22nd ANNUAL SCANDINAVIAN ATHEROSCLEROSIS CONFERENCE

2016

Scandinavian Society for Atherosclerosis Research

April 13-16, Krogerup Højskole, Humlebæk, Denmark

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April 13th-16th, 2016 at Krogerup Højskole, Krogerupvej 13, DK-3050 Humlebæk, Denmark

2016 Program

22nd ANNUAL SCANDINAVIAN ATHEROSCLEROSIS CONFERENCE

April 13th-16th, 2016 at Krogerup Højskole, Krogerupvej 13, DK-3050 Humlebæk, Denmark

SCIENTIFIC COMMITTEE

Katarina Öörni (Finland), Tanja X. Pedersen (Denmark)
Kirsten Holven (Norway), Emil D. Bartels (Denmark)
Vesa Olkkonen (Finland), Patrick Rensen (The Netherlands)
Paolo Parini (Sweden), Jeanine R. Lennep (The Netherlands)

Organized by
SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH

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Anna-Liisa Levonen
Vesa Olkkonen
Vilmundur Gudnason
Gunnar Sigurdsson
Trine Ranheim
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22nd ANNUAL SCANDINAVIAN ATHEROSCLEROSIS CONFERENCE

April 13th-16th, 2016 at Krogerup Højskole, Krogerupvej 13, DK-3050 Humlebæk, Denmark

Wednesday, April 13, 2016

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 2016 NIKKILÄ MEMORIAL LECTURES

19.35 – 19.40 Introduction of the 2016 Nikkilä Lecturer
Alexandra Krettek *(Sweden)*

19.40 – 20.25 **2016 Nikkilä Lecture**
Brown adipose tissue: A novel target to comBAT cardiometabolic disease
Patrick Rensen (*The Netherlands*)

20.25 – 20.45 Discussion

20.45 - Pub will be open

Thursday, April 14, 2016

08.00 – 09.00 **Breakfast**

SESSION I: CARDIOVASCULAR DISEASE
Organized and chaired by Kirsten Holven (*Norway*) and Emil D. Bartels (*Denmark*)

09.00 – 09.25 Cardiovascular disease and dementia – an important relationship in the aging population
Ruth Frikke-Schmidt *(Denmark)*

09.25 – 09.30 Discussion

09.30 – 09.45 Loss-of-function mutations in APOC3, remnant cholesterol, LDL cholesterol, and risk of ischemic vascular disease
Anders Berg Wulff *(Denmark)*

09.45 – 10.00 ACC/AHA guidelines superior to ESC/EAS guidelines for primary prevention with statins: The Copenhagen General Population Study
Martin Bødtker Mortensen (*Denmark*)*****

10.00 – 10.15 Maternal metabolic outcomes six years after pregnancy in women with a history of hypertensive pregnancy disorders
Jeanine Roeters van Lennep (*The Netherlands*)

10.15 – 10.30 A novel long residence time CCR2 antagonist inhibits atherogenesis in apoE deficient mice
Ilse Bot *(The Netherlands)*

10.30 – 11.15 **Coffee, posters and exhibitions**

11.15 – 11.40 Effect of maternal cardiovascular conditions and risk factors on offspring cardiovascular disease
Wulf Palinski *(USA)*

11.40 – 11.45 Discussion

11.45 – 12.00 The water channel AQP1 is associated with human atherosclerotic vascular lesions and attenuates progression of atherosclerosis in males independent of blood pressure
Jane Stubbe (*Denmark*)

12.00 – 12.15 HDL Cholesterol Efflux Does Not Predict Cardiovascular Risk in Hemodialysis Patients
Sanam Ebtehaj (*The Netherlands*)*

12.15 – 12.30 VIsualiZation of Asymtomatic atherosclerotic disease for optimum cardiovascular prevention – VIPVIZA – a RCT nested in routine care in Västerbotten Intervention Programme, Sweden
Ulf Näslund *(Sweden)*

SESSION II: INFLAMMATION AND VASCULAR BIOLOGY
Organized and chaired by Katariina Öörni (*Finland*) and Tanja X. Pedersen (*Denmark*)

12.30 – 12.55 Modelling atherosclerosis in animals – possibilities and limitations
Jacob Bentzon *(Denmark)*

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**

SESSION II: INFLAMMATION AND VASCULAR BIOLOGY
Organized and chaired by Katariina Öörni (*Finland*) and Tanja X. Pedersen (*Denmark*)

19.00 – 19.25 Oxidation, ECM modification and atherosclerosis
Michael Davies (*Denmark*)

19.25 – 19.30 Discussion

19.30 – 19.45	Depletion of the Androgen Receptor in Osteoblasts Protects Against Abdominal Aortic Aneurysms in Male Mice **Marta Lantero Rodriguez** *(Sweden)**
19.45 – 20.00	β-cyclodextrin reduces cholesterol crystal-induced inflammation through modulating complement activation **Siril Skaret Bakke** (*Norway*)*****
20.00 – 20.15	Extracellular lipid particles accumulating in human carotid artery intima induce sterile inflammation in macrophages **Katariina Öörni** *(Finland)*
20.15 – 20.45	**Coffee, posters and exhibitions.**
20.45 – 21.00	Effect of the GLP-1 analogue liraglutide on atherosclerosis and kidney fibrosis in moderate uremia **Line Stattau Bisgaard** *(Denmark)**
21.00 – 21.15	Association between anti -citrullinated protein antibodies and long-term mortality in patients with ST-segment elevated myocardial infarction **Daniël van der Velden** *(The Netherlands)**
21.15 – 21.30	Group IIA secretory phospholipase A2 (sPLA2-IIA) predicts graft failure and mortality in renal transplant recipients by mediating decreased kidney function **Uwe Tietge** (*The Netherlands*)
21.30-21.45	Comprehensive metabolic profiling of statin therapy: longitudinal evidence and mendelian randomization **Peter Würtz** *(Finland)**
21.45 –	Pub will be open

Friday, April 15, 2016

08.00 – 09.00 **Breakfast**

SESSION III: LIPOPROTEINS AND LIPID TRANSPORT
Organized and chaired by Vesa Olkkonen (*Finland*) and Patrick Rensen (*The Netherlands*)

09.00 – 09.25 The role of TM6SF2 in VLDL TG secretion, NAFLD and CVD risk
Stefano Romeo *(Sweden)*

09.25 – 09.30 Discussion

09.30 – 09.45 Unstable LDL - Novel mechanism of atherogenesis and link to cardiovascular deaths
Maija Ruuth (*Finland*)*****

09.45 – 10.00 A loss-of-function variant in OSBPL1A predisposes to low plasma HDL cholesterol levels and impaired cholesterol efflux capacity
Vesa Olkkonen (*Finland*)

10.00 – 10.15 Increased lipoprotein binding to arterial proteoglycans and normal macrophage cholesterol efflux capacity characterize CKD dyslipidemia
Matteo Pedrelli (*Sweden*)

10.15 – 10.30 PCSK9 impacts obesity and metabolic syndrome development in animal models
Giuseppe Danilo Norata (*Italy*)

10.30 – 11.15 **Coffee, posters and exhibitions**

11.15 – 11.40 Making and breaking ubiquitin chains in cholesterol metabolism
Noam Zelcer *(The Netherlands)*

11.40 – 11.45 Discussion

11.45 – 12.00 Ezetimibe in combination with simvastatin reduces remnant-cholesterol without affecting biliary lipid concentrations in gallstone patients
Paolo Parini (*Sweden*)

12.00 – 12.15 BCG lowers plasma cholesterol and delays atherosclerotic lesion progression
Andrea van Dam (The Netherlands)*

12.00 – 12.15 BCG lowers plasma cholesterol and delays atherosclerotic lesion progression
Andrea van Dam *(The Netherlands)**

12.15 – 12.30 FGF21 lowers plasma triglycerides by accelerating lipoprotein catabolism in white and brown adipose tissues
Christian Schlein (*Germany*)*

12.30 – 12.45 Unravelling the mechanism and importance of brown adipose tissue whole lipoprotein uptake
Alexander Fischer (*Germany*)*

12.45 – 14.00 **Lunch**

SESSION IV: OTHER TOPICS
Organized and chaired by Paolo Parini (*Sweden*) and Jeanine R. Lennep (*The Netherlands*)

14.00 – 14.25 Natural history of carriers of PCSK9 mutations
J.C. Defesche (*The Netherlands*)

14.25 – 14.30 Discussion

14.30 – 14.45 The body mass index value associated with the lowest mortality increased from 1976-78 through 2003-13: three cohort studies of the general population
Børge G Nordestgaard (*Denmark*)

14.45 – 15.00 Cold Regulated Bile Acid Synthesis Shapes the Gut Microbiome
Anna Worthmann (*Germany*)*

15.00 – 15.15 Altered cerebral cholesterol homeostasis contributes to cognitive decline in an Alzheimer's disease mouse model with diabetes mellitus
Monique Mulder (*The Netherlands*)

15.15 – 15.30 $LpPLA_2$ in Gestational Diabetes Mellitus – A protective system for placenta and fetus?
Christian Wadsack (*Austria*)

15.30 – 16.15 **Coffee, posters and exhibitions**

16.15 – 16.40 Diabetes in a dish: Human cellular models for the control of glucose homeostasis
Marine Kraus *(Switzerland)*

16.40 – 16.45 Discussion

16.45 – 17.00 Soat2 depletion improves liver steatosis and insulin sensitivity
Osman Ahmed (*Sweden*)*****

17.00 – 17.15 Non-alcoholic fatty liver disease as a cause of ischemic heart disease: a Mendelian randomization study and meta-analysis of 170,998 individuals
Bo Kobberø Lauridsen (*Denmark*)*****

17:15 – 17:30 Liver-humanized mice exhibit lipoprotein-specific phenotypes when grafted with human hepatocytes from different donors
Mirko Enea Minniti (*Sweden*)*****

17.30 – 19.00 **Time free**

19.00 – 19.30 **Cocktail**

19.30 – **Banquet and dancing**

Saturday, April 16, 2016

08.30 – 10.00 **Breakfast**

10.00 **Departure**

Have a nice trip back home!!!

2016 Posters

22nd ANNUAL SCANDINAVIAN ATHEROSCLEROSIS CONFERENCE

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Thursday, April 14, 2016

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.
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: CARDIOVASCULAR DISEASE
Organized and chaired by Kirsten Holven (*Norway*) and Emil D. Bartels (*Denmark*)

Systemic lupus erythematosus flare up is associated with increased carotid intima-media thickness progression
Andrea Baragetti *(Italy)**

Maternal hypercholesterolemia during pregnancy and offspring pre-pubertal cardiovascular risk factors
Jacob Juel Christensen *(Norway)**

Relative deficiency of Apolipoprotein M and Sphingosine 1-phosphate in Type 1 Diabetes
Cecilia Frej *(Sweden)**

Current treatment status and treatment goal attainment among Norwegian FH patients
Kirsten Holven *(Norway)*

A low-carbohydrate, high-fat diet for severe epilepsy: Effects on weight and biomarkers of atherosclerosis
Per Ole Iversen *(Norway)*

Elevated remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease
Anne Marie Kjær Jepsen *(Denmark)* – presented by Anette Varbo

Short-term effect of Low Carbohydrate High Fat diet on Lipid metabolism in heart tissues in female C57BL/6J mice
Masoumeh Motamedi Joibari *(Sweden)*

Is 1691G>A polymorphism within Factor V gene related to arterial ischemic stroke in children?
Ilona Kopyta (Poland) – presented by Beata Sarecka-Hujar

Replacement of dietary SFAs with PUFAs upregulates the mRNA expression levels of the LDL receptor and liver X receptor alpha: a double-blind randomized controlled trial
Lena Leder *(Norway)*

Peculiarities of T-cell immunity in carotid atherosclerosis
Anna Osokina *(Russia)* – presented by Aleksandra Shchinova

Impact of Bifidobacterium bifidum PRL2010 on lipid metabolism in vitro and in vivo
Antonio Piemontese *(Italy)**

