaceutics, LACDR, Leiden University, Leiden, The Netherlands; Department of Surgery, Leiden University Medical Center, Leiden, The Netherlands; Einthoven Laboratory for Experimental Vascular Medicine

Lipocalin-2 (Lcn2) is an antibacterial glycoprotein, which is secreted by activated neutrophils, monocytes and macrophages upon infection. It is associated with different parameters of coronary artery disease, including severity of disease, while its pathophysiological mechanisms remain relatively unexplored. We aimed to examine these in an Lcn2 knock-out (KO) model in experimental diet-induced atherosclerosis .
Lcn2 expression is upregulated during Western-type diet (WTD) in carotid arteries with collar-induced atherosclerosis from female LDLr deficient mice (LDLr KO) as compared to non-atherosclerotic control carotid arteries (t0: 124±12.1, t2: 14822±6936, t10: 1541±925). Female LDLr KO or Lcn2 LDLr-dKO (dKO) mice were fed a WTD for 6 or 12 weeks to examine early and advanced atherosclerosis , respectively. Early lesion development in the 3-valve area was increased in the dKO group (K0: 2.4±0.5*10^5 µm2, dKO: 2.9±0.6*10^5 µm2, p<0.05) while advanced lesion size was not significantly altered. lnterestingly, increased lesion stability as assessed by necrotic core size was observed in advanced lesions from dKO mice (KO: 31.2±6.6, dKO: 24.3±6.2, p<0.01), while intraplaque MMP9 activity appeared decreased. Flow cytometry analysis suggests Lcn2 KO increases monocyte recruitment as circulating pro-inflammatory monocytes were increased during early (K0: 39.6±5.8%, dK0: 50.6±7.3, p<0.05) and advanced lesions (K0: 41.7±4.3%, dKO: 8.4±5.5%, p<0.05). Also, monocyte levels were decreased in bone marrow (KO: 42.6±5.2, dKO:30.0±2.2, p<0.01). lnterestingly, no effects were observed on neutrophil abundance or activation status in the circulation or bone marrow, while Lcn2 is a potent chemokine for neutrophils.
In conclusion, in early-stage experimental atherosclerosis Lcn2 KO increases lesion development, presumably by increasing monocyte recruitment. In advanced stage atherosclerosis Lcn2 KO mainly affects plaque stability. These findings suggest Lcn2 has a stage-dependent contribution to experimental atherosclerosis.

Testosterone is an endogenous regulator of BAFF and splenic B-cell number

Wilhelmson AS, Lantero Rodriguez M, Stubelius A, Fogelstrand P, Johansson I, Buechler MB, Kapoor V, Johansson ME, Fagman JB, Duhlin A, Tripathi P, Camponeschi A, Lianoglou S, Porse BT, Rolink AG, Giwercman A, Nissbrandt H, Turley SJ, Carlsten H, Mårtensson IL, Karlsson MC, Tivesten Å

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Androgen deficiency in men is associated with increased risk of autoimmune disorders through yet unknown mechanisms. Here we show that testosterone is an endogenous regulator of B-cell activating factor (BAFF), a survival factor for B cells and a new treatment target for autoimmune diseases. Male mice with general deletion of the androgen receptor (G-ARKO) had increases in the number of splenic B cells and in autoantibody titers. This was not explained by increased bone marrow B lymphopoiesis; instead, both G-ARKO and castrated mice had increased serum levels of BAFF, and splenic Baff mRNA was regulated by testosterone. Human serum BAFF levels were also higher in hypogonadal young men, supporting this novel connection. In mice, the effect of castration on mature splenic B-cell number was abrogated by concomitant treatment with a blocking BAFF receptor antibody or by the neurotoxic substance 6-hydroxydopamine. Indeed, expansion of BAFF-producing fibroblastic reticular cells (FRCs) in spleen after castration could be coupled to reduced splenic noradrenaline levels as alpha-adrenergic stimulation negatively regulated FRC number in vitro. Our findings have clinical implications for men with subnormal testosterone levels and autoimmune disease and suggest a potential mechanism of the sexual dimorphism in autoimmunity.

Oral presentations – Abstracts

Cardiovascular Disease

SESSION II

Disruption of the biological clock aggravates atherosclerosis

Maaike Schilperoort[1], Rosa van den Berg[1], Sander Kooijman[1], Nienke Biermasz[1], Claudia P. Coomans[2], Johanna H. Meijer [2], Patrick C.N. Rensen[1]

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Background: Accumulating evidence suggests that the biological clock is an important player in cardiometabolic health. Disruption of circadian (i.e. 24 h) rhythm by means of shift work has been associated with obesity, dyslipidemia and cardiovascular disease in humans. In addition, a dominant-negative mutation in the Clock gene, encoding for a transcription factor essential for the generation of circadian rhythms, induces dyslipidemia in ApoE-/- mice. However, causality between circadian disruption upon shift work and atherosclerotic lesion development has not yet been established. Therefore, the aim of this study was to investigate whether mimicking shift work by chronically alternating light cycles directly affects lipid metabolism and atherosclerosis development in mice.

Methods & Results: APOE*3-Leiden.CETP mice were fed a Western-type diet for 15 weeks, during which they were exposed to either a regular light-dark (LD) cycle, or a weekly alternating light-dark (LD-DL) cycle to mimic shift work. Interestingly, food intake was decreased in LD-DL mice as compared to controls (-9.8%; $p<0.01$) while body weight was similar in both groups (24.7±4.08 g in LD vs. 23.5±1.75 g in LD-DL), suggesting reduced energy expenditure. After 15 weeks, plasma cholesterol was determined and histological analysis of the aortic root area was performed to evaluate atherosclerosis. While total plasma cholesterol appeared unaffected (13.5±4.2 mM in LD vs. 15.3±4.5 mM in LD-DL), LD-DL mice had substantially larger lesions (+76%; $P<0.01$) that were more severe (that is, type IV-V; +53%) compared to LD mice, indicating an increased risk for cardiovascular disease.

Conclusion: We are the first to show that disruption of circadian rhythmicity by mimicking shift work directly aggravates atherosclerosis development. Our current focus is on elucidation of underlying mechanisms focused on lipid metabolism and inflammation.

Plasma stem cell factor levels are associated with risk of cardiovascular disease and death

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Objective: Stem cell factor (SCF) is a key growth factor for several types of stem and progenitor cells. There is experimental evidence that such cells are of importance for maintaining the integrity of the cardiovascular system. We investigated the association between circulating levels of SCF and risk for development of cardiovascular events and death.

Approach and results: SCF was analyzed by the Proximity Extension Assay technique in plasma from 4742 subjects participating in the Malmö Diet and Cancer Study. Cardiovascular events and death were monitored through national registers with a mean follow-up time of 19.2 years. Subjects with high baseline levels of SCF had lower risk of all-cause mortality (n=1159), heart failure (n=177), stroke (n=318) and myocardial infarction (n=452). Smoking, diabetes and high alcohol consumption was associated with lower levels of SCF. Single nucleotide polymorphisms in the genes encoding PDX1 C-Terminal Inhibiting Factor 1 (PCIF1) and matrix metalloproteinase -9 were associated with plasma SCF levels. The highest SCF quartile remained independently associated with a lower risk of death (hazard ratio (HR) and 95% confidence interval (CI) 0.73 (0.61-0.88)), heart failure (0.54 (0.33-0.88)) and stroke (0.70 (0.50-0.98)), but not with MI (0.98 (0.73-1.31)) as compared with the lowest quartile when adjusting for traditional cardiovascular risk factors in Cox proportional hazard regression models.

Conclusions: This prospective population-based study demonstrates that subjects with high levels of SCF have a lower risk of cardiovascular events and death. The findings provide clinical support for a protective role of SCF in maintaining cardiovascular integrity.

2.5-fold risk of ischemic stroke in individuals with clinical familial hypercholesterolemia: The Copenhagen General Population Study with 102,961 individuals.

Sabina Beheshti, Christian M. Madsen, Anette Varbo and Børge G. Nordestgaard.

Background: Familial hypercholesterolemia(FH) results in very high concentrations of low-density lipoprotein cholesterol, and high risk of ischemic heart disease. However, there is limited and contradictory information on whether these individuals have high risk of ischemic stroke.

Objectives: To test the hypothesis that individuals in the general population with clinical FH have a high risk of ischemic stroke.

Methods: 102,961 individuals from the Copenhagen General Population Study were followed for up to 11 years and 1,554 individuals developed ischemic stroke during follow-up, which was 100% complete. We used modified Dutch Lipid Clinic Network(DLCN), Make Early Diagnosis to Prevent Early Death(MEDPED), and Simon Broome criteria to diagnose clinical FH.

Results: Using DLCN criteria, individuals with possible FH had a multivariable-adjusted hazard ratio for ischemic stroke of 1.40(95%confidence interval:1.17-1.67; 140 events) and individuals with definite or probable FH a hazard ratio of 2.45(1.31-4.58; 10 events), when compared to individuals with unlikely FH. The cumulative incidences at age 80 years were 9 %, 12 % and 22 % for unlikely, possible and probable or definite FH(according to DLCN criteria), respectively. Premature ischemic heart disease at baseline was associated with an even higher risk of ischemic stroke. There was no association between FH and ischemic stroke using MEDPED or Simone Broome criteria. In addition, mutations causative of FH showed no association with ischemic stroke.

Conclusions: Individuals in the general population with clinical FH according to DLCN criteria have a high risk of ischemic stroke, with even higher risk in those with premature ischemic heart disease.

Participate in the young investigator's award.

Association of apolipoprotein M with cardiovascular risk factors and kidney injury in patients with chronic kidney disease

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Background: Plasma apolipoprotein M (apoM) is bound to HDL-particles and has anti-atherogenic effects. The present study explored whether plasma apoM is reduced in patients with chronic kidney disease (CKD), and associated with risk factors of cardiovascular disease (CVD). Secondly, whether urine apoM was increased in patients with kidney disease.

Materials and methods: Plasma samples were collected from a cohort of patients with CKD stage 1 to 5D (N=409), and healthy controls (N=71). Plasma apoM was measured by a sandwich ELISA and urine apoM by a competitive ELISA.

Results: Plasma apoM was reduced in patients with CKD as compared to healthy controls (0.87+/-0.02 vs. 0.97+/-0.03 umol/l, $p<0.05$). Also, plasma apoM correlated with risk factors of CVD. Patients with CKD and CVD had significantly lower levels of plasma apoM than CKD patients without CVD (0.80+/-0.02 vs. 0.92+/-0.02 umol/l, $p<0.001$). Moreover, CKD patients with diabetes type 2 displayed even further reduction in plasma apoM levels than CKD patients without diabetes (0.69+/-0.03 vs. 0.93+/-0.02 umol/l, $p<0.001$). Urine apoM/creatinine ratio was not significantly increased in patients with proteinuria, CKD and AKI compared to healthy control subjects.

Conclusion: Patients with CKD have reduced plasma apoM concentrations. Both CVD and diabetes type 2 add further to reduction of plasma apoM in patients with CKD. Whether apoM plays a role in human uremic atherogenesis or will be useful as a marker of accelerated CVD in CKD warrants further studies.

Participate in the young investigator´s award

Genetic variants in CYP7A1 and risk of myocardial infarction and symptomatic gallstone disease

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Aim: Myocardial infarction(MI) and gallstone disease are intrinsically linked via cholesterol metabolism. We tested the hypothesis that inactivating variants in the gene encoding cholesterol 7 alpha-hydroxylase(CYP7A1), the rate-limiting enzyme in the conversion of cholesterol to bile acids in the liver, are associated with an increased risk of MI and gallstone disease in the general population.

Methods: We performed tests of association between lipid levels and eight rare nonsynonymous mutations and two common variants, rs2081687 and rs3808607, in CYP7A1 in 100,149 individuals from the general population. We further tested whether weighted allele scores for rs2081687 and rs3808607 which were associated with increased plasma levels of LDL cholesterol(LDL-C), were associated with an increased risk of MI and symptomatic gallstone disease. During follow-up, MI developed in 2,326 individuals and gallstone disease in 2,007.

Results: For rare mutations, CYP7A1 allele count was associated with an increase in LDL-C of 12%(0.4 mmol/L) for individuals with the highest versus the lowest allele count(P for trend=3x10-4). For common variants, CYP7A1 weighted allele scores in individuals with a score >0.04 versus ≤0 were associated with stepwise increases in LDL-C of up to 2.4%(0.08 mmol/L), and with corresponding multifactorially adjusted hazard ratios of 1.25(95% confidence interval(CI):1.10-1.41) for MI and 1.39(95% CI:1.22-1.59) for gallstone disease (P for trend=5x10-4 and 2x10-7, respectively).

Conclusions: Genetic variants in CYP7A1 which are associated with increased levels of LDL-C, cause an increased risk of MI and gallstone disease. These data therefore suggest that increasing CYP7A1 activity may reduce risk of both MI and gallstone disease.

Participate in the young investigator´s award.

Incidence of acute myocardial infarction and coronary heart disease in patients with genotyped familial hypercholesterolemia in Norway during 2001-2009

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Aims: It is not known to which extent statin treated familial hypercholesterolemia (FH) increases the risk for cardiovascular disease (CVD). The primary aim of this study was to investigate incidence of acute myocardial infarction (AMI) and coronary heart disease (CHD) relative to the total Norwegian population of about 5 million people in the period 2001-2009 in a large sample of genotyped FH patients stratified by sex and age groups.

Methods and Results: 4164 genotyped FH patients without previous AMI and CHD were included in the analyses. All AMI and CHD hospitalizations and coronary out-of-hospital deaths in all Norwegian genotyped FH patients were obtained from the Cardiovascular Disease in Norway project. An incident AMI was defined as a hospitalization or out-of-hospital death due to AMI with no prior hospitalization for AMI. Standardized incidence ratios (SIRs) with 95% confidence intervals (95% CIs) were calculated by indirect standardization with AMI and CHD incidence rates for the total Norwegian population during 2001-2009 stratified by sex, calendar year and one-year age groups as reference rates. In FH women total AMI SIR (95% CI) was 2.3 (1.6-3.2), in young women 25-39 years SIR (95% CI) was 13.6 (5.1-36.2). In FH men total AMI SIR (95% CI) was 2.3 (1.8-3.0), and in young men 25-39 years SIR (95% CI) was 7.5 (3.7-14.9). The corresponding CHD SIRS (95% CI) were in women total 4.7(3.9-5.7), and in men: 4.2 (3.6-5.0), and in the age group 25-39 years in women: 17.3(9.6-31.2) and in men: 11.1(7.1-17.5).

Conclusion: Both women and men with genetically verified FH have significantly higher incidence of AMI and CHD compared to the general Norwegian population. Highest incidences were found in the young age groups 25-39 years where the AMI and CHD incidences were more than 13- and 7-fold, and 17-and 11-fold increased respectively.

Body Mass Index and Risk of Alzheimer Disease: A Mendelian Randomization Study of 399,536 Individuals

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Background: Recently, data on two million people established that low body mass index (BMI) is associated with increased risk of dementia. Whether this reflects a causal association is unknown.

Methods: Using a Mendelian randomization approach, we studied 95,578 individuals from the Copenhagen General Population Study and consortia data on 303,958 individuals. We first tested the observational association between low BMI and risk of Alzheimer disease. Secondly, we tested whether five genetic variants in FTO, MC4R, TMEM18, BDNF, and GNPDA2 combined into a weighted allele score were associated with BMI and risk of Alzheimer disease. Finally, we calculated causal estimates.

Results: In observational analyses, risk of Alzheimer disease increased stepwise as a function of lower BMI (P: 5*10-7). The estimated causal risk ratio for Alzheimer disease for a 1kg/m2 decrease in genetically determined BMI, was 0.98 (95% confidence interval 0.77-1.23). The corresponding observational hazard ratio was 1.07 (1.05-1.09). Using 32 BMI decreasing variants from the Genetic Investigation of Anthropometric Traits (GIANT) and the International Genomics of Alzheimer's Project (IGAP) the causal odds ratio for Alzheimer disease for a one standard deviation decrease in genetically determined BMI was 1.02 (0.86-1.22).The corresponding observational hazard ratio was 1.32 (1.20-1.46).