Cardiovascular disease in patients with FH in Norway
Kjertil Retterstøl *(Norway)* – presented by Per Ole Iversen

SESSION II: INFLAMMATION AND VASCULAR BIOLOGY
Organized and chaired by Katariina Öörni (*Finland*) and Tanja X. Pedersen (*Denmark*)

Pentraxin 3 deficiency is associated with increased arterial thrombosis in animal models
Fabrizia Bonacina *(Italy)*

IL10 secreting B cells decrease during progression of atherosclerosis and modulate the inflammatory response in LDLR-/- mice
Hidde Douna *(The Netherlands)*

The increased blood Th17 frequencies may contribute to carotid atherosclerosis progression
Anastasia Filatova *(Russia)*

Biological consequences of Endonuclease V ablation in mice
Xiang Yi Kong *(Norway)*

Cellular model for studying the atherogenic mechanisms
Olga Maltseva *(Russia)*

The role of the DNA repair enzyme Neil3 in atherosclerosis
Ana Quiles-Jimenez *(Norway)*

The NLRP3 inflammasome mediates oxidative stress-induced pancreatic islet dysfunction
Trine Ranheim *(Norway)*

Depletion of conventional dendritic cells in atherosclerosis using the Zbtb46-DTR mouse model
Miche Rombouts *(Belgium)*

ER stress conditions lead to an upregulation of TFPI and is associated with an anti-apoptotic macrophage phenotype
Sandra Espada Serrano *(Norway)*

Friday, April 15, 2016

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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 III: LIPOPROTEINS AND LIPID TRANSPORT
Organized and chaired by Vesa Olkkonen (*Finland*) and Patrick Rensen (*The Netherlands*)

Oxidation of LDL by iron at lysosomal pH leads to the formation of tryptophan radicals which is not inhibited by probucol
Feroz Ahmad *(United Kingdom)*

Effects of moderate and excess alcohol consumption on the reverse cholesterol transport in vivo
Simone Battista *(Italy)**

Disturbed skin lipid composition and organization in hyperlipidemic ApoE KO mice
Renata Martins Cardoso *(The Netherlands)*

Evaluation of HDL cholesterol efflux capacity (CEC) after consumption of an innovative food enriched with bioactive components and functional probiotics
Eleonora Cipollari *(Italy)**

Role of a Cytoprotective Protein on Cholesterol Efflux in Human Macrophage Foam Cells
Burcin Gungor *(Finland)**

Mitochondrial function in brown adipose tissue is rapidly diminished by high fat diet
Eline Kuipers *(The Netherlands)**

Short-term cold exposure modulates human VLDL and HDL metabolism
Kimberly Nahon *(The Netherlands)**

Regulation of HDL-c levels via ABCA1: a role for LRP1
Federico Oldoni *(The Netherlands)**

Brown and beige adipocyte activity controls metabolic flux through the HDL compartment
Nicola Schaltenberg (Germany)*

G protein-coupled receptor 120 signaling activates brown adipocytes
Maaike Schilperoort *(United Kingdom)**

Mechanisms behind ANGPTL3-deficiency induced hypolipidemia
Anna Tikka *(Finland)**

SESSION IV: OTHER TOPICS

Organized and chaired by Paolo Parini (*Sweden*) and Jeanine R. Lennep (*The Netherlands*)

Energy balance method and the adequate analysis of lipid content in the study of body composition
Gustavo Abreu-Vieira *(The Netherlands)**

Impact of fetal oxidative stress on the development of metabolic disease in adulthood
Lidiya Dimova *(The Netherlands)**

Influence of mothers´ knowledge, attitude and behavior on diet and physical activity of their children: a cross-sectional study from Nepal
Alexandra Krettek *(Sweden)*

Using genetics to explore whether the cholesterol-lowering drug ezetimibe may cause an increased risk of cancer
Bo Kobberø Lauridsen *(Denmark)**

Evaluation of novel apoA-I two-site immunoassays
Janita Lövgren *(Finland)*

MBOAT7-TMC4 rs641738 variant increases the risk of NAFLD in individuals of European descent
Rosellina M. Mancina *(Sweden)**

Beta-cyclodextrin increases fecal sterol excretion and reverse cholesterol transport
Rima Mistry *(The Netherlands)*

Impact of ezetimibe on lipid profiles in cardiac transplant recipients receiving statin: a meta-analysis
Aneta Ostróżka-Cieślik *(Poland)*

Enzymatic degradation of 7-Ketocholesterol-A new strategy for the treatment of atherosclerosis
Irum Perveen *(Pakistan)*

Anti-apoptotic effect of Apolipoprotein M (ApoM) associated Sphinsosine 1-phosphate (S1P)
Mario Ruiz *(Sweden)**

Relation between 20210G>A polymorphism within Factor II gene and arterial ischemic stroke
Beata Sarecka-Hujar *(Poland)*

Oral presentations – Abstracts

Session I

Cardiovascular Disease – oral presentations

Loss-of-function mutations in *APOC3,* remnant cholesterol, LDL cholesterol, and risk of ischemic vascular disease

Anders Jørgensen[1], Børge G. Nordestgaard [1,2,3], Anne Tybjærg-Hansen[1,2,3].

[1]Departments of Clinical Biochemistry, Rigshospitalet and Herlev Hospital; [2]The Copenhagen General Population Study; [3]The Copenhagen City Heart Study.

Background: Loss-of-function mutations in *APOC3* associates with low risk of ischemic vascular disease(IVD). We tested to what extent this was mediated by low plasma levels of remnant cholesterol or low-density lipoprotein cholesterol(LDL-C).
Methods: We first determined the levels of remnant cholesterol and LDL cholesterol in *APOC3* heterozygotes versus noncarriers in meta-analyses of more than 137,000 individuals. Second, we determined whether the contribution of LDL cholesterol to IVD risk was masked by lipid-lowering therapy. Finally, using mediation analysis we determined the fraction of the observed lower risk of IVD and ischemic heart disease(IHD) in heterozygotes vs noncarriers which was mediated by remnant cholesterol and LDL-C, respectively.
Results: In meta-analyses, *APOC3* heterozygotes(n=776) had 43%(95% CI: 39%-46%) lower levels of remnant cholesterol, and 4%(2%-6%) lower levels of LDL-C compared with noncarriers. In the general population, LDL-C observed in heterozygotes vs noncarriers were 3% lower in individuals overall, 4% lower in individuals corrected for lipid-lowering therapy, and 3% lower in untreated individuals(P=0.06 to 0.008). Remnant cholesterol mediated 37%(31%-43%) of the observed 41% lower risk of IVD and 54%(45%-62%) of the observed 36% lower risk of IHD; corresponding values mediated by LDL-C were 1%(0%-2%) and 2%(0%-3%).
Conclusions: The lower risk of IVD observed in *APOC3* heterozygotes is mainly explained by the associated low levels of remnant cholesterol, and not by low LDL-C. This suggests that *APOC3* and remnant cholesterol are important new targets for reducing residual cardiovascular risk.

ACC/AHA guidelines superior to ESC/EAS guidelines for primary prevention with statins: The Copenhagen General Population Study

Martin B. Mortensen[1], Børge Nordestgaard[2], Shoaib Afzal[2], Erling Falk[1]

[1]*Department of Cardiology, Aarhus University Hospital*
[2]*The Department of Clinical Biochemistry, Herlev and Gentofte Hospital*

Aim: We compared the 2013 American College of Cardiology/American Heart Association (ACC/AHA) and the 2012 European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines on prevention of atherosclerotic cardiovascular disease (ASCVD) using different risk prediction models (US Pooled Cohort Equations (US-PCE for any ASCVD) and European Systematic COronary Risk Evaluation system (European-SCORE for fatal ASCVD)) and different statin eligibility criteria.

Methods and results: We examined 44,889 individuals aged 40-75 in the Copenhagen General Population Study. We detected 2217 any ASCVD events and 199 fatal ASCVD events through 2014. The predicted-to-observed event ratio was 1.2 using US-PCE for any ASCVD and 5.0 using European-SCORE for fatal ASCVD. The US-PCE, but not the European-SCORE, was well-calibrated around decision thresholds for statin therapy. For a class I recommendation, 42% of individuals qualified for statins using the ACC/AHA guidelines versus 6% with the ESC/EAS guidelines. Using ACC/AHA- versus ESC/EAS-defined statin eligibility criteria led to a substantial gain in sensitivity with a smaller loss in specificity: for class I recommendations, the binary net reclassification index (to treat or not to treat) was +0.27 for any ASCVD and +0.40 for fatal ASCVD. Similar differences between the ACC/AHA and ESC/EAS guidelines were found for men and women separately, and for class IIa recommendations.

Conclusions: The ACC/AHA guidelines were superior to the ESC/EAS guidelines for primary prevention of ASCVD, that is, for assigning statin therapy to those who would benefit the most.

Participates in Young Investigator Award

Maternal metabolic outcomes six years after pregnancy in women with a history of hypertensive pregnancy disorders

Jeanine Roeters van Lennep[4],Laura Benschop[4],Sarah Schalekamp -Timmermans[4], Vincent Jaddoe[2,3],Monique Mulder[1],Nienke Bergen[4], Eric Steegers[4]

[1]Dep of Internal Medicine, [2]Dep of Epidemiology, [3]Dep of Paediatrics [4]Dep of Obstetrics and Gynaecology, Erasmus MC, Rotterdam, the Netherlands

Aims: To identify metabolic risk factors six years after pregnancy in women with gestational hypertension (GH) and preeclampsia (PE).
Methods and results: In this population-based cohort study 4933 women were prospectively followed from pregnancy to six years postpartum. Weight and blood pressure were measured during pregnancy. Six years after pregnancy we measured weight, blood pressure, plasma lipid concentrations (triglycerides, Apolipoprotein-B (Apo-B), total cholesterol, glucose, HDL-c, LDL-c , lipoprotein (a) (lp (a)), total body and abdominal fat distribution. Compared with normotensive pregnancies, women with GH had higher levels of triglycerides (0.08mmol/L;95%CI 0.02, 0.15), Apo-B (0.05g/L;95%CI 0.02, 0.08), total-cholesterol (0.14mmol/L;95%CI 0.002, 0.28) and LDL-c (0.12mmol/L;95%CI 0.03, 0.21) and lower concentrations of HDL-c (-0.06mmol/L;95%CI -0.11, -0.01) after pregnancy. No differences were observed in lipid levels between normotensive and PE women. Women with GH and PE were at increased risk of diagnosis of metabolic syndrome (MS) (OR 2.6; 95%CI 1.7, 4.0 and OR 3.6; 95%CI 1.9, 6.8, respectively) compared with controls. Early pregnancy systolic and diastolic blood pressure and weight were independently associated with increased odds of diagnosis of the MS.
Conclusion: Hypertensive pregnancy disorders, especially GH, are associated with increased odds of diagnosis of metabolic risk factors after pregnancy compared to women with normotensive pregnancies. Early pregnancy blood pressure and weight can be novel predictors of diagnosis of the MS after pregnancy.

A novel long residence time CCR2 antagonist inhibits atherogenesis in apoE deficient mice

I. Bot[1], N. Ortiz Zacarias[2], H. de Vries[2], D. van der Velden[1], J. Kuiper[1], D. Stamos[3], A. IJzerman[2], L. Heitman[2]

Divisions of [1]Biopharmaceutics and [2]Medicinal Chemistry, LACDR, Leiden, The Netherlands. [3]Vertex Pharmaceuticals, San Diego, USA.

CC Chemokine Receptor 2 (CCR2) and its ligand CCL2 are involved in the development of atherosclerosis. CCR2 antagonists blocking the CCL2-CCR2 interaction have been developed as potential therapeutic agents, however to date the *in vivo* efficacy of these antagonists is very limited. Characterization of the drug-target residence time (RT) in early phases of research has been suggested to lead to a better prediction of *in vivo* efficacy. In this study, we thus aimed to determine whether the long RT CCR2 antagonist 15a is effective in inhibiting atherogenesis.