Conclusions: Genetic and hence lifelong low BMI is not associated with increased risk of Alzheimer disease in the general population. These data suggest that low BMI is not a causal risk factor for Alzheimer disease, and that the corresponding observational association likely is explained by reverse causation or confounding.

Oral presentations – Abstracts

Lipoprotein and Lipid Transport

SESSION III

Effect of weight reduction on Lipoprotein(a) levels in obese and Type 2 diabetes patients

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Elevated levels of lipoprotein(a) [Lp(a)] are an independent cardiovascular disease (CVD) risk factor, in particular in type 2 diabetes (T2D). While weight loss improves conventional CVD risk factors in type 2 diabetes, effects on Lp(a) are unknown and possibly influencing the long term CVD outcome of diet-induced weight loss. The aim of this clinical study was to determine the effect of diet-induced weight loss on Lp(a) levels in obese individuals with T2D.

Plasma Lp(a) levels were determined immunoturbidimetrically in plasma obtained before and after 3-4 months of a calorie-restricted diet in four independent study cohorts. The primary cohort consisted of 131 predominantly obese patients with T2D (cohort-1).The secondary cohorts consisted of 30 obese patients with type 2 diabetes (cohort-2) and 37 obese subjects without type 2 diabetes (cohort-3), and 26 obese subjects without T2D who underwent bariatric surgery (cohort-4).

In the primary cohort, the calorie-restricted diet resulted in a weight loss of 9.9% (95%CI 8.9, 10.8) and improved conventional CVD risk factors such as LDL cholesterol. Lp(a) levels increased by 7.0 mg/dl (95%CI 4.8, 9.7). In univariate analysis, the change in Lp(a) correlated with baseline Lp(a) levels ($r=0.38$, $p<0.001$) and change in LDL cholesterol ($r=0.19$, $p=0.033$). In cohorts 2 and 3, the weight loss of 8.5% (95%CI 6.5, 10.6) and 6.5% (95%CI 5.7, 7.2) was accompanied by a median Lp(a) increase of 6.4 mg/dl (95%CI 1.1, 14.2) and 5.6 mg/dl (95%CI 2.7, 9.0), respectively (all $p<0.001$). When the cohorts 1-3 were combined, the diet-induced increase in Lp(a) correlated with weight loss ($r=0.178$, $p=0.012$). In cohort-4, no significant change in Lp(a) was found (-3.3 mg/dl (95%CI -8.9, 2.5)) despite considerable weight loss (14.0% (95%CI 12.2, 15.7)).

In conclusion diet-induced weight loss was accompanied by an increase in Lp(a) levels in obese subjects with and without T2D while conventional CVD risk factors improved. This increase in Lp(a) levels may potentially antagonize the beneficial cardio-metabolic effects of a diet-induced weight reduction.

High lipoprotein(a) and low risk of major bleeding in the general population: a Mendelian randomization study

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Aim: The physiological role of lipoprotein(a) is unclear; however, lipoprotein(a) may play a role in wound healing. We tested the hypothesis that high lipoprotein(a) levels are associated with low risk of major bleeding, observationally and causal, genetically.

Methods: We examined 109,169 individuals from the Copenhagen City Heart Study and the Copenhagen General Population study, two similar prospective studies conducted in the Danish general population. Individuals had information on plasma lipoprotein(a) levels (n=59,980), LPA kringle-IV type 2(KIV-2) number of repeats (n=98,965), and/or LPA single-nucleotide polymorphism rs10455872 (n=109,169), and all register information on hospital contacts or death due to major bleeding, that is, bleeding in the central nervous system and airways.

Results: Using extreme phenotypes or genotypes, the multifactorially adjusted hazard ratio for major bleeding was 0.84(95%CI: 0.71-0.99) for lipoprotein(a) >80mg/dL versus <11mg/dL, 0.83(0.73-0.96) for KIV-2 <24 versus >35 number of repeats, and 0.89(0.81-0.97) for rs10455872 carriers versus non-carriers. Also, for a one standard deviation higher lipoprotein(a) (=31mg/dL) the hazard ratio for major bleeding was 0.95(95%CI: 0.91-1.00) observationally, 0.89(0.80-0.98) genetically based on LPA KIV-2 number of repeats, and 0.94(0.87-1.02) genetically based on the LPA rs10455872. The low risk of bleeding in individuals with high lipoprotein(a) appeared to be most pronounced at high alcohol intake, above age 60, and in the presence of hypertension.

Conclusion: High lipoprotein(a) levels were associated with low risk of major bleeding observationally and causal, genetically. This indicates that lipoprotein(a) may play a role in wound healing.

Complex functions of High Density Lipoproteins (HDL), HDL increases inflammatory and bacterial clearance responses in macrophages

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Aim: Membrane cholesterol is known to modulate a variety of cell signaling pathways and functions. While cholesterol depletion by High-Density-Lipoproteins (HDL) has potent anti-inflammatory effects in various cell types, its effects on inflammatory responses in macrophages remain ill defined.
Methods: Using various forms of HDL and different sources of macrophages we investigated the inflammatory effects of HDL in vitro as well as in vivo.
Results: Pre-incubation of human and murine macrophages in vitro with human reconstituted (apolipoproteinA-I/phosphatidylcholine) or native HDL significantly decreased LPS-induced anti-inflammatory IL-10 production, while the opposite was observed for the pro-inflammatory mediators IL-12 and TNF. We show that these effects are mediated by passive cholesterol depletion and lipid raft disruption, without involvement of ABCA1, ABCG1, SR-BI or CD36. These pro-inflammatory effects are confirmed in vivo in peritoneal macrophages from ApoA-I transgenic mice, which have high circulating HDL levels. In line, innate immune responses required for clearance of P. aeruginosa bacterial infection in lung were compromised in mice with low HDL levels. Expression analysis, ChIP-PCR and combinatorial pharmacological/genetic intervention studies unveiled that native and reconstituted HDL enhance Toll-like receptor-induced signaling by activating a PKC-NFkB/STAT1-IRF1 axis, leading to increased inflammatory cytokine expression. Native and reconstituted HDL enhances Toll-Like-Receptor-induced signaling by activating protein kinase C (PKC), since inhibition of PKC ablated the observed HDL effects.
Conclusions: HDL exerts pro-inflammatory effects on macrophages which could support proper functioning of macrophage immune responses. These pro-inflammatory activities on macrophages could at least partly underlie the disappointing therapeutic potential of HDL raising therapy in current cardiovascular clinical trials.

Participate in the young investigator´s award.

Lysosomal oxidation of LDL and its implications for atherosclerosis

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Background: We have shown that LDL aggregated either mechanically by vortexing or enzymatically by sphingomyelinase (SMase-LDL) is internalised by macrophages and oxidised in lysosomes by redox active iron. We have now studied the effects of lysosomal oxidation of LDL on various lysosome-associated functions and also shown how the lysosomotropic antioxidant cysteamine is able to prevent these effects.

Approach and Results: LDL was incubated for 4 hours with sphingomyelinase, which increased its average particle diameter from 27 to 209 nm. Intralysosomal lipid peroxidation was studied in human THP-1 macrophages using the ratiometric dye Foam-LPO. SMase-LDL treatment caused increased lysosomal lipid peroxidation in THP-1 cells compared to native LDL, which was decreased by cysteamine. Intralysosomal ceroid was detected by Oil Red O. Little ceroid was seen after incubation with native LDL for 7 days, but large amounts were seen with sphingomyelinase-aggregated LDL and cysteamine considerably inhibited the ceroid formation. Lysosomal oxidation of LDL increased the lysosomal pH and decreased the overall function of lysosomes, whereas cysteamine was able to restore the pH and normal function. It is believed that decreased lysosomal proteolytic activity and increased lysosomal pH occurs as a consequence of ageing in long-lived post mitotic cells. Both native LDL and especially SMase-LDL induced senescent-like properties in human THP-1 macrophages and treatment with cysteamine significantly decreased the SMase-LDL- induced senescence. Inflammation plays a key role in the initiation, progression and rupture of atherosclerotic lesions. Both native LDL and SMase-LDL treatment increased the LPS-induced secretion of TNF-α by THP-1 macrophages. Pre-incubation with cysteamine for 24 h decreased the secretion of TNF-α by macrophages induced by SMase-LDL.

Conclusion: Therapeutic strategies to target lysosomes with antioxidants like cysteamine to prevent the intralysosomal oxidation of LDL seems a plausible avenue to decrease the development of atherosclerosis.

Participate in the young investigator´s award.

Loss of Function of GALNT2 Lowers High-Density Lipoproteins in Humans, Nonhuman Primates, and Rodents

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Human genetics studies have implicated GALNT2, encoding GalNAc-T2, as a regulator of high-density lipoprotein cholesterol (HDL-C) metabolism, but the mechanisms relating GALNT2 to HDL-C remain unclear. We investigated the impact of homozygous GALNT2 deficiency on HDL-C in humans and mammalian models. We identified two humans homozygous for loss-of-function mutations in GALNT2 who demonstrated low HDL-C. We also found that GALNT2 loss of function in mice, rats, and nonhuman primates decreased HDL-C. O-glycoproteomics studies of a human GALNT2-deficient subject validated ANGPTL3 and ApoC-III as GalNAc-T2 targets. Additional glycoproteomics in rodents identified targets influencing HDL-C, including phospholipid transfer protein (PLTP). GALNT2 deficiency reduced plasma PLTP activity in humans and rodents, and in mice this was rescued by reconstitution of hepatic Galnt2. We also found that GALNT2 GWAS SNPs associated with reduced HDL-C also correlate with lower hepatic GALNT2 expression. These results posit GALNT2 as a direct modulator of HDL metabolism across mammals

Protein destabilization as a regulating factor in intravascular lipolysis

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Intravascular triglyceride hydrolysis is mediated by lipoprotein lipase (LPL). This process is controlled by endogenous inhibitors (eg ANGPTL4) and is focalized on capillary endothelial cells by the glycolipid-anchored GPIHBP1. LPL is synthesized and secreted from myocytes and adipocytes and is sequestered in the sub-endothelial space by its interaction with heparan sulfate proteoglycans. Trafficking of LPL from the sub-endothelial space to the capillary lumen is mediated GPIHBP1. The LPL•GPIHBP1 complex secures margination of lipoprotein particles at the endothelial cell surface. Missense mutations impairing the functions of LPL or GPIHBP1 lead to severe familial chylomicronemia. Genetic ablations of Gpihbp1 cause the secreted LPL to remain mislocated in the sub-endothelial space. Genome-wide association studies demonstrate that a polymorphic variant of ANGPTL4 (E40K) is associated lower of plasma triglyceride levels and lower risk of developing coronary artery disease.

Despite advanced insights into the genetics and physiology of intravascular lipolysis the underlying protein structure-function relationships remain largely enigmatic. With highly purified proteins and contemporary biophysical methods such as SPR, HDX-MS, and SAXS, we study the molecular interplay between LPL, GPIHBP1 and ANGPTLs. Based on these studies we propose that regulation of intravascular lipolysis is driven by intrinsic protein destabilization of LPL (refs below). Some of our key observations are: 1) intrinsic unfolding of the N-terminal hydrolase domain in LPL accounts for its unstable activity; 2) ANGPTLs 3 and 4 inactivate LPL by catalyzing this unfolding; 3) GPIHBP1 renders LPL largely refractory to ANGTPLs; 4) cooperativity between the LU domain and the intrinsically disordered acidic domain of GPIHBP1 are required for this protective effect; and 5) the E40K variant of ANGPTL4 has lower inhibitory activity due a pronounced destabilization of its first α-helix. PROTEIN DISORDER bring ORDER to lipid metabolism.

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Cidea improves the metabolic profile through expansion of adipose tissue and modulation of lipid metabolism

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In mice, the expression of Cidea (cell death-inducing DNA fragmentation factor alpha-like effector A) is limited to brown adipose tissue (BAT) where it is classically used as a marker for BAT recruitment status. In contrast, in humans, it is also highly but variably expressed in white fat, and the expression correlates with metabolic health. However, it remains elusive whether this is only correlative, or a functional, causative association.

We generated transgenic mice expressing human Cidea in adipose tissues (aP2-hCidea mice) and housed them under humanized conditions (thermoneutrality, mature age and prolonged exposure to high-fat diet) to study the effects of the presence of Cidea in white adipose tissue on metabolic health and on lipid and glucose metabolism.

The aP2-hCidea mice displayed a strong increase in adipose tissue expandability compared to WT littermates, leading to increased obesity. However, insulin sensitivity was improved and the malfunctioning of visceral fat (VAT) normally caused by massive obesity was fully overcome - perilipin 1 and AKT expression were preserved, tissue degradation was prevented, macrophage accumulation was decreased and adiponectin expression remained high. This was linked to increased de novo lipogenesis in white fat and reduced LPL activity. Importantly, the association between VAT Cidea levels, de novo lipogenesis and insulin sensitivity was also found in a cohort of lean and obese humans.

Our data establish a functional role for Cidea and suggest that, in humans, the association between Cidea levels in white fat and metabolic health is not only correlative but also causative. The beneficial effects of Cidea on adipose tissue function involve a fine-tuning of de novo lipogenic, lipolytic and lipid uptake processes, thereby preserving adipose tissue function. Since Cidea is a lipid droplet associated protein that also has been shown to act as a transcriptional coregulator, the data presented here suggests that Cidea functions as a hub integrating adipocyte nutrient status and uptake/production of lipids.

Participate in the young investigator´s award.

Butyrate via the gut-brain circuit reduces appetite and activates brown adipose tissue

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Background: Butyrate has beneficial metabolic effects, but underlying mechanism(s) have not been delineated as yet. Therefore, the aim of this study was to verify the metabolic effects of butyrate in mice and to investigate underlying mechanisms.

Methods and Results: Oral administration of butyrate to C57Bl/6J mice rapidly activated anorexigenic POMC neurons and suppressed orexigenic NPY neurons within the hypothalamus, and reduced food intake. In contrast, i.v. infusion of butyrate did not affect food intake, suggesting butyrate induces satiety via acting on the gut-brain circuit thereby activating hypothalamic satiety signalling. Next, APOE*3-Leiden.CETP mice were fed a high-fat diet (HFD) without or with butyrate for 10 weeks. A third group of mice received the same amount of HFD as that of the butyrate group (pair fed group). Dietary butyrate caused a persisting reduction in food intake and prevented diet-induced obesity and hepatic steatosis, mainly attributed to reduced food intake. Additionally, butyrate increased the sympathetic outflow towards BAT as evident by increased protein expression of tyrosine hydroxylase. As a result, butyrate increased the thermogenic capacity of BAT, as it decreased the intracellular lipid vacuole size, increased the UCP-1 content, and increased the triglyceride-derived fatty acid uptake by BAT. To investigate the role of gut-brain neuronal circuit, APOE*3-Leiden.CETP mice received either vagotomy or sham surgery, after which they were fed a HFD with or without sodium butyrate for 7 weeks. Vagotomy completely abolished the effect of butyrate on food intake and impaired butyrate-induced BAT activation.

Conclusion: Butyrate acts on the vagal nerve between gut and the hypothalamus to improve energy metabolism, via reducing energy intake and enhancing fat energy expenditure by activating BAT, thereby preventing diet-induced metabolic syndrome.

Oral presentations – Abstracts

Other Topics

SESSION IV

Disruption of the cholesterol efflux transporters ABCA1 and ABCG1 alters megakaryocyte proplatelet production

Amber B. Ouweneel[1]*, Marco van der Stoep[1,2]*, Harry F. Heijnen[1], Miranda Van Eck[1], Suzanne J.A. Korporaal[1,2]

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Platelets, produced by megakaryocytes in the bone marrow, play an important role in the onset and development of atherosclerosis and atherothrombosis. Platelet numbers and functionality are affected by hypercholesterolemia in mice and humans. In this study, we investigated whether disruption of cholesterol efflux influences megakaryocyte proplatelet production, using bone marrow explants from mice lacking ATP-Binding cassette transport A1 (ABCA1 KO, G1 (ABCG1 KO) or both (ABCA1/ABCG1 double KO (dKO)) and wildtypes.

Explant cultures of both ABCA1 KO and ABCG1 KO bone marrow showed lower amounts of megakaryocytes at the periphery of the explants (-52±4% ($p<0.01$) and -32±3% ($p<0.001$) resp.), indicating that reduced cholesterol efflux capacity negatively influences either megakaryocyte maturation from progenitor cells, or their migratory capacity. Interestingly, ABCA1 and ABCG1 deletion had differential effects on proplatelet formation. ABCA1 deficiency inhibited proplatelet formation (-67±16%, $p<0.001$). In contrast and despite the lower amount of megakaryocytes, a clear increase in proplatelet formation was observed in ABCG1 KO explants (+243±77%, $p<0.001$).

In line with the notion that ABCA1 and ABCG1 independently modulate megakaryocyte maturation and/or migration, the amount of visible megakaryocytes was even further reduced in bone marrow explants from ABCA1/ABCG1 dKO mice (-75±3%, $p<0.001$). In further support, the marked increase in proplatelet formation resulting from ABCG1 deficiency was diminished upon additional deletion of ABCA1 function, leading to only a mild increase in proplatelet formation (+42±15%, $p<0.05$) in dKO compared to wildtype megakaryocytes.

In conclusion, absence of the cholesterol efflux transporters ABCA1 and ABCG1 alters proplatelet production by megakaryocytes. Moreover, our studies suggest that modulation of the function of individual cholesterol efflux transporters may translate into an overall differential effect on platelet production.

Participate in the young investigator´s award.

Biological clock strongly regulates brown adipose tissue activity: implications for postprandial triglyceride metabolism

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Background: Disturbed circadian rhythms associate with dyslipidemia, and disruption of the biological clock reduces the capacity of brown adipose tissue (BAT) to combust triglyceride (TG)-derived fatty acids (FA) into heat (PNAS USA 2015). Here, we hypothesized that a circadian rhythm in BAT activity determines circadian plasma triglyceride metabolism.

Methods: Wild-type mice and dyslipidemic APOE*3-Leiden.CETP mice were exposed to short (8h), normal (12h), or long (16h) days during 5 weeks. BAT rhythm was determined by its capacity to take up plasma TG-derived FA at 6 consecutive time points. Postprandial lipid excursions were determined following an oral olive oil bolus at different time points and at thermoneutrality (30°C). In 37 healthy volunteers, postprandial lipid excursions following three identical isocaloric meals were determined in 24h blood samples taken every 30 min.

Results: In both wild-type and dyslipidemic mice, BAT FA uptake displayed a pronounced circadian rhythm as compared to other metabolically organs. The highest uptake was observed at wakening, which was independent of light exposure regimes and persistent at thermoneutrality. This rhythm coincided with circadian expression patterns of thermogenic and lipid genes. Interestingly, circadian rhythmicity in BAT activity dictated circadian plasma lipid clearance by BAT as well as plasma lipid levels. Strikingly, in mice as well as humans postprandial lipid excursions were nearly absent at wakening and high before sleep, consistent with circadian BAT activity patterns.

Conclusion: BAT displays a strong diurnal rhythm in TG-derived FA uptake, which determines differences in postprandial TG metabolism throughout the daily light cycle. Since BAT activity is highest at wakening, accompanied by lowest postprandial lipid excursions, we anticipate that restriction of food intake to the early wakeful period improves metabolic health.

Participate in the young investigator´s award.

Cold-triggered bile acid synthesis shapes the gut microbiome

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Cold-stimulated activation of thermogenic adipocytes results in a higher uptake of dietary carbohydrates and lipids as well a cold-associated gut microbiota composition, which provokes an intestinal reorganization in order to fuel adaptive thermogenesis. Here we show, that cold-triggered increased intake of cholesterol is compensated for by elevated fecal bile acid loss finally causing alterations in gut microbial composition. In mice, cold-induced activation of thermogenic adipocytes initiates a metabolic program orchestrating intravascular lipoprotein processing and hepatic conversion of excess remnant-derived cholesterol to bile acids via the alternative synthesis pathway. This process, depending on hepatic CYP7B1 induction, results in elevated plasma levels and pronounced fecal excretion of conjugated bile acids accounting for changes in gut microbiota. Pharmacological intervention using ezetimibe, a drug blocking dietary cholesterol uptake, prevented both the rise in bile acid excretion and compositional changes in gut bacteria in response to cold. Additionally, abrogation of cold-induced bile acid synthesis in EZ-treated or Cyp7b1 deficient mice impairs normal brown adipose tissue function.
Together, these results identify the hepatic cholesterol and bile acid metabolism as the determinant of cold-induced gut microbiota and underpin its relevance for brown adipose tissue function.

Participate in the young investigator´s award.

Blood-brain barrier transcytosis genes and risk of dementia -a study of 74,754 individuals

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Apolipoprotein E (apoE) is pivotal for lipid transport in the brain and facilitates clearance of amyloid-β via lipoprotein receptors. The transcytosis of amyloid-β through the blood-brain barrier, as well as the genetic contribution to this pathway, is however not well-known. We tested the hypothesis that genetic variants in PICALM, BIN1, CD2AP and RIN3 –genes that encode proteins that are involved in transcytosis of amyloid-β across the blood-brain barrier via LRP1- causally associate with Alzheimer's disease and all dementia in the general population. We also tested whether this association is independent of, and comparable in size to the population-attributable fraction of the common APOE genotype. In a prospective cohort study of 74,754 individuals we genotyped four variants in PICALM, BIN1, CD2AP and RIN3, and generated a weighted and a simple allele score. All four genetic variants were individually associated with stepwise increases in risk of either Alzheimer's disease, all dementia, or both. Multifactorially adjusted hazard ratios for the fourth quartile versus the first quartile of the weighted allele score were 1.60 (95% confidence interval 1.33-1.92) for Alzheimer's disease and 1.41 (1.24-1.60) for all dementia. Results were similar after further adjustment for ε2/ε3/ε4 APOE genotype, and when analyses were performed exclusively on an APOE ε33 background. The population-attributable fraction of the fourth versus the first quartile of the weighted allele score was 13% for Alzheimer's disease and 9% for all dementia, while corresponding population-attributable fractions were 17% and 12% for APOE ε44 genotype. In conclusion, genetic variants in PICALM, BIN1, CD2AP and RIN3 – genes suggested to be involved in blood-brain barrier amyloid-β transcytosis pathways – are causally associated with increased risk of Alzheimer's disease and all dementia in the general population. These associations are independent of the APOE ε4 allele, and the population-attributable fraction of Alzheimer's disease and all dementia is comparable in size to that of the APOE ε44 genotype.

Participate in the young investigator´s award.

Extreme High High-Density Lipoprotein Cholesterol is Paradoxically Associated with High Mortality in Men and Women: two Prospective Cohort Studies

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Aims: High-density lipoprotein (HDL) cholesterol concentrations are inversely associated with cardiovascular disease and mortality across a range of concentrations, but genetic evidence suggest that extreme high concentrations may paradoxically lead to more cardiovascular disease. We tested the hypothesis that extreme high concentrations of HDL cholesterol are associated with high all-cause mortality in men and women.

Methods and results: A total of 52,268 men and 64,240 women were included from the two prospective population-based studies, the Copenhagen City Heart Study and the Copenhagen General Population Study.
During 745,452 person-years of follow-up, number of deaths from any cause were 5,619 (mortality rate, 17.1/1,000 person-years (95% confidence interval (CI): 16.7-17.6)) in men and 5,059 (mortality rate, 12.1/1,000 person-years (11.8-12.4)) in women. The association between HDL cholesterol concentrations and all-cause mortality was U-shaped for both men and women, with both extreme high and low concentrations being associated with high all-cause mortality risk. The concentration of HDL cholesterol associated with the lowest all-cause mortality was 1.9mmol/L (95% CI: 1.4-2.0) (73mg/dL(54-77)) in men and 2.4 mmol/L (1.8-2.5) (93 mg/dL(69-97)) in women. When compared to the groups with lowest risk, the multifactorially adjusted hazard ratios for all-cause mortality were 1.36 (95% CI: 1.09-1.70) for men with HDL cholesterol of 2.5-2.99 mmol/L (97-115 mg/dL) and 2.06 (1.44-2.95) for men with HDL cholesterol ≥3.0 mmol/L (116 mg/dL). For women, corresponding hazard ratios were 1.10 (0.83-1.46) for HDL cholesterol of 3.0-3.49 mmol/L (116-134 mg/dL) and1.68 (1.09-2.58) for HDL cholesterol ≥3.5 mmol/L (135 mg/dL).

Conclusion: Men and women in the general population with extreme high HDL cholesterol paradoxically have high all-cause mortality. These findings need confirmation in future studies.

Participate in the young investigator´s award.

Protein components of intracellular membrane contact sites in endothelial cells: Roles of ORP2 and protrudin in angiogenesis

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Membrane contact sites (MCS) mediate the exchange of molecules and signals between cell organelles apposed at a short distance. In this study we characterized MCS components in primary human umbilical vein endothelial cells (HUVECs).

We visualized by imaging prominent MCSs in HUVECs and investigated the subcellular localization of known MCS protein components, including the oxysterol-binding protein-related proteins (ORPs). MCS components were knocked down or overexpressed, and impacts on HUVEC morphology and angiogenesis were analyzed in vitro.

ORP2, a protein previously associated with ER-lipid droplet (LD) junctions, was also observed on late endosomes (LE) in HUVECs. Protrudin/ZFYVE27 is a protein associated with ER-LE junctions, playing a key role in neurite outgrowth. Overexpression of ORP2 induced protrusions and ORP2 knock-down inhibited angiogenesis. Similarly, protrudin overexpression induced protrusions and its knock-down inhibited angiogenesis. Overexpression of ORP2 interfered with the LE localization of protrudin, and a mutant protrudin altered the subcellular distribution of ORP2. ORP2 was excluded from LE labeled by a PI3P probe but not from those labeled by a PI3,5P2 probe; The LE association of protrudin depends on phosphoinositides. The cross-talk of ORP2 and protrudin may thus involve the transport or metabolism of phosphoinositides on LE.

Our findings suggest that ECs abundantly employ MCSs for their physiologic functions. ORP2 not only regulates neutral lipid metabolism at ER-LD junctions but also angiogenesis in HUVECs. Likewise, protrudin is not only active in neurite outgrowth but also in angiogenic tube formation. The work generates new information on the molecular machineries governing angiogenesis, and will provide potential new therapeutic targets for the treatment of cardiovascular diseases and cancer.

Low LDL-cholesterol, genetic variation, and risk of Alzheimer's dementia and Parkinson's disease

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Objectives: Low levels of LDL-cholesterol due to statin use or inhibition of PCSK9 might be associated with increased risk of Alzheimer's dementia and Parkinson's disease. We tested the hypothesis that low LDL-cholesterol due to genetic variation in PCSK9 and HMGCR genes, involved in LDL-cholesterol metabolism and biosynthesis, respectively, is associated with high risk of Alzheimer's dementia, vascular dementia, any dementia, and Parkinson's disease in the general population.

Methods: We examined observational associations of low plasma LDL-cholesterol with risk of disease; genetic variants with low plasma LDL-cholesterol levels; and genetic variants with risk of disease as an indication of a causal effect of low LDL-cholesterol. For this purpose we used a Mendelian randomization design in 111,194 individuals from two Danish general population studies, the Copenhagen General Population Study and the Copenhagen City Heart Study.

Results: In observational analyses, the multifactorially adjusted hazard ratio for Parkinson's disease in individuals with an LDL-cholesterol<1.8mmol/L versus ≥4.0mmol/L was 1.70(1.03-2.79), while the corresponding hazard ratios for Alzheimer's dementia, vascular dementia, or any dementia did not differ from 1.0. PCSK9 and HMGCR variants combined were associated with 9.3% lower LDL-cholesterol. In genetic, causal analyses adjusted for age, sex, and year of birth, a 1mmol/L lower LDL-cholesterol had risk ratios of 0.57(0.27-1.17) for Alzheimer's dementia, of 0.81(0.34-1.89) for vascular dementia, of 0.66(0.34-1.26) for any dementia, and of 1.02(0.26-4.00) for Parkinson's disease. Summary level data accounting for effect of weak instruments from the International Genomics of Alzheimer's Project gave a risk ratio for Alzheimer's dementia of 4.66(0.57-38) for 26 PCSK9 and HMGCR variants, and of 0.97(0.91-1.02) for 380 LDL-cholesterol lowering variants.

Conclusion: Low LDL-cholesterol levels due to genetic variants had no causal effect on high risk of Alzheimer's dementia, vascular dementia, any dementia, or Parkinson's disease.

Poster presentations – Abstracts

Inflammation and Vascular Biology

SESSION I

The effect of adipose tissue products on monocyte adhesivity to the endothelium

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Adhesion of monocytes to the endothelium is one of the initial steps in the development of atherosclerosis. Adipose tissue (AT) secretes a number of cytokines which may affect the endothelium; in particular, it may stimulate monocyte adhesion and infiltration which initiate and accelerate atherosclerosis development.

Adipose tissue-conditioned media (ATCM) were prepared by culturing visceral adipose tissue obtained from living kidney donors. The effect of ATCM on endothelial cells (ECs) was analyzed by changes in adhesiveness of calcein AM-labelled monocytes to the affected ECs using ATCM and quantified by intensity of fluorescence of the adhered monocytes. Concentrations of cytokines in ATCM were measured by Luminex assay.

Increasing concentrations of IL-1β, TNF-α, MCP-1, IL-10, and RANTES (CCL5) gradually increases monocyte adhesivity to ECs (correlation coefficients $r = 0.69$, $p < 0.0001$; $r=0.45$, $p<0.0001$; $r=0.45$, $p<0.0001$; $r=0.44$, $p<0.0001$ and $r=0.27$; $p=0.003$, respectively). No such relationship was found for IL-4, IL-5 and CXCL5. Cytokine concentrations in ATCM were not related to individual atherosclerosis risk factors (age, sex, menopause, and BMI).

Adhesion of monocytes to ECs was significantly influenced by IL-1β, TNF-α, MCP-1, IL-10 and RANTES released from adipose tissue. The concentrations of these cytokines in ATCM were not related to individual BMI and other risk factors.

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Participate in the young investigator´s award.

Altered leukocyte distribution under hypercholesterolemia: a cross-sectional study in children with familial hypercholesterolemia

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Background and aims: Children with familial hypercholesterolemia (FH) have elevated LDL cholesterol from first year of life, and represent a model of early-stage atherosclerosis. Data suggest that adults with FH have alterations in circulating monocyte subpopulations towards a more pro-inflammatory phenotype, but it is not known whether FH children have similar perturbations. Also, there are no data on the distribution of lymphocyte subpopulations in FH children. The objective of the present study was to characterize the distributions of circulating monocyte and lymphocyte subpopulations in children with FH and healthy, normocholesterolemic children.

Methods: Using flow cytometry analysis, we analyzed whole blood B- and T-cell subpopulations and monocyte subpopulations in FH (n = 23) and healthy (n = 20) children. Also, we measured serum markers of leukocyte and endothelial cell activation using EIA.

Results: We found that FH children had monocytosis as well as a shift in the monocyte subpopulations. This shift was characterized by higher circulating pro-inflammatory and non-classical monocytes, and lower levels of classical monocytes, and seemed to be present only in FH children with low HDL cholesterol (HDL-C, below 1.3 mmol/L). Additionally, monocytes expressing CD18 and serum E-selectin were higher in FH children, in particular FH children with low HDL-C.