Carotid artery atherosclerosis was induced by collar placement in apoE$^{-/-}$ mice, followed by treatment with 15a (RT=714 min, 150 µg/day) or control. After 4 weeks, atherosclerotic plaques were analyzed.

At sacrifice, circulating CCR2^{+} monocyte numbers were reduced in the 15a-treated mice (controls: 14.9±3.2*10^3, 15a: 4.5±1.0*10^3 cells/mL, P<0.05). Carotid artery plaque size was reduced from 64.4±11.8*10^3 µm^2 in control mice to 17.6±4.1*10^3 µm^2 in 15a-treated mice (-73%, P<0.01). In the aortic root, 15a inhibited atherosclerosis from 252±25*10^3 µm^2 in controls to 157±15*10^3 µm^2 (-38%, P<0.01). Relative plaque macrophage content in the carotids was significantly decreased in 15a-treated mice (controls: 46±4%, 15a: 25±8%, P<0.05), and in the aortic root from 35±4% to 23±4% (P<0.05).

In conclusion, we have established that the long RT CCR2 antagonist 15a displays therapeutic efficacy in inhibiting atherosclerotic plaque development in mice. These data render 15a a promising compound for drug development and establish that RT is a key parameter to include in the drug development process.

The water channel AQP1 is associated with human atherosclerotic vascular lesions and attenuates progression of atherosclerosis in males independent of blood pressure

Pamela Wintmo*, Søren H. Johansen*, Pernille B.L. Hansen*; Jes S. Lindholt#, Sigitas Urbonavicius†, Lars M. Rasmussen*#, Peter Bie*, Boye L. Jensen*, & Jane Stubbe*

*University of Southern Denmark; # University Hospital of Odense; †Viborg Hospital

The water channel aquaporin 1 (AQP1) supports tumor growth by facilitating endothelial cell migration in tumor neovascularization. We tested the hypothesis that AQP1 promotes neovascularization of growing vascular lesions and aneurysms using human vascular tissue and AQP1-/-Apolipoprotein E (ApoE)-/-mice. AQP1 was localized in the neovasculature in human atherosclerotic lesions and in human abdominal aortic aneurysm wall, while in internal thoracic arteries AQP1 was restricted to endothelial cells and adventitial vasa vasorum. In female ApoE-/- mice with lesion burden aortic AQP1 mRNA expression was augmented. AQP1 was localized in endothelial cells, vasa vasorum and intralesional cells at the aortic root. Lesion burden did not differ between AQP1-/-ApoE -/- and ApoE -/- after 8 and 16 weeks on western diet (WD, n =13-15). Male AQP1-/-ApoE-/- mice fed WD and treated with angiotensin II (ANGII) for 4 weeks had elevated atherosclerotic lesion burden compared to ApoE-/- (11± 1 vs. 4 ± 2 %, n =10-11, $p<0.02$). Freely moving AQP1-/-ApoE-/- and ApoE-/- mice showed similar blood pressure responses to ANGII i.v. infusion (60ng/kg*day for 7 d) measured by indwelling catheters (nighttime: 129 ± 4 and 128 ± 5 mmHg, respectively). It is concluded that AQP1 is associated with intralesional neovasculature in humans and mice. AQP1 accelerates ANGII-induced atherosclerosis in male mice independent of blood pressure.

HDL Cholesterol Efflux Does Not Predict Cardiovascular Risk in Hemodialysis Patients

S. Ebtehaj[1], C. Kopecky[2], B. Genser[3], C. Drechsler[4], V. Krane[4], M. Antlanger[2], J.J. Kovarik[2], C.C. Kaltenecker[2], J. Werzowa[2], M. Hecking[2], M. Parvizi[1], C. Wanner[4], T. Weichhart[5], M.D. Säemann[2], U.J.F. Tietge[1]

[1]Dept. of Pediatrics, Univ. Medical Center Groningen, Groningen, The Netherlands; [2]Dept. of Internal Medicine III, Div. of Nephrology and Dialysis, [5] Inst. of Med. Genetics, Medical Univ. of Vienna, Austria; [3]BGStats Consulting, Vienna, Austria; [4]Dept. of Medicine, Div. of Nephrology, Univ. Hospital Würzburg, Germany

The cardioprotective effect of HDL is largely determined by its cholesterol efflux capacity, which was shown to correlate inversely with atherosclerotic cardiovascular (CV) disease in populations with normal kidney function. Patients with end-stage renal disease suffer an exceptionally high CV risk not fully explained by traditional risk factors. Here, we investigated in a *post-hoc* analysis in 1147 patients with type 2 diabetes mellitus on hemodialysis participating in the 4D Study (The German Diabetes Dialysis Study), if the HDL cholesterol efflux capacity is predictive of CV risk. Efflux capacity was quantified by incubating human macrophage foam cells with apolipoprotein B-depleted serum. During a median follow-up of 4.1 years n=423 patients reached the combined primary endpoint (composite of cardiac death, nonfatal myocardial infarction and stroke), n=410 experienced cardiac events and n=561 died (all-cause mortality). Strikingly, in Cox regression analyses we found no association of efflux capacity with the combined primary endpoint (hazard ratio [HR], 0.96; 95% confidence interval [CI], 0.88 - 1.06, *p*=0.417), cardiac events (HR, 0.92; CI, 0.83-1.02; *p*=0.108) or all-cause mortality (HR 0.96; 95% CI, 0.88-1.05; *p*=0.390). In conclusion, HDL cholesterol efflux capacity is not a prognostic CV risk marker in diabetic patients on hemodialysis.

Participates in Young Investigator Award

Oral presentations – Abstracts

Session II

Inflammation and Vascular Biology - oral presentations

Depletion of the Androgen Receptor in Osteoblasts Protects Against Abdominal Aortic Aneurysms in Male Mice

Marta Lantero Rodriguez[1], Anna S Wilhelmson[1], Inger Johansson[1], Elin Svedlund[1], Camilla Alexandersson[1], Johan B Fagman[2], Åsa Tivesten[1]

[1]*Wallenberg Laboratory, Institute of Medicine;* [2]*Dept of Surgery, Sahlgrenska Academy, Göteborg, Sweden*

Background and Aim: Male sex is a strong risk factor for abdominal aortic aneurysms (AAA), which are 4-5 times more common in men vs. women. The male sex hormone testosterone increases AAA frequency in experimental studies and we have shown that mice with a general androgen receptor (AR; the receptor for testosterone) knockout (G-ARKO) mice are protected from AAA, but the mechanism is unclear. G-ARKO mice show reduced neutrophil migration to sites of inflammation. Osteoblasts play an important role in bone marrow neutrophil homeostasis. To test the hypothesis that testosterone affects AAA formation via an osteoblast/neutrophil-mediated mechanism, we studied neutrophil migration and AAA in mice with depletion of the AR specifically in osteoblast-lineage cells (O-ARKO mice).
Methods and Results: In a sterile peritonitis model, mice were given thioglycollate i.p.; FACS analysis after 4h revealed a 40% reduction in migrated peritoneal neutrophils in G-ARKO vs. controls. This effect was mirrored in O-ARKO mice (36% reduction). To study AAA formation, mice on ApoE-deficient background received 1000 ng/kg/min of angiotensin II between 8 and 12 weeks of age. G-ARKO mice were protected against AAA formation, showing an AAA frequency of 19% vs. 62% in the control mice. This protection was mimicked in O-ARKO, which showed an AAA frequency of 22% vs. 64% in controls.
Conclusions: Depletion of the AR in osteoblast-lineage cells protects against experimental AAAs in male mice. These data suggest that testosterone may increase AAAs in males by modulating neutrophil biology that, in turn, is mediated by osteoblasts in the bone marrow.

Participates in Young Investigator Award

β-Cyclodextrin reduces cholesterol crystal –induced inflammation through modulating complement activation

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Aim
Cholesterol crystals (CC) are found to be abundant in atherosclerotic plaques and we have previously shown that CC initiate an inflammatory response via the complement system and inflammasome activation. Cyclic oligosaccharide 2-hydroxypropyl-β-cyclodextrin (BCD) is a compound that solubilizes lipophilic substances and is commonly used in pharmaceuticals. BCD is reported to increase cholesterol solubility and to promote the removal of cholesterol from foam cells. However, it remains unknown whether BCD has any effect on crystalline cholesterol.
Results
BCD attenuated the CC-induced inflammatory cytokine response (e.g. CCL3, TNF, IL-6) as well as regulated a range of CC-induced genes in human peripheral blood mononuclear cells. BCD binds to CC and this may be the mechanism behind the reduced deposition of complement factors C1q, C3c and TCC on CC observed in human plasma in presence of BCD. Furthermore, BCD decreased complement activation as measured by terminal complement complex (TCC) and the expression of complement receptors in response to CC stimulation in human whole blood. BCD did not affect formation of TCC by mono sodium urate crystals or zymosan. Of interest, after 1 hour of incubation, BCD started to dissolve the CC.
Conclusions
These data demonstrate that BCD is an inhibitor of CC-induced inflammation, which might be explained by BCD-mediated attenuation of complement activation. Thus, BCD is a potential candidate for treatment of atherosclerosis.

Participates in Young Investigator Award

Extracellular lipid particles accumulating in human carotid artery intima induce sterile inflammation in macrophages

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Rationale: Carotid atherosclerosis is a risk factor for ischemic stroke. Accumulation of extra- and intracellular lipid deposits is characteristic to atherosclerotic lesions. This study aimed at characterization of the extracellular lipid particles in carotid atherosclerotic plaques and examining their proinflammatory potential.
Methods and Results: We isolated and characterized extracellular lipid particles from human carotid atherosclerotic plaques. The extracellular particles were found to contain apoB and they were larger than plasma VLDL, yet smaller than the intracellular lipid droplets of foam cells. The particles contained oxidized epitopes and they were enriched in unesterified cholesterol. We also visualized the carotid intima with 3D-electron microscopy (3D-EM). 3D-EM showed cholesterol crystals, which were connected to the extracellular lipid particles. The extracellular lipid particles, and LDL modified *in vitro* to resemble the composition and morphology of the particles in carotid plaques, induced activation of the NLRP3 inflammasome.
Conclusions: The extracellular lipid particles show signs of modifications by oxidation and lipolysis and connect with cholesterol crystals in the lesions. The lipid particles can induce inflammasome activation, and may so actively contribute to the inflammatory component of atherogenesis.

Effect of the GLP-1 analogue liraglutide on atherosclerosis and kidney fibrosis in moderate uremia.

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Chronic kidney disease (CKD) leads to accumulation of waste products in plasma, i.e. uremia. CKD is characterized by progressive formation of kidney fibrosis and loss of kidney function, which in turn leads to a progressive increase in risk of atherosclerosis and cardiovascular death. To enable treatment and prevention of both kidney fibrosis and atherosclerosis in CKD patients, insight into `risk-increasing-effects' of uremia on both parameters is warranted. The GLP-1 analogue liraglutide improves glucose homeostasis and is approved for treatment of type 2 diabetes. Animal studies suggest that GLP-1, in addition, can dampen both inflammation and atherosclerosis.
The aim of the present study was to examine the effect of liraglutide treatment on kidney fibrosis and atherosclerosis in a mouse model of moderate uremia.
Moderate uremia was induced by 5/6-nephrectomy in LDLr-/- mice (n=29) and sham-operated controls (n=14). 4 weeks later, mice were treated with liraglutide (270 nmol/kg, s.c. once daily) or vehicle for 12 weeks.
Liraglutide significantly attenuated plaque formation by 40 % ($p < 0.05$) in the uremic mice. Examination of the remnant kidneys by flow cytometry and gene expression suggested that moderate uremia induced kidney inflammation by increasing the number of monocyte-like cells ($CD68^+F4/80^-$), $CD4^+$, $CD8^+$ T-cells. Furthermore, markers of fibrosis (i.e. Col1a1 and Col3a1) were upregulated indicating a fibrotic environment. Liraglutide treatment attenuated the increase in immune cells, but did not affect fibrosis marker expression. This study in mice suggests that liraglutide has beneficial effects in moderate uremia by reducing atherosclerosis and attenuating kidney inflammation.