Conclusions: FH children with low HDL-C had monocytosis as well as a shift in the monocyte subpopulations towards a more pro-inflammatory phenotype. Our results suggest activation of monocytes at a very early stage of atherosclerosis in humans.

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Detection of pyruvate kinase isoform M2 (PKM2) in arterial wall cells, intact arteries and human atherosclerotic lesions

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Pyruvate kinase, the final rate-limiting enzyme in glycolysis, catalyzes phosphate transfer from phosphoenolpyruvate to ADP yielding pyruvate and ATP. This enzyme is widely distributed and exists in multiple isoforms, with the high activity PKM1 isoform predominating in many tissues. The PKM2 isoform is associated with the Warburg effect, and switching of glucose metabolism from the tricarboxylic acid cycle to the pentose phosphate pathway, with this conversion ascribed to an increased demand for biosynthetic materials in cells undergoing rapid division. PKM2 is expressed in embryonic tissues, but is replaced by the non-allosterically-regulated PKM1 isoform, during differentiation. In tumours reversion to PKM2 occurs, but recent data suggest that this isoform may persist in other tissues. In the current study, we show that PKM2 is present in primary human coronary arterial endothelial and smooth muscle cells, in normal mammary artery specimens and in carotid and aortic atherosclerotic lesion samples by use of activity measurements, activators and inhibitors, Western blotting and mass spectrometry. The data obtained is consistent with the presence of PMK2 (or a putative PKM3 isoform) in the arterial wall cells, and suggests that vascular cells have the capacity to regulate glucose metabolism in an allosteric manner, and divert energy use to the generation of NADPH and reducing equivalents, that may protect against damage and stress, as well as a synthetic / proliferative phenotype.

Vascular stiffening: mechanisms and therapeutic options in a mouse model of Hutchinson Gilford Progeria Syndrome.

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We aimed to study the mechanisms underlying vessel stiffening and dysfunction in a mouse model of premature aging. Hutchinson-Gilford progeria syndrome (HGPS) is a rare human genetic disorder caused by the expression of progerin, a mutant form of nuclear lamin A which is also expressed at low levels in cells from aged non-HGPS individuals. Premature aging in HGPS is associated with cardiovascular alterations leading to premature death at an average age of 14.6 yr. HGPS patients exhibit vessel stiffening, loss of vascular smooth muscle cells (VSMCs), arterial fibrosis and thickening. Here, we analyzed vascular structure and mechanics in mutant LmnaG609G/G609G mice with ubiquitous progerin expression. We also generated LmnaLCS/LCSTie2Cre+/tg and LmnaLCS/LCSSM22Cre+/tg mice, which express progerin specifically in endothelial cells (ECs) and in VSMCs, respectively.

We found vessel stiffening and inward remodeling in vessels of LmnaG609G/G609G and LmnaLCS/LCSSM22Cre+/tg, but not in LmnaLCS/LCSTie2Cre+/tg. LmnaG609G/G609G and LmnaLCS/LCSSM22Cre+/tg mice also displayed severe impairment in aortic contractility, which were not seen in LmnaLCS/LCSTie2Cre+/tg. These structural alterations in aorta were associated with decreased amount of muscular tissue as imaged by histology, without decreasing the amount of cells in the vessel wall. These arteries also displayed increased collagen deposition and decreased transversal waving of elastin layers. Wire miography studies revealed that collagen alterations are the main contributor to vessel stiffening in progeroid mice. Sodium nitrite in drinking water prevented stiffening and inward remodeling, improved aortic contractility, and reduced the alterations in elastin structure.

In summary, progeroid mice develop arterial stiffening and contractile impairment mainly due to VSMC alterations. These changes are mechanistically associated with alterations in collagen structure in the vessel wall, which secondarily affect elastin transversal waving and were partially reversed by nitrite dietary supplementation.

Participate in the young investigator´s award.

Targeting B cells with an agonistic BTLA antibody in atherosclerosis

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Cardiovascular diseases remain a major global health issue. The main underlying cause is atherosclerosis, which has been established as a chronic autoimmune-like inflammatory disease.

Although we have made significant improvements in treating cardiovascular diseases in the last decades, there is still an urgent need for novel therapeutic options that intervene in the immune system. Nowadays, both B cell depletion and modulating critical co-stimulatory pathways are approaches that are pursued to treat atherosclerosis. A very promising option that might harness the effects of both of these methods is to target the co-inhibitory molecule BTLA. It is constitutively expressed on B cells, with very low expression on T cells. Importantly, it is mainly expressed by the atherogenic follicular B cells, with only moderate to no expression on the atheroprotective B1 and marginal zone B cells. Stimulation of this pathway leads to an inhibitory signal, rendering B cells anergic or apoptotic and incapable of responding to activating stimuli. Since earlier work has also shown that BTLA KO mice have hyperactive B cells, we proposed that activation of BTLA on B cells by using an agonistic antibody would lead to the inhibition and possibly a reduction of follicular B cells and in doing so, reduce the development of atherosclerosis in LDLR KO mice.

To investigate this, we fed LDLR KO mice a Western type diet and injected them twice a week for 6 weeks in total with the agonistic antibody. We found that this resulted in a dramatic decrease in the numbers and percentages of circulating and splenic B cells. Indeed, only the BTLA-expressing follicular B cells were affected, while other B cell subtypes (e.g. marginal zone, B1 and Breg cells) were relatively increased. In addition we found significant differences in CD4 and CD8 subsets, all pointing towards a less inflammatory state which is usually associated with less atherosclerosis.

Participate in the young investigator´s award.

Role of macrophages in the production of extracellular traps during chronic inflammation – a pathway to lesion development in atherosclerosis?

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Chronic inflammation is the major driving force of atherosclerosis, with the infiltration of leukocytes, particularly neutrophils and macrophages, to the vessel wall intricately linked with lesion development and destabilisation. Neutrophils release extracellular traps (ETs), following a novel mode of cell death called ETosis, which is distinct from apoptosis or necrosis. Structurally, ETs are a mesh of DNA, histones, myeloperoxidase (MPO) and proteolytic enzymes, which trap and kill bacteria. ETs have also been implicated in playing a causal role in the development of atherosclerosis, and are present in human lesions. However, it is not clear how ETs are formed in lesions or how they promote the development of atherosclerosis. The MPO-derived oxidant hypochlorous acid (HOCl) plays a crucial role in lesion development and triggers ET release from neutrophils. In this study, we show for the first time that exposure of human monocyte-derived macrophages (HMDM) to pathophysiological levels of HOCl results in the dose-dependent extrusion of histones and DNA into the cellular supernatant. Further evidence for ET release was obtained by staining HMDM with the cell impermeable fluorescent DNA stain Sytox Green and scanning electron microscopy imaging. Exposure of the HMDM to other pro-inflammatory stimuli, including TNFα and interleukin-8 (IL-8) also resulted in ET release. In each case, ET release was independent of MAP kinase signaling, NADPH oxidase activity or peptidylarginine deiminase (PAD)-mediated citrullination, which is in contrast to the mechanism of ET release from neutrophils. Exposure of HMDM to HOCl also led to the sustained cytosolic accumulation of Ca2+, activation of transcription factors (Egr-1, cJun and NFkB), and increased expression of cytokines/chemokines including IL-8, MCP-1 and TNFα. Taken together, these data indicate a novel role for macrophages in mediating ET formation and propagation of the inflammatory response evident in atherosclerosis.

Effects of Imiquimod-induced psoriasis-like lesions and digoxin in low density lipoprotein receptor-deficient mice

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Background: Psoriasis is a chronic inflammatory skin disorder associated with several comorbidities including atherosclerosis. Activation of T helper (Th)1 and Th17 cells are seen in both psoriasis and atherosclerosis. Topical application of imiquimod (IMQ), a ligand of toll-like receptor 7, is an established mouse model of psoriasis-like skin inflammation that is dependent on the interleukin (IL)-17/IL-23 axis. Digoxin inhibits the master transcription factor of Th17 differentiation, retinoid acid receptor-related orphan nuclear receptor γt, and has been shown to attenuate other IL-17-dependent pathologies in mice.

Aim: To establish whether IMQ-induced psoriasis-like skin inflammation accelerates atherosclerosis in low density lipoprotein receptor-deficient (LDLr-/-) mice, and investigate whether digoxin can reduce both diseases.

Results and discussion: Application of IMQ or vehicle on both ears for three repeated cycles of five days with two-three weeks of pause in-between increased ear thickness, keratinocyte proliferation, and skin accumulation of CD3+ T cells. Topical IMQ also affected the mice systemically with induction of splenomegaly as well as increased plasma IL-17A and serum amyloid A levels. IMQ slightly reduced atherosclerosis in the aortic arch but did not affect atherosclerotic plaque composition in the aortic root. Digoxin significantly reduced the ear thickening, but showed divergent effects on IMQ-induced systemic inflammation and did not affect aortic arch atherosclerosis or plaque composition.

Conclusion: Long-term treatment with IMQ induces psoriasis-like skin lesions but does not accelerate atherosclerosis in LDLr-/-mice. Hence, other models need to be developed to explore the link between atherosclerosis and psoriasis observed in humans.

Quantitative analysis of Monocyte subpopulations and CD3+ iNKT cells in patients with atherosclerotic plaque and dyslipidemia

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Monocytes and Lymphocytes play central roles in vascular inflammatory diseases. The purpose of this study was to investigate the relationship between the number of Lymphocytes express CD3 iNKT, The CD14 (+)Low/CD16Negative as Primary Peripheral Blood Monocytes, the mature circulating monocyte subpopulations, such as (CD14++(Bright) / CD16−(Low)) referred to as classical monocytes, intermediate monocytes (CD14++(Bright)/CD16+(Low)) and non-classical monocytes (CD14+(Low)/ CD16 ++ (Bright))(NCM), with pre-clinical indicator of atherosclerosis Carotid Intima-media thickness, Arterial stiffness, Flow Mediated Dilation, Area of Visceral Fat and Non Alcoholic Fatty Liver Disease In patients with dyslipidemia and atherosclerotic plaque.

We included 31 subjects in the control group and 43 dyslipidemic patients without plaque, and 19 patients diagnosed with plaque. In Our study we found the number of NCM, significantly increased in patients diagnosed with plaque, compared to the controls and Dyslipidemic participants (11358, ± 5492vs Control: 8370±3811 and Dyslipedimic: 8504±4511; P Value<0,05). We also showed that NCM positively correlated with the amount of Area of Visceral Fat and negatively to FMD and Stiffness. Furthermore, There was a meaningful increase in the number of Primary Peripheral Blood Monocytes in Dyslipidemic patients, in comparison to the control and Plaque (17872±20814 vs

Control:7401±10029 and Plaque:4473±5541;P Value<0,05). Finally, We observed also a non-significant higher number of CD3 iNKT cells in patients with carotid plaque.

To sum up, circulating blood NCM may affect the vascular damages and can be used as a vascular damage predictor. Moreover we showed that dyslipidemia has an inhibitory inhibitory effect on monocytes differentiation. We understood still it needs further studies on the CD3 iNKT cells to be clearly warranted to justify the clinical relevance of its role in atherosclerosis.

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Uremia does not affect neointima formation in mice

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Objective: Atherosclerotic cardiovascular disease is a major complication of chronic kidney disease (CKD). CKD leads to uremia, which modulates the phenotype of aortic smooth muscle cells (SMCs). Phenotypic modulation of SMCs plays a key role in accelerating atherosclerosis. We investigated the hypothesis that uremia potentiates neointima formation in response to vascular injury in mice.
Approach and Results: Carotid wire injury was performed on C57Bl/6 wt and apolipoprotein E knockout mice two weeks after induction of uremia by 5/6 nephrectomy. Wire injury led to neointima formation and downregulation of genes encoding classical SMC markers (i.e., myocardin, alpha-smooth muscle actin, SM-22 alpha, and smooth muscle myosin heavy chain. Contrary to our expectations, uremia did not potentiate neointima formation, nor did it affect intimal lesion composition as judged from magnetic resonance imaging and histological analyses. Also, there was no effect of uremia on SMC marker gene expression in the injured carotid arteries, suggesting that there may be different effects of uremia on SMCs in different vascular beds.
Conclusion: Uremia does not accelerate neointima formation in response to wire injury of the carotid artery in mice.

Apolipoprotein E Deficiency Increases Remnant Lipoproteins and Accelerates Progressive Atherosclerosis in Yucatan Minipigs

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Background: Apolipoprotein E (APOE) mediates hepatic uptake of lipoproteins through the low-density lipoprotein receptor and low-density receptor-related protein 1. Defects of APOE causes familial dysbetalipoproteinemia in humans, characterized by accumulations of VLDL, IDL and chylomicron remnants and a higher risk of atherosclerotic disease. In mice, APOE-deficiency results in severe atherosclerosis, but it is unknown to what extent this is unique to mice, because APOE in mice has a more central role for lipoprotein clearance than in humans, primates and pigs. APOE also plays roles in inflammation and controlling smooth muscle cell (SMC) proliferation and migration.

Objective: To create a minipig model with targeted deletion of APOE and study the resulting dyslipidemia and atherosclerosis.

Methods and Results: By (i) recombinant adeno-associated virus mediated gene targeting in pig fibroblasts, (ii) somatic cell nuclear transfer and (iii) embryo transfer, APOE-deficient Yucatan minipigs were born. Minipigs with homozygous APOE deletions displayed increased plasma cholesterol and accumulation of APOB48-containing chylomicron remnants. Inflammatory markers haptoglobin and C-reactive proteins were not affected by the remnant lipoproteinemia. After 1 year on high-fat, high-cholesterol diet, homozygous APOE-deficient minipigs showed increased progressive atherosclerosis in atherosclerosis-prone vascular beds. Lesion formation in vascular beds dominated by xanthoma formation was not increased, suggesting a distinct effect on later plaque types. The acceleration of progressive atherosclerotic lesions could not be explained by loss of inhibitory effect of APOE on SMCs, which appeared to accumulate similarly in APOE-deficient and wildtype minipigs.

Conclusions: Targeted disruption of APOE in Yucatan minipigs causes remnant lipoproteinemia and accelerates progressive atherosclerosis.
Participate in the young investigator´s award.

The role of nrf2 in bone marrow derived macrophages in atherosclerosis

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Atherosclerosis is a disease of large arteries in which oxidative stress and inflammation have a critical role in pathogenesis. In the initial events of atherosclerosis, circulating monocytes migrate into subendothelial space, where they take up modified LDL and become foam cells that constitute the early fatty streak lesion.

The redox-activated transcription factor Nuclear factor E2-related factor 2 (Nrf2) protects cells from oxidative stress via regulation of antioxidant and anti-inflammatory genes. Its role in atherosclerosis is controversial, as it has differential cell-specific functions.

We have previously shown that Nrf2 deficiency in mouse peritoneal macrophages enhances foam cell formation and promotes the pro-inflammatory phenotype. To clarify the cell-type specific role of Nrf2 in atherogenesis, we studied further the mechanism by which Nrf2 affects macrophage lipid loading and cellular bioenergetics in murine bone marrow derived macrophages (BMDM). We show that similar to thioglycollate-elicited mouse peritoneal macrophages, Nrf2 deficiency increases the uptake of acetylated LDL in comparison to wild type controls in BMDM. Pro-inflammatory stimulus by LPS further increases the foam cell formation in both wild type and Nrf2-deficient cells. Additionally, anti-inflammatory stimulus by IL-4 abolishes the enhanced lipid accumulation in Nrf2 deficient foam cells. To define the mechanism by which Nrf2 affects macrophage lipid loading, we performed genome-wide gene expression profile by RNA sequencing. The preliminary results indicate that Nrf2 deficiency drastically influences the gene expression profile of lipid-laden macrophages. In addition, Nrf2 deficiency increases the mitochondrial respiration of macrophages assessed by simultaneous measurement of oxygen consumption and glycolysis (SeahorseBiosience), whereas lipid loading had no impact on oxygen consumption. In conclusion, we show that Nrf2 deficiency and pro-inflammatory stimuli enhance foam cell formation, indicating that Nrf2 has an athero-protective role in macrophages.

Deficiency of the hemoglobin scavenger receptor CD163 in macrophages exacerbates development of atherosclerosis in apolipoprotein E deficient mice

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Aim: The hemoglobin scavenger receptor CD163 is expressed specifically in monocytes and macrophages and upregulated expression of this receptor is one of the hallmarks in the macrophage switch to alternative activated M2 phenotypes in inflammation. We have now analyzed the effect of CD163 deficiency in different inflammatory mouse models. In this study, the development of age-induced atherosclerosis was investigated in apolipoprotein E (ApoE) and CD163 deficient mice.

Methods: CD163-/-ApoE-/-, ApoE-/-, CD163-/- and wildtype mice on standard diet were analyzed at age 33 weeks. Quantification of cellular cholesterol in atherosclerotic plaques was made using a Xenogen IVIS Imaging System. Paraclinical parameters were measured using Roche Hitachi Cobas 600 and standardized ELISA procedures. RT² Profiler™ PCR Arrays with 92 atherosclerosis biomarkers were designed for Fluidigm® BioMark™ gene expression analysis of the aorta.

Results: Aorta plaque development and plasma cholesterol levels were significantly increased in the CD163-/-ApoE-/- mice compared to ApoE-/- mice. Further, gene expression profiles of the aorta showed pronounced up-or down-regulation of a number of genes involved in development of atherosclerosis selectively in the CD163-/-ApoE-/- mice. Of notice, the interleukin 1 gene was up-regulated 114 fold. Further, the low density lipoprotein receptor gene was upregulated, whereas the peroxisome proliferator activated receptors alpha, delta and gamma were downregulated in the CD163-/-ApoE-/- mice.

Conclusion: Deficiency of CD163 in the ApoE-/- model exacerbates development of age-induced atherosclerosis and biochemical as well as gene expression analyses suggest that several important inflammatory mechanisms are affected as a result of CD163 deficiency.

Hematopoietic CARD9 deficiency reduces macrophage number in atherosclerotic plaques within a hyperglycemic atherosclerotic mouse model

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Aim: Diabetes enhances the risk for the development of cardiovascular disease (CVD). High glucose levels associate with enhanced macrophage numbers in atherosclerotic plaques, the major cause of CVD. It remains unclear by which mechanisms hyperglycemia may induce macrophage activation. Pattern recognition receptors (PPRs) on monocytes and macrophages translate danger signals (e.g. pathogens) into cellular responses, but can also be activated by endogenous compounds (e.g. free fatty acids). We propose that C-type Lectin receptors (CLRS) such as Dectin-2, as well as, CARD9, which is the adaptor signaling molecule for multiple CLRs, on plaque formation under hyperglycemic conditions.

Methods: Atherosclerosis prone LDLr-/- mice were transplanted with bone marrow cells of Dectin-2-/-, CARD9-/- or wild-type (WT) control mice. Hyperglycemia was induced by streptozotocin injections. All mice received high-cholesterol diet for 10 weeks to induce atherosclerosis. Plaque formation determined in aortic roots. Immune cell numbers in blood were measured by FACS analysis. qPCR was used to determine the inflammatory phenotype of the liver.

Results: Hematopoietic deficiency of CARD9 or Dectin-2 did not reduce plaque formation under hyperglycemic condition. However, Card9 deficiency reduced macrophages in the atherosclerotic plaques (33.3%, P=0.06). In addition to macrophages, CARD9 deficiency affected T-cell dynamics, as reflected by a reduced number of circulating cytotoxic T-cells (22.5%, $p<0.05$), and the reduced hepatic expression of T helper-17 (TH17) associated transcription factor (Rorc) ($p<0.01$). T-cell numbers in the atherosclerotic plaque are currently investigated.

Conclusion: CARD9, but nor Dectin-2 deficiency reduced macrophage numbers in atherosclerotic plaques in hyperglycemic conditions. This implies CARD9 signaling contributes to the inflammatory plaque phenotype in diabetes patients.

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Mitochondrial dysfunction prevents repolarization of inflammatory macrophages

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Macrophages determine the outcome of atherosclerosis by propagating inflammation, foam cell formation and necrotic core development. Yet, the pathways that regulate their atherogenic functions remain ill-defined. Macrophages are innate immune cells that adopt diverse activation states in response to their microenvironment. Editing macrophage activation is of high interest to dampen inflammatory diseases by promoting the repolarization of inflammatory (M1) macrophages to anti-inflammatory (M2) macrophages. Here, we found that mouse and human M1 macrophages completely failed to convert into M2 cells upon IL-4 exposure in vitro and in vivo. In sharp contrast, M2 macrophages were more plastic and readily repolarized into an inflammatory M1 state. We identified M1-associated inhibition of mitochondrial oxidative phosphorylation as the factor responsible for preventing M1→M2 repolarization. Inhibiting nitric oxide production, a key effector molecule in M1 cells, dampened the decline in mitochondrial function and improved metabolic and phenotypic reprogramming to M2 macrophages. Thus, inflammatory macrophage activation blunts oxidative phosphorylation, thereby preventing repolarization. Therapeutically restoring mitochondrial function might be useful to improve the reprogramming of inflammatory macrophages into anti-inflammatory cells to control cardiovascular disease.

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Participate in the young investigator´s award.

HDL deficiency abrogates lipid lowering-induced plaque stabilization in apolipoprotein E knockout mice

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Reversal of hypercholesterolemia results in the disappearance of macrophages from existing (advanced) atherosclerotic lesions. We anticipate that an improved balance of cholesterol efflux over influx translates into a block of foam cell formation and a concomitant restored migration of macrophages from atherosclerotic lesions. High-density lipoprotein (HDL) facilitates macrophage cholesterol efflux. Here, we investigated the contribution of HDL to the lipid lowering-induced removal of plaque macrophages and the associated lesion stabilization. Hereto, 14 weeks-old apolipoprotein E (APOE) knockout mice were transplanted with APOE containing wild-type bone marrow to reverse hypercholesterolemia. Subsequently, mice were fed either a regular chow diet (n=16) or a chow diet containing the HDL lowering drug probucol (0.025%; n=18) for 9 weeks. Chow-fed animals displayed 3-fold lower ($p<0.005$) plasma total cholesterol levels resulting from a decreased in VLDL/LDL levels. Due to an additional >80% depletion of HDL-cholesterol ($p<0.05$ vs chow), total cholesterol values were 5-fold lower ($p<0.005$) in mice challenged with probucol. No progression of atherosclerotic lesions was observed in the regular chow diet group (1.2x105 µm2) as compared to mice sacrificed before the bone marrow transplantation (1.0x105 µm2). Lipid lowering was associated with a 3-fold decrease ($P<0.05$) in the macrophage over collagen ratio, indicative of the presence of more stable lesions. In contrast, HDL depleted mice did not show stabilization but rather lesion progression as evident from the 1.9-fold ($P<0.01$) increase in lesion size. In line with a less stable plaque phenotype, probucol-treated mice displayed a 2-fold ($p<0.05$) higher macrophage over collagen ratio as compared to chow-fed controls.

In conclusion, we have shown that HDL deficiency abrogates lipid lowering-induced plaque macrophage removal resulting in atherosclerotic lesion destabilization and progression.

Poster presentations – Abstracts

Cardiovascular disease

SESSION II

Relationship between microfibrillar-associated protein 4 (MFAP4) and vascular patency in lower extremity peripheral artery disease (PAD)

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Background Microfibrillar-associated protein 4 (MFAP4) is a matricellular glycoprotein that co-localizes with elastic fibers and is highly expressed in the heart, vessels and lungs of humans. Symptomatic peripheral artery disease (PAD) is an atherosclerotic arterial occlusive disease affecting the lower extremities with severer leg pains. Most patients are undiagnosed and treatment not effective. The aim is to investigate if the serum level of MFAP4 in patients with PAD undergoing reconstructive surgery, could be used as a biomarker for the development or progression of symptomatic PAD.

Methods A total of 507 patients with intermittent claudication (IC) or critical lower-extremity ischemia (CLI) were enrolled prospectively. The serum level of MFAP4 was measured by enzyme-linked immunosorbent assay (ELISA) in blood samples from 343 patients. Hazard ratios of the variables MFAP4, gender age diabetes, hypertension, BMI, stroke and cases of CLI/IC were obtained with survival analysis.

Results Patients with high levels of MFAP4 in serum had a high risk of death than patients with a low level of serum MFAP4. High levels of MFAP4 were associated with an increase in the need for vascular reconstructive surgery in patients with PAD compared with the control group. Low levels of MFAP4 were associated with increased risk of having an occlusion of the reconstructed vessel within the observation period.

Conclusions MFAP4 can be used as a predictor for the need for a vascular reconstructive surgery and for occlusion of reconstructed vessels in PAD patients.

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Elevated Lp(a) levels strongly increase the risk for cardiovascular disease in patients with familial hypercholesterolemia.

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Background: Several studies have suggested that elevated levels of Lp(a) is a strong and independent risk factor for coronary heart disease (CHD) in the general population. We and others have previously demonstrated that high Lp(a) levels is a CHD risk factor in familial hypercholesterolemia (FH) patients. This may suggest that high Lp(a) levels leads to a more aggressive phenotype in presence of a high LDL-cholesterol (LDL-C).

Aim: In patients with FH, to investigate if CHD was more prevalent in patients with Lp(a) ≥ 900 mg/L than in those with Lp(a) levels < 900 mg/L despite otherwise similar cholesterol burden.

Methods: Retrospective collection of data from medical charts of patients with FH followed at lipid clinics in Norway.

Results: All data are presented as mean (standard deviation) unless otherwise stated. In total, 599 adult FH patients with Lp(a) measurements were included in the study. FH patients with Lp(a) levels < 900 mg/L (n=503) was compared to FH patients with Lp(a) ≥ 900 mg/L (n=96). The two groups were otherwise similar in cholesterol burden in terms of age of FH diagnosis (30.2 [15.7] vs. 29.6 [15.2] years, P=0.755), age at start of lipid-lowering treatment (31.3 [12.4] vs. 33.2 [12.2] years, P=0.266), First visit LDL-C (5.4 [1.9] vs. 5.1 [2.1] mmol/L, P=0.286) and on-treatment LDL-C (3.4 [1.3] vs. 3.3 [1.2] mmol/L, P=0.654). The Lp(a) < 900 group had Lp(a) levels of median 195 (range 10-894) mg/L, and the Lp(a) > 900 group had levels of median 1190 (range 900-3180) mg/L, P<0.001. The Lp(a) ≥ 900 group had significantly higher prevalence of CHD (30.2 %) compared with the Lp(a) < 900 group (14.3 %), P<0.001. CHD was defined as clinical diagnosis of angina pectoris (16.7% vs. 7.8%; P< 0.010), and myocardial infarction (13.5% vs. 6.6%; P<0.05), in the Lp(a) ≥ 900 and Lp(a) < 900 group, respectively.

Conclusion: Elevated Lp(a) levels severely aggravates the FH phenotype by increasing the prevalence of CHD. This may suggest that high Lp(a) is more important in presence of a high LDL-C.

Characterization of the antibody immune response in atherosclerosis

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Atherosclerosis is a chronic inflammatory disease with contribution of the adaptive immune response. Given the therapeutic value of antibodies, understanding the contribution of B cells and antibody production during atherosclerosis holds great clinical interest. Antibodies against atherosclerosis model antigens, such as oxLDL, MDA-LDL and hsp60, are found in patients and atherogenic animal models and immunization with these antigens is atheroprotective. However, the precise functional role of B cells and the antibody-mediated immune response in the disease remains controversial. AIM: 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 functional impact.

METHODS: Low density lipoprotein-receptor deficient mice (LDLR-/-) fed with high fat diet (HFD) is used as a pro-atherogenic model for: i) analysis of the humoral response by flow cytometry ii) evaluation of antibodies against atherosclerosis antigens by ELISA, iii) characterization of antibodies generated by single cell sorting, sequencing and cloning of heavy and light chains and iv) functional analysis of the cloned antibodies by expression, ELISA, immunofluorescence and in vivo studies. RESULTS: We found that the proportion of germinal center, plasma and memory B cells was significantly increased in the spleen of LDLR-/- HFD, revealing a B cell response in atherosclerosis. This was accompanied by a progressive accumulation of antibodies against prototypic atherosclerosis antigens in the serum. Single cell sequencing and expression of immunoglobulins from atherogenic mice showed a distinct atherosclerosis antibody repertoire, with an enrichment of IgM+, mutated plasma cells. This allowed the identification of a collection of germinal center-derived expanded clones in LDLR-/- HFD mice that show reactivity against atherosclerosis-associated antigens and atheroma plaque reactivity. Their functional characterization will provided direct evidences for their atherogenic or protective properties.

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Treat to target Familial Hypercholesterolemia: a prospective study on effects from aggressive lipid lowering treatment in an outpatient setting in patients with Familial hypercholesterolemia

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Background: Patients with familial hypercholesterolemia (FH) has high risk of premature cardiovascular disease (CVD). Early initiation of lipid lowering treatment (LLT) and control of CVD risk factors is crucial to reduce the risk. Aim: Describe the effects of aggressive LLT with potent statins and in addition to ezetimibe on free-living FH patients for up to 9 years. Primary study outcomes were lipid levels, glucose parameters and anthropometry. Secondary outcomes were presence of risk factors in CVD patients versus non-CVD patients. Methods: In 2006, 357 adult heterozygous FH patients attended visit 1 (V1). 332 patients conducted visit 2 one year after. During 2014-16 visit 3 (V3) was conducted for 156 patients reported here. At all visits data was collected through an ordinary medical examination and by the medical journals.

Results: The clinical FH diagnosis was set at a mean age of 33.9 years. Total cholesterol (TC) and LDL-cholesterol (LDL-C) improved from the pre-treatment levels to V1 and further to V3. Despite aggressive LLT, only 40% achieved LDL-C <2.5 mmol/L at V3. Further, only 6.3% of 79 patients with the more stringent LDL-C goal of <1.8 mmol/L reached it. Approximately 30% had developed metabolic syndrome (MetS) at V3. Compared to non-CVD patients, CVD patients were older at V3 and at FH-diagnosis, had higher levels of pre-treatment TC, higher glucose parameters, waist circumference and TG at V3 and a higher proportion with MetS, males and former smokers.

Conclusion: We observed changes towards a more favorable cholesterol profile. Almost no FH patients could reach a treatment target of LDL-C <1.8 mmol/L in this time period prior to PCSK9-inhibitors becoming available. Importantly, MetS developed over time in many FH patients despite regular consultations with specialized health care professionals. Patients with CVD had a higher proportion of MetS and former smokers and indication of a higher cholesterol burden due to late diagnosis of FH.

Maternal cholesterol levels may predict ldl cholesterol levels in children with familial hypercholesterolemia

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Background and Aims: Familial hypercholesterolemia (FH) is associated with increased risk of premature cardiovascular disease (CVD). Children with FH, in contrast to adult FH patients, are less influenced by lifestyle factors, hence they represent a unique model system to investigate which factors that may predict their lipid profile. The aim of this study was to investigate the impact of maternal lipid profile and family CVD history on the offspring's pre-treated lipid profile.

Methods and results: Children with FH (n = 1059) aged between 1 and 19 years were included. Information about inheritance, family history of CVD, mutation type and pretreated lipid profile was retrieved from the medical records. Analyses were performed using linear and generalized mixed models, with random effects to account for the dependency between siblings. In a subgroup of children with maternal FH inheritance, untreated total cholesterol levels of their FH mothers (n=178, i.e. 36% of children with maternal FH inheritance) were available. After adjusting for LDLR mutation type we found that when total cholesterol levels were above mean (9.61 mmol/L) in FH mothers, FH children had higher LDL cholesterol compared to FH children with FH mothers with total cholesterol levels below mean. However, there were no significant differences in the FH offspring's lipid profile when investigating the effect of CVD in FH mother, FH father or FH grandparent after adjusting for the LDLR mutation type.