Participates in Young Investigator Award

Association between anti -citrullinated protein antibodies and long-term mortality in patients with ST-segment elevated myocardial infarction

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Background. Cardiovascular (CV) mortality is higher in patients with rheumatoid arthritis (RA), in particular when anti -citrullinated protein antibodies (ACPA) are present. Recently, ACPA have also been described in patients without RA, but with CV disease. It is however unknown if ACPA is associated with mortality in these patients. The purpose of this study was to assess the relation between ACPA and long-term mortality in patients with ST-elevated myocardial infarction (STEMI) without RA.
Methods. All patients with STEMI from the MISSION! Intervention Study in 2004-2005 were analyzed. Patients with RA were excluded. The association between ACPA (anti-CCP3) at baseline and long-term mortality was investigated.
Results. In total, 30 (10%) of 290 included patients were ACPA positive. Increased cumulative cardiac mortality was observed in these patients in comparison with ACPA negative patients. Corrected for age, ACPA positivity was independently associated with long-term mortality [HR 2.4 (CI 1.1-5.4) p-Value= 0.026].
Conclusion. Long-term mortality in STEMI patients without RA was independently associated with the presence of ACPA. ACPA in patients with and without RA might act as an independent pro-atherogenic factor.

Participates in Young Investigator Award

Group IIA secretory phospholipase A_2 (sPLA2-IIA) predicts graft failure and mortality in renal transplant recipients by mediating decreased kidney function

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The acute phase protein sPLA2-IIA is proatherosclerotic. The present study prospectively investigated whether plasma sPLA2-IIA levels associate with graft failure, cardiovascular (CV) and all-cause mortality in renal transplant recipients (RTRs), patients with accelerated atherosclerosis, both in graft and systemic vasculature.
In 495 RTRs (median follow-up 7.0 years) sPLA2-IIA determined at baseline was significantly higher in RTRs than healthy controls (median 384 vs. 185 ng/dL, P<0.001), but lower than in end-stage renal disease (median 1053 ng/mL, P<0.001). Kaplan-Meier analysis demonstrated increased risks for graft failure (P=0.002), CV (P<0.001) and all-cause mortality (P<0.001) with increasing gender-stratified quartiles of sPLA2-IIA. In Cox regression analyses sPLA2-IIA was strongly associated with increased risks of graft failure (hazard ratio(HR)=1.42, P=0.006), CV (HR=1.48, P=0.001) and all-cause mortality (HR=1.39, P<0.001). However, this association was largely explained by kidney function parameters. Further RTRs with higher baseline sPLA2-IIA had a faster decline in renal function during follow-up. In addition, kidney function in human sPLA2-IIA transgenic mice deteriorated more rapid over time compared with wild-type controls (urinary albumin:creatinine ratio at 48 weeks of age, P<0.01).
In summary, this prospective study demonstrates that sPLA2-IIA is a significant predictive biomarker for chronic graft failure, overall and CV mortality in RTRs dependent on kidney function. This dependency is explained by sPLA2-IIA negatively impacting kidney function over time in humans and transgenic mice.

Comprehensive metabolic profiling of statin therapy: longitudinal evidence and mendelian randomization

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Objectives: Statins are first-line therapy for cardiovascular prevention, but their systemic effects across lipoprotein subclasses, fatty acids, and circulating metabolites remain incompletely characterized. We sought to determine the molecular effects of statin therapy on multiple metabolic pathways.
Methods: Metabolic profiles based on serum NMR metabolomics were quantified at 2 time points in 4 population cohorts from the UK and Finland. Concentration changes in 80 lipid and metabolite measures during follow-up were compared between 716 individuals who started statins and 4,874 persistent nonusers. We further used Mendelian randomization to assess the corresponding metabolic associations of a genetic variant mimicking HMG-CoA reductase inhibition for 27,914 individuals.
Results: Starting statin therapy was associated with numerous lipoprotein and fatty acid changes including substantial lowering of remnant cholesterol (80% relative to LDL-C), but only modest lowering of triglycerides (25% relative to LDL-C). Among fatty acids, omega-6 levels decreased the most (68% relative to LDL-C); other fatty acids were only modestly affected. No robust changes were observed for amino acids, ketones, or glycolysis related metabolites. The intricate metabolic changes associated with statin use closely matched the association pattern with rs12916 in the HMGCR gene (R2=0.94, slope 1.00±0.03).
Conclusions: Statin use leads to extensive lipid changes beyond LDL-C and appears more efficacious for lowering remnant cholesterol than estimated based on triglyceride lowering. The results exemplify how detailed metabolic characterization of genetic proxies for drug targets can inform indications, pleiotropic effects, and pharmacological mechanisms.

Participates in Young Investigator Award

Oral presentations – Abstracts

Session III

Lipoproteins and Lipid transport – oral presentations

Unstable LDL - Novel mechanism of atherogenesis and link to cardiovascular deaths

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Aggregation of LDL particles is involved in atherogenesis, but studies providing mechanistic or clinical insights into the role of LDL aggregation are missing. We have previously demonstrated that aggregation-prone unstable LDL has an increased sphingomyelin/phosphatidylcholine (SM/PC)-ratio. By lowering the SM/PC-ratio, we were able to stabilize the particles in vitro, and this type of LDL stabilization in mice resulted in reduced atherosclerosis.
In an attempt to specify the mechanisms of LDL aggregation, we identified a specific domain in the C-terminal part of apoB-100 involved in particle aggregation. In the Finnish participants of the Healthy Nordic Diet -study we found that changes in diet correlated with changes in the SM/PC-ratio of LDL particles and in their stability. Thus, an increase in dietary polyunsaturated fatty acids and fiber decreased the ratio and stabilized LDL, while an increase in dietary sucrose caused the opposite. Finally, patients having >50% coronary stenosis (Corogene study) were followed for up to 2.5 years, and interestingly in them a low SM/PC-ratio in LDL was found to correlate with particle stability and to protect from cardiovascular deaths.
Taken together, measurement of LDL instability may serve as a predictive biomarker that can identify patients who are at high-risk for cardiovascular disease, and help in the development of new targeted treatments for atherosclerosis.

Participates in Young Investigator Award

A loss-of-function variant in *OSBPL1A* predisposes to low plasma HDL cholesterol levels and impaired cholesterol efflux capacity

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In subjects with HDL-C <1st percentile in the general population, we identified a heterozygous variant *OSBPL1A p.C39X* encoding a truncated protein fragment, that co-segregated with low plasma HDL-C. We investigated the composition and function of HDL from the carriers and non-carriers and studied the properties of the mutant protein in cultured hepatocytes.

Plasma HDL-C and apoA-I were lower in carriers versus non-carriers. Sera of the carriers displayed a reduced cholesterol acceptor capacity, whereas the acceptor capacity of their isolated HDL was normal. Fibroblasts from a *p.C39X* carrier showed reduced cholesterol efflux. GFP-OSBPL1A partially co-localized in endosomes with fluorescent apoA-I, suggesting that OSBPL1A may regulate the cellular handling of apoA-I. The GFP-OSBPL1A-39X protein remained cytosolic and failed to interact with Rab7 which recruits OSBPL1A on late endosomes/lysosomes, showing that the mutation represents a loss-of-function.

The present work represents the first report on a human *OSBPL1A* mutation; It suggests that a loss-of-function mutation in *OSBPL1A* affects the first step of the reverse cholesterol transport process associated with the low HDL-C phenotype, and bring up the possibility that rare mutations in *OSBPL* genes may contribute to dyslipidemias.

Increased lipoprotein binding to arterial proteoglycans and normal macrophage cholesterol efflux capacity characterize CKD dyslipidemia

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In chronic kidney disease (CKD), the decline of estimated glomerular filtration rate (eGFR) associates with increased cardiovascular mortality, which is not fully explained by the dyslipidemia developed by these subjects. Here we studied the association of eGFR decline with changes in lipoprotein composition, size and selected functionalities: for HDL, the cholesterol efflux capacity (CEC) and its antioxidant function; for LDL, the binding to arterial proteoglycans (PGs). Non-diabetic CKD patients, dialysis and statin naïve, were divided into 2 groups: eGFR>60 or eGFR≤30 mL/min/1.73 m^2. Patients with eGFR≤30 showed smaller LDL and HDL. In this group whole and apoB-depleted serum CEC via aqueous diffusion and SR-BI was lower, but CEC by ABCA1 higher. This was due to increased proportion of plasma small HDL and prebeta-HDL. Total macrophage CEC and HDL capacity to prevent LDL-oxidation did not differ between groups. The binding of plasma to human arterial proteoglycans was higher in patients with eGFR≤30. Hence, low eGFR associates with decreased size of LDL and depletion of large HDL particles, which in turn increase binding of apoB-containing lipoproteins to PGs. This suggests a potential mechanism for the increased cardiovascular mortality observed in CKD patients.

PCSK9 impacts obesity and metabolic syndrome development in animal models

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Objective - PCSK9, a liver-secreted plasma enzyme, primarily regulates the levels of circulating low-density lipoprotein cholesterol (LDL-C) by enhancing the degradation of the hepatic LDL receptor (LDLR). The emerging importance of PCSK9 inhibition for the treatment of hypercholesterolemia warrants investigation of the physiological role of PCSK9, beyond LDL-C lowering. As PCSK9 targets additional receptors, which could play a critical role in triglyceride-rich lipoproteins metabolism, aim of this study was to investigate the impact of PCSK9 in metabolic syndrome and obesity.
Methods and Results - Metabolic dysfunction was induced in 2-months old PCSK9 KO and WT male mice by HFD or SFD feeding for 20 weeks. As expected, the deletion of PSCK9 resulted in a significant reduction in plasma cholesterol levels (86,1±2,1 mg/dl vs 123,4±5,2 mg/dl and 51,8±12,3 mg/dl vs 79,8±11,0 mg/dl with HFD and SFD respectively, $p<0,05$), but not in plasma triglyceride levels. MRI analysis showed that PCSK9 KO mice accumulated significant more visceral adipose tissue than WT littermates (+50%±17% with HFD, $p<0,05$) with a trend towards increased weight gain. Further assessment of metabolic profile showed that PCSK9 deficiency resulted in impaired glucose tolerance compared to control mice (AUC for GTT +40%±9% for HFD, $p<0,05$), while the response to insulin was not affected.
Conclusion - Taken together our data indicate that, under impaired metabolic setting, PCSK9 deficiency results in reduced glucose tolerance and in increased visceral adipose tissue accumulation.

Ezetimibe in combination with simvastatin reduces remnant-cholesterol without affecting biliary lipid concentrations in gallstone patients

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Remnant-cholesterol, the cholesterol in triglyceride (TG)-rich lipoproteins, has been recognized as a causal risk factor for ischemic heart disease. NPC1L1 in the intestine is the target of ezetimibe, but its function in the liver is still unknown. To clarify the function of hepatic NPC1L1 we performed a randomized, single-blind, placebo-controlled trial and studied the molecular changes in lipoprotein, lipid and carbohydrate metabolism secondary to combined inhibition of cholesterol synthesis and intestinal cholesterol absorption. Forty cholesterol gallstone patients received 80 mg/day simvastatin, 10 mg/day ezetimibe, a combination of simvastatin and ezetimibe, or placebo for four weeks. Plasma, bile and liver biopsies were collected. Ezetimibe alone or in combination with simvastatin did not increase the % molar concentration of cholesterol in bile nor blunted the decrease due to simvastatin. Combination therapy led to a 70% decrease in cholesteryl esters ($p<0.001$) and a 50% decrease in TG ($p<0.01$) in the core of TG-rich lipoproteins. These dramatic changes were associated to a decrease number of TG-rich lipoproteins and were not seen in subjects treated with simvastatin or ezetimibe alone. In conclusion, the expected increase in biliary cholesterol following treatment with ezetimibe does not occur in humans, suggesting different functions of NPC1L1 (or ezetimibe action) in the liver and intestine. The results also help to explain why ezetimibe associated to simvastatin was more effective in reducing CVD events in the diabetic patients (IMPROVE-IT), known to have increased levels of TG-rich lipoproteins.