Conclusion: Untreated maternal total cholesterol levels may predict LDL cholesterol levels in non-statin treated children with FH. Family CVD history did not seem to have an effect on the FH children's lipid profile.

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Inhibition of immunoproteasomal subunit LMP7 attenuates atherosclerosis.

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Introduction: The immunoproteasome was found to be important for the generation of CD8 T-cell epitopes. Now it is known that the immunoproteasome influences more processes, like cytokine production, DC activation,T-cell differentiation and more recently it was reported that triglyceride uptake is lowered in the absence of LMP7. Therefore specific inhibition of LMP7, a subunit of the immunoproteasome, with PR-957 (ONX-0914) could be beneficial for atherosclerosis.

Methods: Female LDLr KO mice (n=15) were put on western type diet for 7 weeks and received 10mg/kg PR-957 or control injections 3 times per week. Aortic root lesion size was assessed with ORO staining, macrophage content of the lesions was assessed with MOMA-2. FACS analysis was performed on multiple organs to determine the effect of LMP7 inhibition on immune cells.

Results: PR-957 reduced body weight, triglyceride and glucose levels in the blood, but did not affect cholesterol levels. Aortic root lesion size was reduced by 30%, however no effect was observed on the macrophage content of the lesions. Lesion size did not correlate with triglyceride levels. A significant increase in neutrophils was seen in the blood, possibly caused by lowered CD62L. The percentage of DCs was lowered in lymphoid tissues and lower expression of CD86 and MHC-II was found on these cells. The percentage of T-cells was not significantly altered, however more naive CD4 T-cells, and less effector and memory T-cells were observed. Reduction of body weight could not be explained by reduced food intake, enhanced metabolism, or differences in faecal content.

Conclusion: LMP7 inhibition decreases atherosclerosis in LDLr KO mice fed a WTD, improves parameters of metabolic syndrome, and reduces inflammatory status. The immunoproteasome plays a central role in atherosclerosis. How inhibition of the immunoproteasome reduces body weight remains to be investigated.

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A microenvironment-specific protective role for CD8+ T cells in advanced atherosclerosis

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Atherosclerosis is a chronic, inflammatory disorder of the arterial wall that causes millions of deaths worldwide each year. CD8+ T lymphocytes may play an important role in atherogenesis, as they represent up to 50% of all lymphocytes in advanced human plaques. However, the contribution of this lymphocyte subset to plaque progression and stability remains debated. In this study, we investigated the role of CD8+ T cells in advanced atherosclerosis using CD8 depletion.

To investigate the effect of CD8 depletion on advanced atherosclerosis in vivo, LDLr-/- mice were fed a Western-type diet (WTD) for 10 weeks to establish initial lesions, after which they were fed WTD for another six weeks combined with twice weekly i.p. injections of either CD8α depleting antibody or isotype control antibody.

Depletion of CD8+ T cells in advanced atherosclerosis altered the lesion composition and stability, although lesion size was not affected. Collagen content in the trivalve area was significantly lower in CD8 depleted mice compared to the isotype-treated group (18% vs. 22%, P=0.02), whereas the necrotic core area was increased upon CD8 depletion compared to controls (27% vs. 19%, P=0.02).

Mechanistically, we observed that the lesions of the CD8 depleted group contained more CD4+ T cells per lesion area than lesions of isotype-treated mice (0.98 cells/mm2 vs. 0.50 cells/mm2, P=0.02).

Moreover, CD8 depletion affected the CD4+ T cell subsets in the aortic lesions, increasing the T-bet/GATA3 ratio (CD8: 1.89, Iso: 0.88, P=0.03), whereas this ratio was decreased in CD4 T+ cells derived from the spleen (CD8: 1.13, Iso: 2.24, P=0.07).

Together, these findings suggest a protective role of CD8+ T cells in advanced atherosclerosis by a micro-environment specific skewing of CD4+ T cells, which results in increased plaque stability.

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Poster presentations – Abstracts

Lipoproteins and Lipid Transport

SESSION III

Activation of lipoprotein lipase and endothelial lipase in thermogenic adipocytes promotes HDL turnover and reverse cholesterol transport:

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Activity of brown adipose tissue (BAT), the primary organ for heat production in small mammals and humans, reduces triglyceride levels via a lipoprotein lipase (LPL) dependent process, decreases remnant cholesterol levels and protects from atherosclerosis. However, the relevance of BAT for the metabolism of high-density lipoprotein (HDL) remains unknown. Therefore, we investigated the impact of BAT activation on HDL concentration, composition and function. By high-resolution, full scan mass spectrometry the composition of isolated lipoproteins from cold-activated wild-type and adipocyte-specific LPL knock-out (aLKO) mice was measured. Metabolic turnover studies were performed in BAT-activated aLKO and endothelial lipase knock-out (eLKO) mice to analyze clearance and cholesterol uptake of radiolabeled HDL into different tissues. Injection of cholesterol-labeled macrophages allowed determination of in vivo reverse cholesterol transport. After BAT activation, HDL present a characteristic lipidomic fingerprint which is accompanied with an increased macrophage-to-feces cholesterol transport. aLKO mice, however, present derogated HDL remodeling and diminished turnover. Furthermore, lack of endothelial lipase also diminishes cholesterol clearance from plasma and abates selective uptake into BAT, normally seen after BAT activation. In summary, the atheroprotective functions of HDL by our findings can be defined by highly active thermogenic adipocytes which regulate metabolic flux systemically.

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Novel ApoA-I derived peptides with anti-diabetic and anti-CVD effect

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Background and Aim: Type 2 diabetes (T2D) patients have an increased risk of cardiovascular disease (CVD) and macrovascular complications such as atherosclerosis are currently a major cause of death in T2D. Therefore, there is an unmet need for new anti-diabetic treatments with meaningful anti-CVD effect. Apolipoprotein A-I (ApoA-I) is the main protein component of high density lipoprotein (HDL) and is a regulator of cholesterol transport. Administration of ApoA-I protein has been shown to reduce atherosclerosis and improve glycemic control in rodent models. A short peptide retaining the beneficial functions of full length ApoA-I is desirable due to lower cost and easier production. Therefore, the active region of ApoA-I has been identified and novel peptides manufactured from this region. The aim of this study is to investigate the anti-diabetic and anti-atherosclerotic activities of these peptides.

Methods and Results: Acute administration of ApoA-I peptides improved glucose tolerance in Diet-Induced Obesity and LepR-/- (db/db) mouse models of T2D. The mechanisms of this effect are being investigated further using in vitro models of glucose-stimulated insulin secretion (INS1E cells) and glucose transport in skeletal muscles (L6 cells). The activity of the peptides in reverse cholesterol efflux was tested. They were able to form rHDL particles in a benchtop assay as expected. They also showed significant cholesterol efflux activity from J774 macrophages. To discover if this will translate into anti-atherosclerotic activity in vivo, we are currently conducting studies using ApoE-/- mice that develop atherosclerotic plaques.

Conclusions: These data demonstrate that ApoA-I derived peptides have anti-diabetic activity in vivo and retain cholesterol transport function in vitro. Thus, these ApoA-derived peptides are interesting clinical candidates for the treatment of T2D patients.

Real-time imaging of vascular brown adipose tissue lipoprotein lipase activity

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Aim: Lipoprotein lipase (LPL) liberates fatty acids and glycerol from the core of triglyceride-rich lipoproteins (TRLs). Delayed postprandial clearance of TRLs is associated with atherosclerosis. Measurement of the effective tissue LPL activity has proven difficult, as not only the intrinsic activity of the enzyme but also the intravascular localization is important for proper TRL processing. We developed an ultrafast confocal in vivo microscopy-based approach for visualizing LPL action in capillaries.

Method:
The method is based on increased fluorescence resulting from LPL-mediated liberation of resorufin from recombinant TRLs containing resorufin-oleate (R-TRL). R-TRL can be used for determining LPL activity in vitro, and for visualizing LPL action in vivo by intravital confocal microscopy.

Results: Enzymatic activity determined by the R-TRL method could be inhibited by the lipase inhibitor tetrahydrolipstatin. It was higher in post-heparin than in control plasma, and elevated in brown adipose tissue (BAT) lysates of cold-exposed vs warm-housed mice, demonstrating specificity of the assay for LPL. This was further confirmed by the observation that LPL activity in BAT lysates from adipocyte-specific LPL knock out mice (aLKO) was markedly decreased compared to wild type (WT) mice. To map the exact anatomical site of LPL activity in BAT, we injected R-TRL intravenously and performed intravital microscopy. The association of R-TRL with capillaries and the release of resorufin were profoundly increased in cold-exposed vs. control mice, and diminished in aLKO vs. WT mice. LPL action in cold-activated BAT was found to be a discontinuous process, showing pronounced fluctuation over time. The flucuations could be prevented by the vasodilator acepromazin.

Conclusion: We developed a novel specific method for determining LPL activity in vitro and visualizing LPL action in an ultrafast manner. Our data identify blood flow as a regulator of LPL substrate availability in cold-activated BAT.

Protein destabilization controls LPL activity and regulation

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Lipoprotein lipase (LPL) mediates the rate-limiting step of intravascular lipolysis, thus controlling the flux of triglycerides from VLDL and chylomicron particles to the underlying tissue. In LPL, several mutations that correlate with increased plasma triglyceride levels, or even development of chylomicronemia, have been identified. In pursuit of understanding the underlying molecular mechanisms of LPL regulation, we have employed highly purified proteins combined with advanced biophysical techniques such as SPR, CD, HDX-MS and SAXS to investigate the molecular structure/function relationship that governs LPL activity. This approach has enabled us to show that LPL undergoes spontaneous unfolding of the catalytic domain, thus losing the hydrolase activity. We have further shown that the mechanism of spontaneous LPL inactivation is identical to the molecular mechanism of how the LPL inhibitor, ANGPTL4, mediates LPL inhibition, by catalyzing protein unfolding. GWAS studies have identified a common polymorphic variant of ANGPTL4 (E40K), which results in lower plasma triglyceride levels and a decreased risk of developing coronary artery disease.

We have shown that the E40K mutation in ANGPTL4 destabilizes the secondary structure of ANGPTL4, and therefore decreases ANGPTL4's unfolding activity towards LPL. Additionally, we have shown that the small LPL-transporter protein GPIHBP1, which also anchors LPL to the surface of the capillary lumen, can rescue LPL from both spontaneous unfolding and ANGPTL4-catalyzed unfolding. The protective effects of GPIHBP1 were found to originate from cooperativity between the folded LU-domain and a highly disordered negative domain of GPIHBP1. The novel insights provided by these studies may aid the understanding of how LPL activity is controlled on a molecular mechanistic level, and may lead to a better understanding of disease-causing mutations within the LPL, GPIHBP1, and ANGPTL4 genes.

Depletion of TM6SF2 disturbs membrane lipid composition and dynamics in HuH7 hepatoma cells

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A polymorphism of TM6SF2 has been associated with non-alcoholic fatty liver disease (NAFLD) and reduction of hepatic triacylglycerol (TAG) secretion. TM6SF2 variant allele carriers also show lower serum TAG and LDL-cholesterol concentrations than non-carriers, and thus have a reduced risk of cardiovascular disease. However, the function of the encoded protein has remained unknown. We studied the effect of stable TM6SF2 knock-down on the lipid content and composition, mitochondrial fatty acid (FA) oxidation and organelle structure of HuH7 hepatoma cells. Depletion of TM6SF2 resulted in intracellular accumulation of TAGs, cholesterol esters, phosphatidylcholine (PC) and phosphatidylethanolamine. In these lipid classes, relative levels of polyunsaturated lipid species were significantly reduced while saturated and monounsaturated species increased their proportions. In PCs, also an absolute decrease in the species containing arachidonic acid (20:4n-6) was seen. Furthermore, the synthesis and turnover of the hepatocellular glycerolipids was enhanced, more lysosome/endosome structures appeared, and mitochondrial capacity for palmitate oxidation was significantly reduced. These observations elucidate the function of TM6SF2 and the mechanism(s) by which the deficiency of the protein disturbs hepatic TAG secretion. We suggest that the impaired secretion is associated with distorted dynamics of membrane phospholipid synthesis/degradation affected by the suboptimal phospholipid FA composition.

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High quality fish oil has a more favourable effect than oxidized fish oil on intermediate and low density lipoprotein subclasses

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BACKGROUND: Fish oil (FO) supplementation reduces the risk of cardiovascular disease (CVD). However, it is not known if FOs of different qualities have different effects on lipoprotein subclasses in humans.

OBJECTIVE: We aimed at investigating the effects of oxidized FO and high quality FO supplementation on lipoprotein subclasses and their lipid concentrations in healthy humans.

METHODS: 54 subjects completed a double-blind randomized controlled intervention study. The subjects were randomly assigned to receive high quality FO (n=17), oxidized FO (n=18) or high-oleic sunflower oil capsules (HOSO, n=19) for 7 weeks. The concentration of marine omega-3 fatty acids was equal in high quality FO and oxidized FO. The peroxide and anisidine values were 4 mEq/kg and 3 in high quality FO and 18 mEq/kg and 9 in oxidized FO. Blood samples were collected at baseline and end of study. Nuclear magnetic resonance spectroscopy was applied for the analysis of lipoprotein subclasses and their lipid concentrations.

RESULTS: High quality FO reduced the concentration of intermediate-density lipoprotein (IDL) particles and large, medium and small low-density lipoprotein (LDL) particles, and the concentrations of total lipids, phospholipids, total cholesterol, cholesterol esters and free cholesterol in IDL and LDL subclasses compared to oxidized FO and HOSO.

CONCLUSION: High quality FO and oxidized FO differently affect lipid composition in lipoprotein subclasses, with a more favourable effect mediated by high quality FO.

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Familial longevity is characterized by preserved circadian rhythmicity of serum cholesterol levels in healthy middle-aged individuals

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Aim: The biological clock determines bodily circadian (i.e. 24h) rhythms, including of cholesterol, and its function deteriorates with increasing age. As dampening of circadian rhythms is associated with disease, we hypothesized that individuals with a genetic predisposition for longevity preserve high amplitude circadian rhythms, which is reflected by a preserved circadian serum cholesterol level rhythm. We aimed to investigate circadian rhythmicity of serum cholesterol levels in offspring of nonagenarian siblings and their partners.

Methods: Offspring from nonagenarian siblings (n=19), and their partners as controls (n=18), were recruited from the Leiden Longevity Study. Participants were studied in a controlled in-hospital setting over a 24h period, receiving three isocaloric meals at 9:00h, 12:00h and 18:00h. Lights were off between 23:00h and 8:00h. Serum total cholesterol (TC), HDL-cholesterol (HDL-c), and non-HDL-c were measured in venous blood every 30 min.

Results: Serum TC levels during night were lower than during day in offspring (5.2 vs. 4.7 mM; $p<0.001$) and in controls (5.3 vs. 5.0 mM; $p<0.001$). In addition, the differences in TC levels between day and night tended to be greater in offspring than in controls ($p=0.094$). In fact, the day-night serum differences of non-HDL-c were 2-fold greater in offspring than in controls ($p=0.041$) and most explicit in females (TC: $p=0.03$; non-HDL-c: $p=0.058$).

Conclusion: Familial longevity is characterized by preserved circadian rhythmicity of non-HDL-c levels in healthy middle-aged individuals, indicating that age-related dampening of circadian rhythmicity could be preserved at middle-age in individuals from long-lived families.

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PNPLA2 selectively mediates the secretion of triglyceride-rich lipoproteins by human hepatoma cells.

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Background. Increased hepatic secretion of triglyceride (TG)-rich lipoproteins and cellular TG-accumulation are associated with enhanced risk for cardiovascular disease and diabetes mellitus, but the factors regulating these processes are largely unknown. KO studies demonstrated that patatin-domain containing protein 2 (PNPLA2), also known as ATGL, is largely responsible for hepatic TG-hydrolysis in mice, but the physiological role of PNPLA2 in human liver metabolism has thus far not been evaluated.

Aim. To investigate the role of PNPLA2 in the secretion of TG-rich lipoproteins and TG-accumulation in human hepatoma Huh7 and HepG2 cell-lines.

Results. Gene-specific siRNA silencing of PNPLA2 generated mRNA reductions of 90% and 77% in Huh7 and HepG2 cells, respectively. PNPLA2-silencing was associated with 30-40% decreases in cellular TG-hydrolysis in both hepatoma cell-lines as measured by an in vivo assay. Secretion of TG-rich lipoproteins was analyzed using an ELISA for apolipoprotein B (APOB) and quantification of C14-TG following incubation with C14-labelled glycerol. It was found that PNPLA2-silencing reduced the secretion of APOB and TG by 35% and 37% in Huh7 cells and by 23% and 29% in HepG2 cells, respectively. Surprisingly, no changes in cellular TG-accumulation were observed following PNPLA2-inhibition in Huh7 and HepG2 cells. Confocal microscopy studies confirmed that PNPLA2 inhibition did not change the lipid-droplet content of the hepatoma cells.

Conclusion. These studies demonstrate that PNPLA2 acts as a TG-hydrolase in human hepatoma cells, is involved in the secretion of TG-rich lipoproteins, but is not influencing hepatic lipid-droplet homeostasis. It is proposed that PNPLA2 selectively mediates the secretion of TG-rich lipoproteins in human liver.

Participate in the young investigator´s award.

The anti-inflammatory function of HDL is impaired in type 2 diabetes

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Background: Assessing the functional properties of high density lipoproteins (HDL) is an emerging topic in cardiovascular research as opposed to merely measuring HDL cholesterol mass levels. Patients with type 2 diabetes mellitus (T2DM) are characterized by low plasma HDL cholesterol, but also functional impairments of HDL might contribute to the increased cardiovascular disease risk in T2DM. Specifically, the effect of chronic hyperglycemia on the anti-inflammatory capacity of HDL is unclear.

Aim: To cross-sectionally determine the anti-inflammatory function of HDL in T2DM patients and controls and characterize potential impacting factors.

Methods: In 40 T2DM subjects (no insulin treatment) and 36 non-diabetic controls the HDL anti-inflammatory capacity was measured as the ability of HDL to suppress TNF-α-induced VCAM-1 mRNA expression in human endothelial cells in vitro. Serum PON-1 (arylesterase) activity, hs C-reactive protein (hsCRP), serum amyloid A (SAA) and circulating TNF-α levels were also determined in all subjects.

Results: The anti-inflammatory activity of HDL was strongly impaired in T2DM (3.18 [2.17-4.33] vs. 1.05 [0.63-1.38], $p<0.001$). In addition, T2DM patients had decreased HDL cholesterol, apolipoprotein A-I and PON-1 activity ($P<0.05$ to $P<0.001$) as well as increased hs-CRP ($P<0.05$) and TNF-α ($P<0.02$). In all subjects combined, age- and sex adjusted multivariable linear regression analysis demonstrated that impaired HDL anti-inflammatory capacity was associated with hyperglycemia ($\beta=0.499$, $P<0.001$), lower PON-1 activity ($\beta=-0.192$, $P=0.030$) and higher hsCRP ($\beta=0.220$, $P=0.016$).

Conclusion: The HDL anti-inflammatory capacity is severely impaired in T2DM, even in metabolically well controlled patients. This decrease in HDL function is, at least partly, attributable to the degree of hyperglycemia, decreased PON-1 activity and enhanced chronic low grade inflammation.

GPR120 as a novel target to reduce obesity in mice

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Background: The free fatty acid receptor GPR120 is highly expressed in brown adipose tissue (BAT), an important player in energy expenditure and lipid metabolism as it combusts fatty acids towards heat through uncoupling by UCP1. The aim of our study was to assess the role of GPR120 in lipid metabolism and substrate utilization, and the therapeutic potential of a selective GPR120 agonist.

Methods: GPR120-/- mice and wild-type littermates (C57BL/6 background) received a high-fat diet (45% kcal) for 8 weeks. In addition, wild-type mice were treated with the GPR120 agonist TUG891 (35 mg/kg) daily for 2.5 weeks. Fully automated metabolic cages were used to measure energy expenditure and substrate utilization.

Results: GPR120-/- mice had higher fat mass (+25%, $p=0.05$) and lower energy expenditure during the dark phase (-5%, $p<0.05$), but no different substrate utilization (similar RER) compared to control mice. GPR120-deficiency reduced expression of Ucp1, Prdm16 and Pparα in BAT (-38 to -59%, $p<0.05$) without affecting uptake of fatty acids derived from intravenously injected lipoprotein-like particles labelled with glycerol tri[3H]oleate ([3H]TO) by BAT. TUG891 effectively reduced fat mass (-73%, $p<0.001$) and body weight (-14%, $p<0.01$). Although TUG891 did not increase energy expenditure, it acutely increased fat oxidation (+331%, $p<0.01$) and reduced glucose oxidation (-18%, $p<0.05$), resulting in a lower RER during the dark phase (-5%, $p<0.01$). TUG891 increased uptake of [3H]TO-derived radioactivity by BAT (+105%, $p<0.05$) and reduced lipid droplet content in BAT (-28%, $p<0.01$), pointing towards more active BAT.

Conclusions: While GPR120-deficiency deteriorates metabolic phenotype with increased fat mass, GPR120 agonism reduces fat mass by increasing fat oxidation and enhancing fatty acid uptake by BAT. Therefore, stimulation of GPR120 holds therapeutic potential to combat obesity.

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A role for the LXR target IDOL in the regulation of intestinal LDLR expression

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Increased levels of Low Density Lipoprotein (LDL) cholesterol in the blood are a major risk factor for the development of atherosclerosis and ensuing cardiovascular disease. The Inducible Degrader of the LDL-receptor (IDOL) is an E3 ubiquitin ligase under transcriptional control of the Liver X Receptor (LXR) transcription factors. IDOL targets several members of the LDL receptor family for degradation, including the LDL receptor itself, and as such has an important role in cholesterol metabolism. Initial studies on IDOL have focused on its role in hepatic cholesterol metabolism. Recent studies implicate intestinal cholesterol metabolism as a central determinant of LXR's anti-atherosclerotic activity.

Whether the LXR-IDOL-LDLR axis is active in the intestine, and whether it contributes to intestinal cholesterol homeostasis remains unknown.

Therefore, as a first step to address this issue we aimed to determine the activity of the LXR-IDOL-LDLR axis in intestinal cells. Our results show that LXR stimulation consistently reduces LDLR expression in three human intestinal cell lines, HCT116, HT29 and SW480. Furthermore, siRNA-mediated silencing of IDOL expression in HCT116 cells attenuated degradation of the LDLR following LXR stimulation, and functionally also increased LDL uptake into cells. Moreover, LDLR expression was increased in colonic derived enterocytes of Idol-/- mice compared to those of wildtype control mice.

In aggregate, these results establish that the LXR-IDOL-LDLR axis is active in intestinal cells. Studies to determine the significance of this axis in vivo are currently underway.

Plasma apolipoprotein M is increased in postmenopausal women

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Introduction: The axis of apolipoprotein M (apoM) and sphingosine-1-phosphate (S1P) controls endothelial function. Menopause is accompanied by impaired endothelial function which can be improved by training. The aim of this study was to explore if these effects are paralleled by changes in the apoM/S1P axis. We investigated the effect of menopausal status and exercise training on the plasma concentrations of apoM and S1P, as postmenopausal women face an elevated risk of developing cardiometabolic diseases, and exercise training reduces this risk.

Methods: Healthy, late premenopausal (n=38, age 49.2(+/-)2.1 years) and early postmenopausal (n=37, age 53.3(+/-)3.0 years) women were included from the Copenhagen Women Study: Menopause. All participants went through a three-month, high-intensity exercise training program. Fasting blood samples collected before and after the training intervention were used for measurements.

Results: Before training, the plasma apoM concentration was 0.817(+/-)0.22 µmol/l in premenopausal women compared to 1.08(+/-)0.23 µmol/l in postmenopausal women ($p<0.0001$). The plasma S1P concentration was similar in the two groups (0.445(+/-)0.087 and 0.464(+/-)0.075 µmol/l, respectively). Hence, the S1P/apoM ratio was 26% lower in pre- than postmenopausal women ($p<0.0001$). After the training intervention, plasma apoM concentrations had increased 8% ($p<0.05$), and plasma S1P concentrations had increased 5% ($p<0.05$) with no difference between pre- and postmenopausal women.

Conclusion: The results suggest that menopause is accompanied by increased plasma apoM but not S1P concentrations, and that exercise training increases plasma apoM and S1P similarly in pre- and postmenopausal women.

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Subjects with familial hypercholesterolemia have larger postprandial increase of small vldl after intake of saturated fat compared to polyunsaturated fat - a randomized controlled trial

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Aim: Postprandial hypertriglyceridemia is associated with increased risk of developing cardiovascular disease (CVD). We aimed to investigate the postprandial response in plasma triglycerides and lipid subclasses after consumption of high-fat meals with different fat quality in subjects with familial hypercholesterolemia (FH) compared to healthy normolipidemic controls.

Methods: A postprandial randomized controlled double-blinded crossover intervention study with two meals and two groups was performed. Thirteen subjects with FH (without current lipid-lowering treatment) and 14 normolipidemic controls were included. All participants were aged 18 to 30 years and had BMI 18.5-30 kg/m2. The meals consisted of one of two muffins with different fat quality, mainly saturated fat (SFA, 42.8 E%) or mainly polyunsaturated fat (PUFA, 39.2 E%), both containing 60 grams (70 E%) of fat. The amount of monounsaturated fat was similar in the two meals. Blood samples were taken at baseline (fasting) and 2, 4 and 6 hours after intake of the meals. Comparisons of area under the curve were analyzed by using a linear mixed model for repeated measures.

Results: Subjects with FH had a larger postprandial response for most lipid subclasses compared to healthy controls, independent of fat quality. Interestingly though, FH subjects had larger postprandial increase of small atherogenic VLDL particles after intake of the SFA meal compared to the PUFA meal, when adjusted for baseline values (P=0.007). No significant difference in the postprandial apolipoprotein B48 or plasma triglyceride concentration was found between the two groups (P>0.05).

Conclusions: Subjects with FH have a significantly larger postprandial increase of small VLDL particles after intake of SFA compared to PUFA. These particles may enter the arterial wall more readily and promote atherosclerosis.

Poster presentations – Abstracts

Other Topics

Session IV

Impaired fatty acid synthesis affects immune cells activation: focus on sterol regulatory element binding factor-1c on t lymphocytes

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Background: Intracellular metabolism has recently been recognized as a key determinant of immune cells differentiation and activation; indeed, whereas activated T cells rely on glycolysis, naïve, memory and regulatory Tcells use fatty acids oxidation (FAO) for their metabolic demand.

Aim: to study how key proteins regulating intracellular fatty acid (FA) metabolism (SREBP1c) affected the polarization of CD4 Tcells.

Material and Methods: A detailed immunophenotyping through flow cytometry and metabolic profiling of isolated Tregulatory(CD4+CD25+) and Tconventional(CD4+CD25-) cells was performed in SREBP1c KO and WT littermates.

Results: SREBP1c deficiency resulted in a significant reduction of T cells fueled by FAO: CD4+CD44+ (18.48±1.03% vs 22.85±0.54%, p<0,01), CD8+CD44+ (11.63±0.82% vs 14.86±1.24% ,p<0,05), and Tregulatory CD4+CD25hiFoxP3+ cells (2.61±0.25% vs 3.38±0.10%, p<0,01). To unrevail the role of FA in Tcells, metabolomic analysis was performed on isolated cells. SREBP1cKO Treg showed a reduction of FA synthesis with accumulation of acetilCoA(2.73±0.3vs4.52±0.59 ng/µgprotein,p<0,01) which led to an accumulation of medium-chain acetylcarnitines (C8: 3.8 ±0.6 vs 6.2±0.07; C10 :2.4±0.04 vs 4.6±0.05; C14: 1.02±0.1 vs 1.8±0.2,pg/µgprotein,p<0,01), suggesting an incomplete FAO; glycolisis was also affected with accumulation of lactate(13.47±2.53 vs 36.25±7.48,ng/µgprotein, p<0,01) in Treg of SREBP1cKO. This phenotype was peculiar of Treg as metabolites of Tconv were similar between SREBP1cKO and WT, thus addressing a key role of FA metabolism in Treg but not other CD4Tcells.

Conclusion: Data collected suggest that Treg may rely on a "futile" cycle by producing and burning FA. SREBP1c represents a key player of FA metabolism and indeed its deficiency affects Treg metabolism leading to reduced levels of these cells in secondary lymphoid organs. Therefore, reprogramming Tcell FA metabolism may represent a therapeutic target for diseases characterized by dysregulations of immune activation.

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Milk cholesterol content is tightly regulated and remains stable regardless of strong dietary or genetic manipulations in hypo- and hypercholesterolemic mice

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**These authors contributed equally.*

Aim: Breast milk contains high cholesterol concentrations, which is important for establishing long-term cholesterol homeostasis in the offspring. However, origin and regulation of milk cholesterol content are largely unknown. Here we investigated the mechanisms for cholesterol excretion and regulation of its content in the milk of the mouse by using dietary and genetic models resulting in maternal hyper- or hypocholesterolemia.

Methods: Milk and plasma lipids were analyzed at L14 postpartum in wild type, LDLR -/- and ABCG8 -/- mice fed chow or high-cholesterol diet; to target a possible HDL-delivery pathway to the mammary glad we used probucol-treated C57BL6 mice. Cholesterol synthesis in mammary gland and liver was assessed via the deuterium incorporation method.

Results: In all three models (C57BL6, LDLR -/-, ABCG8 -/-) we observed an increase in plasma LDL-C in the high-cholesterol-fed condition. However, the corresponding milk cholesterol content remained largely unaffected (1.5±0.27 mmol/L). Probucol treatment reduced the total plasma cholesterol (-80%, $p<0.001$), which resulted in decreased output of cholesterol in the milk (chow -48%, $p<0.001$; high-cholesterol -41%, $p<0.01$) and a corresponding increase in endogenous synthesis.

Conclusions: Our findings suggest an important role for milk cholesterol concentration, which is tightly regulated to prevent overexposure or deficiency of the offspring to dietary cholesterol. Utilizing cholesterol from multiple independent lipoprotein sources and local synthesis insure the high buffering capacity of the mammary gland against dietary influences on cholesterol content of breast milk.

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T-cell activation is associated with artery stiffness in patients with breast cancer.

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Objective. The immune system is suggested to play an essential role in vascular remodeling. We hypothesized that immune disturbances occurring in patients with cancer predispose to vascular stiffening reflecting subclinical cardiovascular disease (CVD).

Materials and methods. 17 women with newly diagnosed untreated breast cancer (HER2-positive, II-III stage) and 20 women with no anamnesis of CVD were enrolled. Groups were comparable in age, blood pressure level and body mass index. Circulating CD4+IFNgamma+ T-helper (Th) 1, CD4+IL4+ Th2, and CD4+CD25lowCD127high activated T-cell (T-act) subsets were evaluated. Pulse wave velocity (PWV) in ankle-brachial segment (PWVab) and in carotid-femoral segment (PWVcf) were assessed via sphygmography and applanation tonometry, respectively.