BCG lowers plasma cholesterol and delays atherosclerotic lesion progression

Andrea D. van Dam[1], Siroon Bekkering[2], Malou Crasborn[1], Lianne van Beek[1], Susan M. van den Berg[3], Vanessa van Harmelen[1], Menno P.J. de Winther[3], Esther Lutgens[3], Mariëtte R. Boon[1], Niels P. Riksen[2], Patrick C.N. Rensen[1], Jimmy F.P. Berbée[1]

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Background: Bacille-Calmette-Guérin (BCG), prepared from attenuated live Mycobacterium bovis, modulates atherosclerosis development as currently explained by immunomodulatory mechanisms. However, its effect on plasma cholesterol, the main driver of atherosclerosis, has remained underexposed. We aimed to elucidate the effect of BCG on inflammation, cholesterol metabolism and atherosclerosis in APOE*3-Leiden.CETP mice, a model of human-like lipoprotein metabolism.
Methods and Results: APOE*3-Leiden.CETP mice were fed a 0.1% cholesterol-containing diet, i.v. injected with BCG, and terminated 6 weeks thereafter. BCG induced mycobacterial infection and hepatomegaly. The enlarged liver (+53%, $p<0.001$) coincided with severe immune cell infiltration and a higher cholesterol content (+31%, $p<0.05$). Circulating and tissue immune cells showed a more inflammatory phenotype. Moreover, BCG reduced plasma cholesterol levels (-34%, $p<0.01$) and accelerated plasma clearance of cholesterol from i.v. injected [14C]cholesteryl oleate-labeled VLDL-like particles (+41%, $p<0.01$) as a result of elevated hepatic uptake (+25%, $p=0.05$). Ultimately, BCG decreased foam cell formation (-22%, $p<0.05$) and delayed atherosclerotic lesion progression in the aortic root of the heart. BCG tended to decrease lesion area (-59%, $p=0.08$) and tended to increase the percentage of undiseased segments (+155%, $p=0.09$), and reduced lesion severity as indicated by an increased number of mild type 1 lesions (+99%, $p<0.05$).
Conclusion: Despite inducing immune activation, BCG delays atherosclerotic lesion formation by reducing plasma cholesterol levels.

Participates in Young Investigator Award

FGF21 lowers plasma triglycerides by accelerating lipoprotein catabolism in white and brown adipose tissues

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Aim: FGF21 decreases plasma triglycerides (TGs) in rodents and humans, however, the underlying mechanism(s) are unclear. In the present study, we examined the role of FGF21 in production and disposal of TG-rich lipoproteins (TRL) in mice.
Methods: To study the role of FGF21 in lipoprotein and glucose metabolism, recombinant FGF21 protein was injected acutely and chronically. Lipid and glucose organ distribution was investigated by the use of radiolabelled TRL, glucose or fatty acids as well as different genetic mouse models lacking either the fatty acid transporter CD36 or lipoprotein lipase specifically in adipose tissue. VLDL production was studied by i.v. injection of the lipase inhibitor Tyloxapol.
Results: Treatment with pharmacological doses of FGF21 acutely reduced plasma non-esterified fatty acids (NEFA), liver TG content and VLDL-TG secretion. In addition, metabolic turnover studies revealed that FGF21 facilitated the catabolism of TRL in white adipose tissue (WAT) and brown adipose tissue (BAT). FGF21-dependent TRL processing was strongly attenuated in CD36-deficient mice and transgenic mice lacking lipoprotein lipase in adipose tissues. Notably, insulin resistance in diet-induced obese and ob/ob mice shifted FGF21 responses from WAT towards energy combusting BAT.
Conclusions: FGF21 lowers plasma TG through a dual mechanism; first, by reducing NEFA plasma levels and consequently hepatic VLDL lipidation and second, by increasing CD36 and LPL dependent TRL disposal in WAT and BAT.

Participates in Young Investigator Award

Unravelling the mechanism and importance of brown adipose tissue whole lipoprotein uptake

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Aim:
Brown adipose tissue (BAT) has been shown to be a major player of lipid metabolism contributing to the effective reduction of plasma lipid levels. BAT does not only take up postprandial lipids via LPL-mediated uptake of fatty acids, but is also able to take up whole lipoprotein particles. However, the exact mechanism and physiological significance of this process still remain elusive.
Methods:
We used radioactive uptake experiments in genetically modified mice as well as marker-based cellular fractionation of BAT to investigate the role different BAT cell types in lipid handling. In addition, we used both real-time intravital microscopy as well as immunofluorescence and transmission electron microscopy to decipher the fate of internalized lipoprotein particles.
Results:
Cold exposure led to a marked, CD36 dependent, increase in uptake of both fatty acids as well as whole lipoprotein particles into BAT endothelial cells. However, while fatty acids were further transported to the adipocytes, lipoprotein core labels remained within the endothelial cells indicating intracellular processing of the lipoprotein particles. Microscopic analysis suggested lysosomal processing of lipoprotein particles.
Conclusion:
BAT endothelial cells appear to play a major role in the regulation of lipid transport in BAT. Whole lipoprotein particles can be taken up by endothelial cells, were they presumably undergo intracellular lysosomal degradation. This process may be required to supply BAT with PPARγ agonists such as PUFAs as well as cholesterol required for membrane plasticity during BAT expansion.

Participates in Young Investigator Award

Oral presentations – Abstracts

Session IV

Other Topics – oral presentations

The body mass index value associated with the lowest mortality increased from 1976-78 through 2003-13: three cohort studies of the general population

Shoaib Afzal, Anne Tybjærg-Hansen, Gorm B. Jensen, and Børge G. Nordestgaard.

Aim: We tested the hypothesis that the body mass index (BMI) value that is associated with the lowest all-cause mortality has increased in the general population over a period of 3 decades.
Methods: We analysed three cohorts from the same general population: the Copenhagen City Heart Study 1976-1978 (N=13704) and 1991-1994 (N=9482), and the Copenhagen General Population Study 2003-2013 (N=97362). BMI was modelled using splines and in categories, and models were adjusted for age, sex, smoking status, cumulative tobacco consumption, alcohol consumption, leisure-time physical activity, income, and plasma cholesterol.
Results: The BMI value that was associated with the lowest all-cause mortality was 23.7 kg/m^2 (95% confidence interval, 23.4-24.3) in 1976-1978, 24.6 kg/m^2 (24.0-26.3) in 1991-1994, and 27.0 kg/m^2 (26.5-27.6) in 2003-2013. The corresponding estimates for cardiovascular mortality were 23.2 kg/m^2 (22.6-23.7), 24.0 kg/m^2 (23.4-25.0), and 26.4 kg/m^2 (24.1-27.4), and for other mortality 24.1 kg/m^2 (23.5-25.9), 26.8 kg/m^2 (26.1-27.9), and 27.8 kg/m^2 (27.1-29.6). The multivariable adjusted hazard ratios for all-cause mortality for BMI ≥30 vs. 18.5-24.9 kg/m^2 were 1.31 (1.23-1.39) in 1976-1978, 1.13 (1.04-1.22) in 1991-1994, and 0.99 (0.92-1.07) in 2003-2013.
Conclusion: The BMI value that was associated with the lowest mortality increased 3 kg/m^2 over 3 decades. Further, the hazard ratio for all-cause mortality that was associated with BMI ≥30 kg/m^2 decreased from 1.3 to 1.0 during this period.

Cold Regulated Bile Acid Synthesis Shapes the Gut Microbiome

Anna Worthmann[1], Clara John[1], Miriam Baguhl[1], Malte Rühlemann[2], Femke-Anouska Heinsen[2], Alexander Bartelt[3], Andre Franke[2], Ludger Scheja[1], Joerg Heeren[1]

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Objective:
Brown Adipose Tissue (BAT) activation increases energy expenditure and stimulates food intake. While some nutrients are directly metabolized by BAT, increased intake of cholesterol may be harmful and therefore requires alternative metabolic routes. Here, we evaluate the effects of BAT activation on systemic cholesterol and bile acid (BA) metabolism.
Methods:
Mice were fed a Western type diet (0.2% cholesterol) and housed at 28°C or 4°C for 7 days to activate BAT. Cholesterol flux and gene expression studies, quantitative LC-ESI-MS/MS-based analysis of BAs as well as fecal microbiome profiling were performed.
Results:
After gavage of radiolabelled cholesterol, we observed a decrease in plasma and an increase in liver and BAT cholesterol indicative of an accelerated cholesterol flux. Notably, we found a 6-fold increased hepatic expression of genes responsible for the alternative pathway of BA formation accompanied by a 50-fold elevation of BAs in the liver and the feces. Elevated fecal BA levels were dependent on functional Cyp7b1, and hepatic lipoprotein clearance. 16S rRNA sequencing of the fecal microbiome revealed a distinct clustering pattern dependent on host housing conditions and fecal BA availability.
Conclusion:
Despite higher cholesterol uptake as a consequence of increased food intake, a systemic BAT-mediated program maintains cholesterol homeostasis by increased hepatic BA synthesis as well as fecal BA excretion. Hence, we assume a functional interplay between altered host BA levels, energy metabolism and gut microbiota.

Participates in Young Investigator Award

Altered cerebral cholesterol homeostasis contributes to cognitive decline in an Alzheimer's disease mouse model with diabetes mellitus

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Diabetes mellitus (DM) is associated with progressing cognitive decline and an increased risk of Alzheimer's disease (AD). How these complex disorders are interconnected remains unclear. Emerging evidence points towards alterations in cholesterol metabolism during aging and in Alzheimer's disease. The ε4 allele of APOE, the major genetic risk factor of AD, increases the prevalence of AD in patients with DM. Apolipoprotein (Apo) E is best known for its role in cholesterol trafficking in circulatory lipoproteins and is also thought to fulfil such a role within the brain. We hypothesize that impediments in cholesterol metabolism provide the biological basis for cognitive decline and AD in DM patients.
Wild-type and AD (APPswePS1ΔE9) mice were treated daily with vehicle or with alloxan (60m/kg) to induce experimental T1DM. Subsequently, T1DM mice were or were not treated with long-acting insulin (Levemir, 24 and 48 nmol/kg) for a period of 2 weeks. Sterol analyses using LC-MS/MS and Q-RT-PCR was performed in hippocampus, cortex and cerebellum. Levels of cholesterol metabolites and precursors were reduced in the hippocampus and cerebellum of the T1DM mice, as compared to wild-type mice. Moreover, mRNA levels of key genes in lipid metabolism were affected by alloxan treatment. Treatment with Levemir partly restored the sterol profile and mRNA levels. Finally, cognitive decline in AD mice with T1DM could be partially restored by Levemir treatment. Results from this study strengthen the hypothesis that alterations in cerebral cholesterol metabolism contribute to the increased risk of AD in DM subject and that cognition of AD mice with T1DM can be restored by treatment with long-acting insulin.

LpPLA2 in GDM - A protective system for placenta and fetus?

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Aim: Gestational Diabetes Mellitus (GDM) and obesity are pregnancy conditions not only associated with low-grade inflammation, but also with oxidative stress in the placenta and fetus alike. In order to protect the feto-placental unit, anti-oxidative defense systems may have evolved. Placental macrophages (HBCs) may contribute to these systems. Lipoprotein-associated phospholipase A2 (LpPLA2) is an enzyme with unique substrate preference towards oxidized phospholipids (oxPL). Whether cleavage of oxPL by LpPLA2 results in inflammation is currently under discussion. Here we tested the hypothesis: LpPLA2 is secreted by HBCs, acts in the fetal circulation and is regulated by the inflammatory environment of the mother. **Methods:** LDL and HDL were isolated from cord blood plasma, LpPLA2 activity, ELISAs assessing oxidative stress and protein modifications were determined. LpPLA2 mass/activity was measured in HBC supernatant in control/diabetic cells and subsequently used in a Multiplex approach to identify cytokines. **Results:** In contrast to adults LpPLA2 activity was higher on fetal HDL than LDL. Fetal plasma LpPLA2 activity was increased by GDM, HDL-LpPLA2 positive correlated with fetal cord blood insulin. HBCs showed an increase in LpPLA2 protein/activity upon insulin treatment, glucose had no effect. In placental tissue LpPLA2 was found to be significantly elevated in GDM, this increase was paralleled by an increase in PAFR expression. **Conclusions:** LpPLA2 -upregulation by oxPL may represent a positive feedback loop. Maternal low grade pro-inflammatory conditions, i.e. GDM are associated with increased LpPLA2 activity in HBCs and offspring. We speculate that LpPLA2 represents an anti-oxidative defense system for placenta and fetus.

Soat2 depletion improves liver steatosis and insulin sensitivity

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Objective: The cholesterol esterifying enzyme acyl-Coenzyme A: cholesterol acyltransferase 2 (ACAT2), encoded by the *Soat2* gene, is exclusively expressed in enterocytes and hepatocytes. *Soat2-/- mice* do not develop atherosclerosis or diet-induced hypercholesterolemia, and accumulate less triglycerides and cholesteryl esters in the liver (hepatic steatosis) when fed a high fat-cholesterol diet.This together with the association between hepatic steatosis and insulin resistance, prompted us to investigate the role of ACAT2 in hepatic steatosis and its potential association with insulin sensitivity and to assess the effect of different diets.
Methods: Wild type and *Soat2-/-* mice were either fed a high-fat (but low in cholesterol), a high-carbohydrate, or a chow diet. Serum, liver, adipose and muscle tissues were analyzed. Oral glucose and insulin tolerance tests were performed. Plasma from humans bearing a rare exonic variant was analyzed.
Findings: *Soat2-/-* mice fed a high-fat diet for eight weeks developed less hepatic steatosis, were more insulin sensitive, and had lower expressions of hepatic membrane glucose transporter 2 (GLUT2) and fat specific protein 27 (FSP27, named Cidec in humans). Similar findings were present in *Soat2-/-* mice fed a high-carbohydrate diet for two weeks. Carrier of a rare exonic variant in Cidec (rs140125102, Pro73Thr) had lower insulin and HOMA-IR compared to non-carriers.
Conclusion: Genetic depletion of *Soat2* dramatically reduces hepatic steatosis and improves insulin sensitivity via downregulation of hepatic FSP27/Cidec expression independently of sex and diets.Targeting *Soat2* may thus be a strategy to treat cardiometabolic diseases.

Participates in Young Investigator Award

Non-alcoholic fatty liver disease as a cause of ischemic heart disease: a Mendelian randomization study and meta-analysis of 170,998 individuals

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Aim: We tested the hypothesis that a life-long increase in liver fat content and non-alcoholic fatty liver disease(NAFLD) is a causal risk factor for ischemic heart disease(IHD).
Methods: We tested whether increasing liver fat content(CT scan) and NAFLD(ICD-code) was associated with an increased risk of IHD in studies totaling 94,708 individuals of which 10,897 had IHD. We then tested whether a genetic variant in *PNPLA3* I148M(rs73809), a strong genetic risk factor for NAFLD, was associated with an increased risk of IHD.
Results: Risk of IHD increased with increasing liver fat content up to an odds ratio(OR) of 2.41(1.28-4.51) in individuals in the highest versus lowest quartile of liver fat (P-trend: 0.004). The corresponding OR for IHD in individuals with NAFLD was 1.65(1.34-2.04)($P=3x10^{-6}$). *PNPLA3* genotype was associated with increased liver fat content up to 5% in MM versus II homozygotes(P-trend: 0.0001), and with corresponding ORs of 2.03(1.52-2.70) for NAFLD, 3.28(2.37-4.54) for cirrhosis, but 0.95(0.86-1.04) for IHD(P-trend: $3x10^{-7}$, $4x10^{-12}$, 0.46). Furthermore, genetically increased liver fat did not associate with IHD in instrumental variable analysis(P=0.47). In meta-analysis totaling 29,064 IHD cases and 141,934 controls, the OR for IHD was 1.01(0.98-1.03) per *PNPLA3* I148M M-allele.
Conclusions: Lifelong, genetically increased liver fat content, does not associate with an increased risk of IHD.

Participates in Young Investigator Award

Liver-humanized mice exhibit lipoprotein-specific phenotypes when grafted with human hepatocytes from different donors

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Animal models are commonly used to study human metabolic diseases despite major differences in hepatic lipid metabolism. Liver-humanized mouse (LHM) is an improved model to study human lipoprotein metabolism, but details on donor-specificity are still lacking. Thus, we aimed to further investigate lipoprotein metabolism in LHM and to assess whether different human donors can affect the metabolic phenotype of this model.
Immune-deficient, fumarylacetoacetate hydrolase (Fah)−/−, Rag2−/− and Il2rg−/− deficient mice on the non-obese diabetic background were repopulated with human hepatocytes from two different female donors. Separation of serum lipoproteins by size exclusion chromatography showed significant differences in total cholesterol, triglyceride and phospholipid levels depending on the human donor. LHM had lower HDL and higher LDL and VLDL levels compared to wild types, thus shifting the LDL:HDL ratio towards a human-like lipoprotein profile. Moreover, we observed circulating levels of Lp(a) in LHM. Surprisingly, LHM was lacking serum CETP activity. Serum cholesterol efflux capacity to mature HDL was tested in macrophages, showing statistical differences based on the human donor. No differences were found between the two groups when macrophages were cultured with cAMP to assess the efflux via ABCA1.
Detailed characterization of lipoprotein metabolism in LHM showed imprinting of the human donor. This suggests LHM as a useful model for translational medicine and personalized studies on hepatic lipid metabolism.

This work was supported by the EU, grant agreement no. Health-2013-INNOVATION-1-602757 (HUMAN).

Participates in Young Investigator Award

Poster presentations – Abstracts

Session I

Cardiovascular Disease – poster presentations

Systemic lupus erythematosus flare up is associated with increased carotid intima-media thickness progression

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AIM
To investigate in SLE patients the association of immunoinflammatory disease activity and of classical cardiovascular risk factors (CVRF) with 5 years c-IMT progression (deltac-IMT).
METHODS
Clinical history, anthropometric, biochemical parameters were collected at baseline and follow-up in 50 SLE patients and age-gender matched controls. SLEDAI score and disease flare-up during follow-up were noted. Ultrasound c-IMT was available.
RESULTS
SLE patients with high basal SLEDAI score presented faster deltac-IMT versus controls (0.007 (0.006) mm/year vs 0.003 (0.001) mm/year respectively, P= 0.026); deltac-IMT was not associated neither with CVRF, nor with lupus serology. During the 5 years follow up, disease flare-up were more frequent with high SLEDAI (P= 0.037) and with faster deltac-IMT compared to low disease activity (0.008 (0.004) mm/year vs -0.006 (0.004) mm/year, P= 0.021).Elevated LDL-C levels were the only CVRF associated with disease flare-up; this might be consequent to aggressive immunosuppressant therapy.
CONCLUSIONS
SLE patients present increased deltac-IMT, which is associated more to disease activity rather than CVRF, supporting a role for the inflammatory response during vascular disease progression in patients with autoimmune diseases.

Participates in Young Investigator Award

Maternal hypercholesterolemia during pregnancy and offspring pre-pubertal cardiovascular risk factors

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Background: Direct evidence of in utero and childhood atherogenesis in relation to maternal gestational hypercholesterolemia has been described by Napoli and co-workers. Purpose: To investigate the associations between an unfavorable intrauterine environment characterized by high maternal levels of plasma lipids and markers of cardiovascular disease risk in children 6-13 years after birth. Methods: We measured cardiovascular risk factors in children of healthy women with hypercholesterolemia or hypocholesterolemia during pregnancy (Low-density lipoprotein (LDL) cholesterol above or below the 90th or 10th percentile, respectively). Results: Sixty-one mothers were included, of which 27 and 34 had hypercholesterolemia and hypocholesterolemia during pregnancy, respectively. There were no differences in mean birth weight of the children in the two groups. Children of hypercholesterolemic mothers were younger, 9.4 versus 10.4 years ($P < 0.05$). There were no significant differences in body weight, BMI, visceral fat, hip fat or total body fat, blood pressure or pulse between groups. Interestingly, LDL cholesterol was significantly higher in the children whose mothers had hypercholesterolemia during pregnancy, 2.3 versus 1.9 mmol/L ($P < 0.01$). Serum fatty acid composition of the children at follow-up is presently being analyzed. Conclusions: Children of women with high LDL cholesterol in early and late pregnancy have high LDL cholesterol levels at age of 10 years.

Participates in Young Investigator Award

Relative deficiency of Apolipoprotein M and Sphingosine 1-phosphate in Type 1 Diabetes

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Type 1 diabetes (T1D) patients have an increased risk of heart disease despite high prevalence of increased HDL-concentration (HDL-C), suggesting the possibility that HDL may be dysfunctional. ApoM is the main carrier of sphingosine 1-phosphate (S1P), a phospholipid believed to exert many of the cardio-protective effects of HDL. The aim of this study was to measure apoM and S1P and their relationship to HDL in T1D as novel indices of HDL functionality in this disease. We measured levels of apoM by ELISA and S1P by LC-MS/MS in 43 controls and 89 T1D patients in plasma and isolated HDL subpopulations. There was no difference in plasma levels of apoM or S1P between controls and patients. However, when expressed per unit HDL-C (apoM/HDL-C) the values were lower in both T1D men and women as compared to controls. In T1D cases with high HDL-C (>85 % percentile of normal HDL-C), the apoM levels were increased but the apoM/HDL-C ratio was decreased compared to those having HDL-C <85 % percentile. In addition, the ratio between S1P and apoM was lower in patients with high HDL-C. In controls, apoM and S1P were mostly present in the smaller and medium sized HDL-particles whereas in T1D there was a shift of apoM and S1P towards larger particles. In conclusion, these data indicates that HDL particles in T1D patients have a relative deficiency of apoM and S1P per unit HDL-C, especially in those with high HDL-C. The functional significance of this deficiency remains to be determined.

Participates in Young Investigator Award

Current treatment status and treatment goal attainment among Norwegian FH patients

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Introduction: Subjects with Familial hypercholesterolemia (FH) have increased cholesterol levels and increased risk of premature CVD and early mortality. Treatment target for FH subjects are guided by LDL cholesterol levels, which in adults should be treated ≤ 2.5 mmol/L or ≤ 1.8 mmol/L in high-risk individuals.

Aim: The aims of the project were to investigate if currently available treatment modalities achieve recommended treatment targets in FH patients.
Method: A retrospective registration of medical journal data from FH patients treated at the Lipid Clinic, from January through October 2014, was performed.
Results: Data from 400 adult FH subjects were included in the present study. Ninety-six patients were categorized as high-risk according to EAS guidelines. High-risk patients were older, diagnosed later and started statin treatment at an older age than non-high-risk patients. High-risk patients more often received potent statin treatment and ezetemibe. Overall, treatment reduced LDL cholesterol from 6.5 to 3.5 mmol/L and from 7.5 to 2.9 mmol/L in non-high-risk and high-risk, respectively. Twenty-two percent and 9 % reached treatment goal of LDL cholesterol in the non-high-risk group, and high-risk group, respectively. Thirty-seven percent patients received maximal- or maximally tolerated treatment. Twenty-eight and 8 % of these reached treatment goal of LDL cholesterol in the non-high-risk group and high-risk group, respectively.
Conclusion: Overall treatment goal attainment was very low, but may partly be explained by patients not having reached maximal treatment yet, as illustrated by 41 and 51% of the patients had their lipid lowering therapy increased at their last visit, in high-risk and non-high-risk, respectively.