Results. The levels of Th1, Th2 and T-act (% lymphocytes) were increased in breast cancer patients as compared with the control group (11 (9.5-16.4) vs. 9 (5.8-11.4) and 1.5 (0.7-1.7) vs. 0.7 (0.4-0.8), $p<0.05$, and 25 (23-31) vs. 21 (19-25), $p=0.05$, respectively). PWVab and PWVcf (m/s) were elevated in breast cancer patients as compared to controls (13.0 (12.1-14.8) vs. 12.0 (11.4-12.7) and 8.1 (7.7-8.7) vs. 7.2 (6.9-7.9), respectively, $p<0.05$). The level of T-act positively correlated with PWVcf and PWVab ($R= 0.72$ and 0.48, respectively, $p<0.05$), and this association was observed both in patients with breast cancer and controls. Th1 and Th2 levels correlated with PWVcf ($R= 0.39$ and 0.47, respectively, $p<0.05$). While the correlation between age and PWVab ($R= 0.64$) was observed, the investigated immune parameters did not correlate with age.

Conclusion. We speculate that vascular wall remodeling in patients with breast cancer may be associated with persistent T-cell activation. Further studies should be held to confirm this hypothesis.

Familiar hypercholesterolemia – treatment and quality of life

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Background and Aims: Familiar hypercholesterolemia (FH) is an autosomal dominant heritable disease that leads to increased levels of the LDL cholesterol, which again causes increased risk of premature coronary heart disease. A healthy life style are important and insight in the quality of life and the challenges and worries these patients often face in their everyday life are significant.

Materials and methods: A questionnaire with 47 questions and assertions was answered by 216 persons diagnosed with FH at the Lipid Clinic in Oslo. Questions are a mix of multiple choice questions, questions that the patient shall explain and answer with his or her own words and different assertions. All the contestants have the HeFH diagnosis.

Results: The patients have reduced their cholesterol level considerably since they were diagnosed. The majority uses statins as drug treatment and many patients experience side effects of this treatment. 50 percent feel that it is difficult to follow recommended advices for diet and life style. More than half of the patients say that life would have been easier without FH, and several are fearful of developing cardiovascular diseases. Of 216 participants, 185 gave information on relatives. Out of 441 relatives, 329 are dead. As many as 53 % died at the age of 60 or younger, wherein 11 % died at the age of 40 or younger and 42 % died between the age of 41-60. 47% died after the age of 60. The average life span was 59.3 years of patients' relatives with FH.

Conclusion: The mean age at time of death was 59.3 years in deceased FH patients who were relatives to the study participants. Smoking was common in those who died at age of 40 or below (80 %) compared to those who died at age of 41 - 60 (43,2 %) and > 60 years old (37,4 %). The treatment of FH, side effects of drug treatment, necessary life style changes, anxiety related to course of disease, and fatal consequences of the disease, often have impact on the patients' quality of life.

Participate in the young investigator´s award.

Common genetic variation in ABCA7 and risk of dementia, ischemic heart and cerebrovascular disease

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ABCA7 is suggested to be implicated in both lipid metabolism and in Alzheimer's disease pathology by clearing amyloid-β in the brain.

We tested whether a common genetic variant in ABCA7 (rs4147929), identified from genome-wide association studies, was associated with risk of Alzheimer's disease, other types of dementia and atherosclerosis-related diseases such as ischemic heart disease and cerebrovascular disease in ~104,000 individuals from the general population. We included consortia data from International Genomics of Alzheimer's Projects (IGAP) including 74,046 individuals to use in a meta-analysis. Lastly we conducted an analysis of lipid, lipoprotein and apolipoprotein levels as a function of ABCA7 genotype.

Multifactorially adjusted hazard ratios for Alzheimer's disease were 1.04 (95% confidence interval 0.93-1.23) for rs4147929 GG versus GA genotype and 1.72 (1.23-2.39) for GG versus AA genotype. Results were similar when further adjusting for APOE genotype. Multifactorially adjusted hazard ratios for myocardial infarction were 1.01 (0.95-1.08) for GG versus GA genotype and 1.21 (1.02-1.44) for GG versus AA genotype. This association remained after further adjustment for total cholesterol, LDL and HDL cholesterol, triglycerides and APOE genotype. We found no association between ABCA7 genotype and risk of vascular dementia, unspecified dementia, all dementia, ischemic heart disease or cerebrovascular disease. We found no association between rs4147929 genotype and lipid, lipoprotein, or apolipoprotein levels. Meta-analysis including consortia data supported our findings of the association between ABCA7 and risk of Alzheimer's disease.

A common genetic variant in ABCA7 was associated with high risk of Alzheimer's disease independent of APOE genotype in the general population. No association was observed between ABCA7 and other types of dementia, atherosclerosis-related endpoints or lipid and lipoprotein levels. Our findings support the suggested role of ABCA7 in Alzheimer's pathology and its function in phagocytic clearance of amyloid-β in the brain.

Participate in the young investigator´s award.

Investigating the underlying mechanisms in high risk CAD by studying patient specific iPSCs derived hepatocyte models in vitro

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Coronary artery disease (CAD) remains asymptomatic for decades and at least 10% of the chronic events occur in apparently healthy individuals in the absence of traditional risk factors. In addition, while some of the atherosclerotic plaques remain stable during the lifetime, others can be highly vulnerable and lead to heart attack and sudden death. Hepatocyte-like cells (HLCs) differentiated from human induced pluripotent stem cells (hiPSCs) offer an alternative model for primary human hepatocytes (PHH) to study lipid aberrations. Here, we aim to develop an in vitro model of CAD patient's hepatocytes to study key lipids involved in formation of vulnerable atherosclerotic plaques. To achieve our aim, induced pluripotent stem cells (iPSCs) have been developed from the skin biopsies of three patient groups: acute, stable CAD and control. Then iPSCs have been differentiated to functional hepatocyte-like cells. Five hepatic differentiation methods were tested to find the best protocol for our purpose and results have been compared. Using mass spectrometry, we monitored the detailed quantification of alteration in cellular lipidome during the entire differentiation of hiPSC to HLC. Lipidomic outputs were utilised in conjunction with biochemical (LDL, HDL, TG, albumin, and urea secretion), functional (LDL uptake) and gene (SOX17, FOXA2, AFP, and ALB) and protein (SOX17, AFP, ALB, ASGPR) expression measurements to identify a suitable cell model that can be applied in exploring lipid abnormalities. The results from HLCs were compared to PHH and a hepatoma-derived cell line (HepG2). In conclusion, we have successfully set up an in vitro patient specific hepatocyte model which is functional and is capable of secreting lipids. This model can be an invaluable platform to investigate the underlying mechanisms in CAD which can lead us to discover novel lipid biomarkers for high risk CAD.

Gut-derived bacterial lps links western diet with adipocyte dysfunction through mitochondrial damage

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Background and aim: Systemic metabolic disease is initiated by the inability of white adipose tissue to buffer lipids away from other organs during chronic over-nutrition. Increased gut-derived bacterial lipopolysaccharide (LPS) absorption after a high-fat meal may be a factor in reaching this threshold, as it has been shown to set the inflammatory tone and trigger systemic insulin resistance. However, the direct effect of LPS on adipose mitochondrial handling of excess calories remains unknown.

Material and methods: Serum and subcutaneous adipose biopsies were collected from 44 morbidly obese T2DM women before and six months after weight loss surgery. Mitochondrial functional assays were performed on human obese subcutaneous adipocytes ChubS7 treated for 24 hours with (10ng/mL; 100ng/mL) or without LPS.

Results: A negative correlation was observed between serum LPS and mitochondrial number in adipose tissue biopsies (r2= -0.485, p=0.005**, n=32). Patients with lower serum LPS levels also exhibited greater weight, HbA1c and lipidaemia reduction in tandem with improvements in mitochondrial gene regulation. Furthermore, LPS resulted in 8 to 15% mitochondrial DNA deletion (p=0.008), mitochondrial protein depletion (p=0.007) and reduced mitochondrial number (p=0.034) compared with controls. This mitochondrial damage functionally manifested in a shift from aerobic to anaerobic respiration (p=0.03), an impaired ability to cope with an energetics stress test (seahorse; p<0.01) and reduced glucose uptake (p<0.001).

Conclusion: Increased circulating LPS levels (often present as a result of a western diet) can directly trigger mitochondrial DNA damage, a dysfunctional shift from lipids towards glucose as energy substrate and an impaired lipid buffering capacity.

Participate in the young investigator´s award.

Regulation of ANGPTL8 by microRNA 221 and inflammation in adipocytes

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Angiopoietin like 8 (ANGPTL8), the most insulin sensitive gene in human white adipose tissue, plays a major role in lipid and glucose metabolism. Recent studies have shown that this molecule is emerging as a potential biomarker for obesity and both type 1 and 2 diabetes. In this study we analyzed the regulation of ANGPTL8 expression by microRNA 221 (miR-221, which is upregulated in adipose tissue of insulin resistant subjects) and inflammation. We observed that ANGPTL8 is a predicted target of miR-221, and direct binding of miR-221 to the ANGPTL8 3'UTR was confirmed using luciferase reporter assays. A negative correlation trend between ANGPTL8 gene expression and miR-221 levels was observed in fat biopsies of a human cohort. Consistently, overexpression of miR-221 mimics in cultured human SGBS adipocytes resulted in reduced levels of ANGPTL8 protein expression. Further experiments in cultured primary adipocytes revealed that inflammation drastically reduces ANGPTL8 and increases miR-221 expression. In conclusion, ANGPTL8 gene expression is regulated by miR-221 and inflammation.

Glucose handling and white adipocyte phenotype are altered in proteoglycan 4 deficient mice

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ChIP sequencing data and in vitro stimulation assays identified proteoglycan 4 (PRG4) as a novel peroxisome proliferator-activated receptor gamma (PPARγ)-responsive gene. PPARγ is a master regulator of lipid metabolism and is associated with adipogenesis and obesity. Therefore, we aimed to investigate the role of PRG4 in diet-induced obesity in mice.

PRG4 is mainly expressed in metabolically active tissues in wild-type (WT) C57Bl/6 mice. Moreover, mice lacking PRG4 display improved glucose handling on chow, providing further support for a potential metabolic function. To induce obesity, male PRG4 knockout (KO) mice and WT littermates were fed a high fat diet (HFD) for 12 weeks. Although body weight gain did not differ, PRG4 deficient adipocytes from the gonadal fat pad showed a significant increase in cell diameter (+11%, p=0.04) compared to WT which translated in a trend towards an increase in adipocyte volume (+29%, p=0.09). Interestingly, PRG4 deficient adipocytes showed significantly reduced lipolysis (2way ANOVA genotype p=0.03). Basal lipolysis did not differ significantly. However, the maximum lipolytic capacity was significantly reduced in PRG4 deficient adipocytes explaining the effect on lipolysis (-28%, p=0.04). A change in adipocyte function could lead to disturbances in glucose metabolism. In line, the improved glucose handling by PRG4 KO mice under normolipidemic conditions was lost upon HFD feeding. Importantly, in a human cohort of obese women with Type 2 Diabetes (T2D) compared to obese normal glucose tolerant controls PRG4 expression was significantly increased (+11%, p=0.03), underlining also a potential role for PRG4 in glucose handling in humans.

We show for the first time that PRG4 deficiency affects glucose handling and adipocyte phenotype in mice. Our human data highlight a potential role for PRG4 in human glucose metabolism.

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Carbohydrate response element binding protein regulates de novo lipogenesis in brown adipose tissue

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Aim: Carbohydrate response element binding protein (ChREBP) is a key transcriptional regulator of hepatic de novo lipogenesis (DNL) and glycolysis. The aim of the study was to investigate the impact of the transcription factor ChREBP on DNL and energy metabolism in adipose tissues.

Methods: To study the relevance of ChREBP in DNL, we investigated mouse line with a global knockout of Mlxipl, the gene that encoding for ChREBP. The mice were adapted to cold or thermoneutrality and lipid and energy metabolism were studied by either analysis of gene and protein expression, as well as histology and organ uptake studies of radioactively labeled tracers.

Results: The effect of ChREBP deficiency on DNL genes in adipose tissue was highly variable, depending on type and anatomical location. Expression of DNL enzymes was markedly reduced in brown adipose tissue (BAT) of ChREBP-/- mice, whereas only modest to no reduction of DNL genes was observed in inguinal and gonadal white adipose tissue. Radioactive tracer studies revealed an increased disposal of triglyceride-rich lipoproteins in BAT of ChREBP-/- mice. Notably, uptake of glucose into BAT of knockout animals was diminished, demonstrating a fuel switch in brown adipocytes. Despite a compensatory mechanism leading to increased uptake of fatty acids from the circulation, ChREBP-/- exhibited significantly impaired BAT lipid storage at thermoneutrality.

Conclusion: We demonstrate a central role of ChREBP for DNL in BAT, emphasizing the importance of DNL-derived fatty acids for efficient adaption to environmental temperatures.

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NLRP3 inflammasome promote myocardial remodeling during diet-induced obesity.

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Introduction and purpose: Obesity has a direct effect on myocardium and can affect cardiac remodeling. Others and we have demonstrated that NLRP3 Inflammasome is functional in the heart with the potential contribution to cardiac function and cell death regulation. The aim of the study was to investigate whether NLRP3 inflammasome play a role in development of myocardial remodeling during diet-induced obesity.

Methods: Wt(C57BI/6J), NLRP3 and ASC deficient male mice were fed a high-fat diet(HFD;60% cal from fat) or control diet for 52 weeks .Echocardiography and MRI were performed to assess cardiac structure and function.

Results: NLRP3 and ASC deficient mice gained significantly less body weight and liver weight (steatosis) during HFD compare to the Wt mice. Wt-HFD mice had significantly higher plasma glucose and lipids levels. Long-term exposure to HFD induced left ventricle(LV) hypertrophy in all mice, but only Wt mice had significantly increased relative wall thickness(RWT), indicating consentric type of hypertrophy. LV systolic and diastolic function was preserved in NLRP3 and ASC deficient mice but significantly reduced in Wt mice.

Conclusion: NLRP3 and ASC deficiency modulate concentric remodeling and preserves LV systolic and diastolic function during diet-induced obesity.

Evaluation of Aortic Valve Morphology in Hyperlipidemic Mice by Magnetic Resonance Imaging (MRI)

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Aims: In aortic stenosis (AS), valvular calcification makes valves thicker and stiffen causing impairment of the valve function, leading to obstruction of the blood flow and eventually to left ventricular hypertrophy. The non-invasive cardiac magnetic resonance imaging (MRI) has been found potential in imaging AS but there is a room for improvement in the image resolution to be able to accurately detect composition and function of the valves. We used high-resolution cine-imaging to study changes in morphology of the aortic valves in a mouse model of AS.

Methods: LDLR-/-ApoB100/100 mice were fed a high-fat diet (HFD) (42 % kcal from fat) or a normal chow diet (control) for 5 months. The mice heart were imaged at MRI and echocardiography, and the ejection fraction (EF), end diastolic and end systolic volumes were determined. MRI imaging was correlated with the histological staining: Masson trichrome for fibrosis, Alizarin Red for the calcification and Mac-3 for the macrophages.

Results; From the MRI cine-images, the aortic wall and the atherosclerotic plaque were clearly separated and echocardiography revealed a marke reduction in EF in HFD mice compared to controls (36 % vs. 52% $p<0.001$). The end systolic images of the aortic valves showed atheroma plaque and valve dysfunction. Preliminary data shows a trend of narrowing (12%) of the orifice area suggesting more severe AS after HFD when compared to controls. In the histological analysis, area of aortic sinus was dilated 71% ($p<0.001$) and enlargement of area of aortic cusps (91%, $p<0.001$) was seen in HFD mice compared to controls.

Conclusions: In hyperlipdemic mice valvular atherosclerotic plaque and stenosis as well as reduction of EF was noted. Dysfunction and morphological changes of the aortic valves were clearly visualized by the cine MRI providing a non-invasive tool to monitor aortic valve morphology in mice.

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