A low-carbohydrate, high-fat diet for severe epilepsy: Effects on weight and biomarkers of atherosclerosis

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The modified Atkins diet, a low-carbohydrate, high-fat diet, is a treatment option for adults suffering from drug resistant epilepsy (Kverneland et al. Epilepsy Behav 53: 197; 2015). The purpose of this study was to examine biomarkers for risk of atherosclerosis imposed by this diet as an adjuvant therapy to anti-epileptic drugs. Thirteen patients (12 w; range 16 – 57 yrs) were prospectively included from 2011-2014. They were instructed to eat maximum 16 g/day of carbohydrate and encouraged to eat high fat foods, and no intake limitation of protein or total energy. Body weight and blood samples were collected before diet start and after 12 weeks. Macronutrient intakes were obtained from a 3 days' weighed record in week 10 of the diet period. Apart from one study subject with congestive heart failure and atrial fibrillation, the subjects were otherwise healthy; in particular none had heart disease or diabetes. Six of the 13 participants completed the 12 weeks study period. The median daily energy intake was 1600 (750–1800) Kcal comprising a protein intake of 64 (36–90) g, a fat intake of 145 (61–155) g, and a digestible carbohydrate intake of 14 (13–18) g. For the 6 completers of the 12 week diet period, median weight reduction was 7.0 (4.3 - 8.1) kg while the median LDL cholesterol increased by 0.45 (0.0 – 0.7) mmol/L. Median HbA1c was reduced by 0.35 (-0.60 - -0.10) %. D-dimer, CRP, total cholesterol, HDL cholesterol and triglycerides were all unchanged. In conclusion, 6 patients completed the modified Atkins diet for 12 weeks. Median weight reduction was 7.0 kg. LDL cholesterol increased, while HbA1c was reduced.

Elevated remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease

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Aim: We tested the hypothesis that elevated remnant cholesterol is a risk factor for all-cause mortality in patients with ischemic heart disease.

Methods: We included 5414 Danish patients diagnosed with ischemic heart disease. Patients on statins were not excluded. Calculated remnant cholesterol was total cholesterol minus LDL and HDL cholesterol. During 35,836 person-years of follow-up, 1319 patients died.

Results: Multivariable adjusted hazard ratios for all-cause mortality were 1.5(95%CI: 1.2-2.0) for patients with calculated remnant cholesterol ≥1.5mmol/L compared to patients with remnant cholesterol <0.5mmol/L. Corresponding values were 1.2(95%CI: 1.0-1.5) for patients with measured remnant cholesterol ≥0.3mmol/L versus <0.05mmol/L and 0.9(95%CI: 0.8-1.2) for patients with LDL cholesterol ≥4mmol/L versus <2mmol/L. The cumulative survival was reduced in patients with calculated remnant cholesterol ≥1 versus <1mmol/L (log-rank, $p=9\cdot10^{-6}$; hazard ratio 1.3(1.2-1.5)), but not in patients with measured LDL cholesterol ≥3 versus <3mmol/L (p=0.76; hazard ratio 1.0(0.9-1.1)).

Conclusions: Elevated concentrations of both calculated and measured remnant cholesterol were associated with increased all-cause mortality in patients with ischemic heart disease, which was not the case for elevated concentrations of LDL cholesterol. This suggests that elevated concentrations of remnant cholesterol explain part of the residual risk of all-cause mortality in patients with ischemic heart disease.

Short-term effect of Low Carbohydrate High Fat diet on Lipid metabolism in heart tissues in female C57BL/6J mice

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Aim: Lipid accumulation in heart tissue correlates with cardiac dysfunction in metabolic diseases. Mechanism behind High-fat diets is not well understood. The present study aimed to determine whether short term of LCHF diet would impact on lipid metabolism in heart tissues in mice.
Method: Mice fed a LCHF diet containing 69% fat, 9% carbohydrate and 22% protein or chow diet for 2 weeks. They were pair fed with control. Body mass composition was measured daily by echoMRI. Hearts were collected for future analysis after 2 weeks.
Results: Fat mass increased while lean mass decreased remarkably in mice treated with LCHF compared to chow group. LPL activity decreased in the LCHF group. PPARGC1 (Peroxisome proliferator activated receptor gamma coactivator1) and ANGPTL4 (Angiopoietin-like 4) mRNA expression increased in LCHF group while LPL (lipoprotein lipase) and CD36 (Cluster of differentiation 36) were unchanged. TNFα (Tumor necrosis factor alpha) mRNA decreased in mice subjected LCHF diet.
Conclusion: Lipolysis is important to provide the heart with fatty acids which is catalysed by LPL, and regulated by ANGPTL4. PPAR-γ regulates lipid storage by controlling genes like LPL, CD36 and ANGPTL4. An increased PPAR-γ mRNA accompanied with higher ANGPTL4 expression and lower LPL activity when subjected to LCHF diet. Moreover TNF-α, regulated by PPAR-γ, decreased in LCHF group. Those findings suggest that the down regulation of lipolysis in heart play as a protective mechanism against lipid accumulation in cardiac myocytes may be regulated by PPAR-γ, and point towards an early onset of diabetic cardiomyopathy.

Is 1691G>A polymorphism within Factor V gene related to arterial ischemic stroke in children?

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Background: The Factor V 1691G>A polymorphism (Leiden mutation) causes activated protein C resistance which in turn limits clot formation. Most often studies analysing the role of genetic polymorphisms in paediatric arterial ischemic stroke (AIS) are performed on small groups of patients due to the rarity of the disease in children. Small sample size may lead to false positive or false negative results that is why we made a review and meta-analysis of available data addressing the association between FV 1691G>A polymorphism and AIS in children and compared the results to the adult patients.
Methods: We included to a study 12 case-control studies with a total number of 1665 patients with AIS (410 paediatric; 1255 adults) and 2297 controls (938 children; 1359 adults). Statistical analyses were conducted with MedCalc.
Results: The pooled analysis showed that carrier-state of 1691A allele is associated to AIS in children (p=0.036, OR=2.64 95%CI 1.07-6.52). There was no relation between *FV* 1691G>A polymorphism and the disease observed in case of adult patients (p=0.799).
Conclusions: The results based on a sizeable groups of subjects with arterial ischemic stroke, showed that 1691G>A polymorphism in *FV* gene is risk factor for paediatric ischemic stroke.

Replacement of dietary SFAs with PUFAs upregulates the mRNA expression levels of the LDL receptor and liver X receptor alpha: a double-blind randomized controlled trial

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Background and aim: Solid evidence indicates that replacing saturated fatty acids (SFAs) with polyunsaturated fatty acids (PUFAs) reduces total cholesterol (total-C) and LDL cholesterol (LDL-C) and thereby coronary heart disease events. The molecular mechanisms of the LDL-C lowering effects are however not completely elucidated. To further understand the molecular mechanisms, we examined the gene expression level of lipid related genes in peripheral blood mononuclear cells (PBMCs) in a human dietary intervention study.
Design: In an 8-week double blinded study, healthy adults (n=95) aged 25-70 years with moderate hypercholesterolemia were randomly assigned to **an** experimental diet group low in SFAs but high in n-6 PUFAs (Ex-group) or a control diet group high in SFAs but low in n-6 PUFAs (C-group). PBMCs were isolated at baseline and end of the study, and the mRNA gene expression analysis was performed using TaqMan Array Micro Fluidic Cards (Applied Biosystems) for RT-qPCR amplification.
Results: Exchanging SFAs with PUFAs reduced plasma total-C and LDL-C, increased LDL receptor (*LDLR),* liver X receptor alpha (*LXRA),* fatty acid synthase *(FASN)* and ATP binding cassette subfamily G member 1 *(ABCG1)* mRNA expression and reduced uncoupling protein 2 *(UCP2)* mRNA expression in PBMCs.
Conclusions: The main effect of replacing SFAs with PUFAs seems to be mediated through an upregulation of *LDLR* mRNA expression, subsequently increasing the mRNA expression of *LXRA* and *LXRA* target genes.

Peculiarities of T-cell immunity in carotid atherosclerosis

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Objective: Common carotid artery (CCA) is an elastic artery while the proportion of muscular component of artery wall increases towards the distal segments (internal carotid artery, ICA). The different incidences of atherosclerotic plaques with varying histological characteristics of elastic and muscular arteries were reported in pathological studies. We aimed to estimate the relationship between T-cell blood subsets and the abundance of carotid atherosclerosis in different segments of CA.
Materials and methods: 96 patients underwent duplex sonography to determine the degree of stenosis of distal segment of the CCA, CCA bifurcation or ICA. CD4+FoxP3+ and CD4+CD25highCD127low regulatory T cells (Treg), CD4+IFNgamma+ T-helpers (Th) 1, CD4+IL17+ Th17 blood frequencies were analyzed via direct immunofluorescence and flow cytometry.
Results: Patients with ICA stenosis >35% had increased Th17 (%CD4) and decreased CD4+FoxP3+ Treg/Th17 ratio vs. patients with minimal lesions of ICA (1.6% (1.0-1.8) vs. 1.0% (0.6-1.1) and 2.4 (1.1-3.4) vs. 4.5 (3.0-5.2), respectively, $p<0.05$), 50% stenosis and above was associated with decreased CD4+CD25highCD127low Treg levels (%CD4) compared to initial atherosclerosis (4.0% (2.8-5.5) vs. 5.4% (4.3-6.4), $p<0.05$). CD4+FoxP3+ Treg/Th17 ratio was lower in patients with CCA bifurcation stenosis >35% vs. patients with initial atherosclerosis (2.3 (1.1-2.9) vs. 3.5 (1.9-4.5), $p<0.05$). No differences in Th1 blood content were observed. The degree of CCA stenosis was associated with the conventional risk factors (body mass index) but not with the investigated immunological parameters.
Conclusion: The disturbances in the balance of pro- and anti-atherogenic T-lymphocyte subpopulations may promote atherosclerosis in distal (myoelastic) segments of carotid arteries.

Impact of Bifidobacterium bifidum PRL2010 on lipid metabolism in vitro and in vivo

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Background and Aim: In the recent years, besides the classical pharmacological strategies to reduce atherosclerosis disease, there are new approaches to decrease plasma cholesterol levels as the use of probiotic bacteria. Probiotics are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host. We investigated the cholesterol lowering activity *in vitro* and *in vivo* of several gut bifidobacterial strains aimed to identify the bacteria with high cholesterol uptake. Then we evaluated the cholesterol uptake trend of alive vs thermic inactivated *Bb*PRL2010, in order to investigate if the cholesterol uptake capability is transported mediated.
Methods: We detected the cholesterol uptake of 17 Bifidobacteria strains, after 3h incubation with ^{3}H-cholesterol into bacterial cultures. The cholesterol uptake trend is analysed, incubating alive and thermally inactivated bacteria with ^{3}H-cholesterolfor 28h. Afterwards we analysed the change of lipid profile in ApoE-/- mice after daily administration of 10^{9}cells of PRL2010 for 6 weeks.
Results and Discussion: A significant uptake capacity is observed for *Bb* PRL2010. The values of ^{3}H-cholesterol found in alive bacteria are between 0,101µCi(t=3h) and 0,131µCi(t=28h), while those one of thermic inactivated are between 0,066µCi(t=3h) and 0,093µCi(t=28h). In the lipid profile of ApoE-/- mice, we observe a decrease of total cholesterol (-27%) in the treated group, instead HDL and triglyceride did not change significantly. *Bb*PRL2010 could be a potential nutraceutical tool to modify the cholesterol levels in the humans.

Participates in Young Investigator Award

Cardiovascular disease in patients with FH in Norway

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We describe incidence and prevalence of CVD in statin treated FH patients. 5 538 FH patients were linked to data on all Norwegian CVD hospitalizations. During 1994-2009 a total of 1 411 FH patients were hospitalized. CHD, acute coronary syndrome or acute myocardial infarction was reported in 90% of all hospitalized FH patients. Mean (SD) age at first hospitalization and first re-hospitalization was 45.1 (16.5) and 47.6 (16.3) years, respectively, with no significant sex differences (p=0.66 and p=0.93, respectively). Median (25th and 75th percentile) number of hospital admissions was 4 (2-7) per FH patient, with no significant sex differences (p=0.87). The FH diagnosis was registered in only 45.7% of the patients at discharge. CONCLUSION: There were no sex differences in age at first hospitalization or re-hospitalization which is an important and novel finding. FH patients were about 20 years younger at first time hospitalization as compared to the general Norwegian population. The awareness and registration of the FH diagnosis during the hospital stays were low.

Poster presentations – Abstracts

Session II

Pentraxin 3 deficiency is associated with increased arterial thrombosis in animal models

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PTX3 is a biomarker of cardiovascular diseases and exerts protective functions in acute myocardial infarction and atherosclerosis. We investigated the role of PTX3 in arterial thrombosis.
PTX3KO showed a 60% reduction in carotid blood flow after FeCl3-induced thrombosis with a greater thrombus formation compared to 20% of WT mice, an effect mediated by PTX3 derived from non-hematopoietic cells:KO transplanted with BM from WT or KO presented a significant increased carotid occlusion compared to WT transplanted with BM from WT or KOmice.PTX3 plasma levels were not increased after arterial thrombosis and the protein localized with fibrin within the border of the damaged artery and the thrombus.The pro-thrombotic phenotype observed was independent on altered hemostasis, impaired platelet activation (P-sel and integrin αIIbβIII expression) and aggregation, modulation of P-sel activity as P-selKO/PTX3KO mice showed a significant increased arterial thrombosis compared to P-selKO. Platelet aggregation induced by collagen and fibrinogen incubated with PTX3 was significantly decreased, an effect depended mainly on the C-and N-terminal domain respectively of PTX3.Finally,exogenous administration of hrPTX3 reverted the pro-thrombotic phenotype in PTX3KO and improves the outcomes in WT mice after thrombosis.
PTX3 deficiency is associated with increased arterial thrombosis via modulation of collagen and fibrinogen thrombogenicity.

Participates in Young Investigator Award

IL10 secreting B cells decrease during progression of atherosclerosis and modulate the inflammatory response in LDLR$^{-/-}$ mice.

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Regulatory B cells (B_{regs}) can dampen the activation of T cells and dendritic cells and have shown great promise in a number of murine models of auto-immune disorders. A time-dependent decrease of B_{regs} and proB_{regs} was found when LDLR$^{-/-}$ mice were put on a western type diet, indicating that B_{regs} might also play a role in the development of atherosclerosis. Although it has been shown that depletion of B-cell derived IL10 does not result in more pronounced atherosclerosis, it was also accompanied by an increase in IL10 gene expression in the aorta. Indicating that the loss of B-cell specific IL10 might be compensated by other cell types. Therefore, we aimed to investigated the effects of adoptively transferred B_{regs} in LDLR$^{-/-}$ mice. While a large number of distinct markers have been attributed to B_{regs}, to date there is no definitive B cell subset in which all IL10-producing B cells are enriched. We therefore used a specialized IL10-secretion assay to isolate highly pure, live IL10-producing cells from splenic B cells that were activated for 48h with an agonistic CD40 antibody. LDLR$^{-/-}$ mice were put on a western type diet for 9 weeks and either received an i.v. injection with PBS, non IL10-secreting B cells or IL10-secreting B cells every three weeks. Preliminary results show that injection of IL10-secreting B cells resulted in a strong reduction in circulating lymphocytes, monocytes and neutrophils compared to control groups ($p<0.01$, $p<0.05$ and $p<0.001$ respectively). Additionally, circulating inflammatory Ly6C^{hi} monocytes and T helper 1 cells were also significantly reduced ($p<0.05$) all pointing towards a less inflammatory state which is usually associated with less atherosclerosis.

Participates in Young Investigator Award

The increased blood Th17 frequencies may contribute to carotid atherosclerosis progression

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T-cell-mediated immune responses were demonstrated to play a significant role in atherosclerosis (AS) initiation and progression. Regulatory T cells (Treg) are supposed to have a protective effect while proinflammatory IL-17-producing T-helper lymphocytes (Th17) may accelerate AS progression. We **aimed** to evaluate the predictive significance of Treg and Th17 frequencies and other blood lymphocyte subpopulations, in carotid AS.
Methods: 33 males were enrolled. Carotid ultrasound was performed at admission and in 1 year after the enrollment. AS plaques in the common (CCA) and internal (ICA) carotid arteries as well as the intima-media thickness (IMT) in the plaque-free distal segments of CCA were estimated. The progression of carotid AS was identified as a “newly” acquired AS plaque or the increase of stenosis in CCA or/and ICA by 5% or above. Peripheral blood lymphocyte phenotyping was performed by direct immunofluorescence and flow cytometry at the enrollment. Th1 were identified as CD4+IFNgamma+ cells, Th2 - CD4+IL4+, Treg - CD4+FoxP3+, Th17 - CD4+IL17a+ cells.
Results: In patients with carotid AS progression (n=18) the basal level of Th17 (% CD4+) was higher vs. patients without AS progression (1.4 (1.0-2.4) vs. 1.1 (0.8-1.5), respectively, $p<0.05$). The basal Treg/Th17 ratio was lower in patients with carotid AS progression than in patients without AS progression (4.0 (2.4-7.8) vs. 7.0 (5.1-10.8), respectively, $p<0.05$). However the estimated immune parameters did not show predictive value for IMT growth. No differences in Th1 or Th2 frequencies between groups were observed.
Conclusion: The imbalance between Treg and Th17 with relative prevalence of Th17 may reflect a predisposition to carotid AS plaques emergence and progression but not to increase of IMT.

Biological consequences of Endonuclease V ablation in mice

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Endonuclease V (EndoV) is a highly conserved protein that in prokaryotes is involved in removal of deaminated adenosine (inosine) from DNA. In contrast, studies of the human homologue identified human EndoV as a ribonuclease highly specific for inosine-containing RNA. Inosine is a natural component of RNA introduced by specific enzymes necessary for proper function of these transcripts. Several congenital disorders have been associated with imbalance in this adenosine-to-inosine editing in RNA. In order to study the biological importance of EndoV, we have created a mouse model with disrupted *EndoV* expression (*EndoV*$^{-/-}$ mice). Initial studies reveal a slight increase in triglycerides in the livers of adult *EndoV*$^{-/-}$ mice. Preliminary results from gene expression analysis and studies using primary cells from the *EndoV*$^{-/-}$ mice and human cell lines, also suggested disturbance in metabolic, inflammatory and immunological pathways. Furthermore, we found altered *EndoV* expression in human carotid atherosclerosis and NASH, two diseases characterized by metabolic imbalance, chronic inflammation and altered immune cell function.
Ongoing studies aim at characterizing the biological consequences following EndoV ablation in a novel mouse model. Here we will present some preliminary data and future perspectives, with emphasis on metabolism, inflammation and immune responses.

Participates in Young Investigator Award

Cellular model for studying the atherogenic mechanism

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One of the key aspects of atherogenesis is enhanced transport of lipoproteins, from the blood plasma into the arterial intima. Despite high interest of researchers all over the world to the study of the cellular and molecular background of atherosclerosis, the mechanisms of transendothelial transport are not well understood, thus the aim of our study was in vitro investigation of transendothelial transport.
Human EA.hy926 endothelial cells were cultured in DMEM medium in TransWells (BD Falcon) coated with type I collagen to form a confluent monolayer. The upper chamber of the system was filled with 5% normal human serum. The penetration of serum proteins into the lower chamber through the monolayer of endothelial cells was the subject of study. Incubations was carried out for 24 hr with sampling from the upper and lower chambers at certain intervals for analysis. Cellular uptake of the macromolecules has been evaluated by using a confocal microscope.
Using this system, we detected apoB, apoA-1, IgM protein molecules in the lower chamber, whose concentration constantly increased over time. The intensity of transendothelial transport was found to be influenced by the presence of histamine, C-reactive protein, ouabain in the upper chamber. Ouabain decreased, while histamine, CRP appeared to increase this measure. According to morphological studies and enzyme immunoassay, cells have been intensely uptaking labeled LDL and albumin. Chlorpromazine suppressed the uptake indicating that the mechanism of transport may implicate clathrin-dependent endocytosis.
These results suggest the adequacy of our in vivo model of transendothelial transport to investigation of endothelial barrier functioning and the mechanisms of protein and lipoprotein transendothelial transport during atherogenesis.

Participates in Young Investigator Award

The role of the DNA repair enzyme Neil3 in atherosclerosis

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Oxidative stress, most importantly reactive oxygen species (ROS), induces both nuclear and mitochondrial DNA damage. Atherosclerotic plaques are abundant in ROS which, if not counteracted, promote cellular damage and apoptosis leading to plaque instability. Attenuated DNA repair mechanisms may also be important contributors to non-resolving plaque inflammation and therefore influence the pathogenesis of atherosclerosis. Recently our group has shown that DNA glycosylases involved in the Base Excision Repair pathway (e.g. NEIL3) that remove oxidized DNA bases are differentially regulated during atherogenesis in human carotid atherosclerosis. In a follow-up study, we have shown in a mouse model that Neil3 deficiency in an *ApoE*$^{-/-}$ background decreases atherosclerosis in mice fed chow diet while atherosclerosis was increased in mice fed a high-fat diet. This increase was the result of increased amounts of cells accumulating in the vessel wall through influx or proliferation. Furthermore, an *in vitro* assay using non-stimulated aortic tissue of mice fed high-fat diet showed increased proliferation of vascular smooth muscle cells (VSMC) in *ApoE*$^{-/-}$*/Neil3*$^{-/-}$, as compared to *ApoE*$^{-/-}$. We hypothesize that NEIL3 has dual function acting both as a DNA repair enzyme, as well as, a checkpoint sensor for cell proliferation. Pathway analysis will be performed on microarray sequencing data to look further into molecular mechanisms causing these differences in expression patterns. The expression and function of NEIL3 will be further investigated in a culture-based model for proliferative and contractile VSMC.

The NLRP3 inflammasome mediates oxidative stress-induced pancreatic islet dysfunction

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The NLRP3 inflammasome has been linked to different types of oxidative stress, including that inflicted by ischemia and hypoxia, also to the development of diabetes, related to the central role of IL-1beta in the destruction of pancreatic islets. We therefore investigated how lack of inflammasome proteins influence survival of the beta-cells during oxidative stress.

Wild type (WT), $ASC^{-/-}$ and $NLRP3^{-/-}$ mice were exposed to Alloxan which is expected to cause oxidative stress when administered as multiple low dose injections, thereby selectively destroying insulin-producing beta-cells in the pancreas. We found that blood glucose was significantly reduced in both ASC and NLRP3 deficient mice compared to WT. Pancreata from the three genotypes were harvested for histological analysis. Macrophage staining was significantly reduced in $ASC^{-/-}$ and $NLRP3^{-/-}$ mice compared to WT, suggesting that the beneficial effect of NLRP3 inflammasome deficiency on oxidative stress-mediated beta cell damage could involve reduced macrophage infiltration and decreased apoptosis. We therefore, to confirm this hypothesis, injected WT mice with Clodronate encapsulated in liposomes which causes macrophage reduction. We found that injections of clodronate liposomes caused macrophage depletion in WT mice islets. Additionally, the systemic levels of of inflammatory monocytes were reduced.

Conclusion: Deletion of the NLRP3 inflammasome protects beta-cells against oxidative stress-induced cell death. We hypothesize that the NLRP3 inflammasome acts as a sensor of oxidative stress in pancreatic islets and a mediator of destruction of insulin producing cells.

Depletion of conventional dendritic cells in atherosclerosis using the Zbtb46-DTR mouse model

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The precise role of conventional dendritic cells (cDC) in atherosclerosis remains unclear because selective cDC depletion models are lacking. Recently, a unique transcription factor was described for cDC (Zbtb46), enabling us to target this cell type in mice. We depleted cDC in Ldlr-/- mice transplanted with Zbtb46-DTR bone marrow and fed them a Western type diet for 18 weeks. To maintain cDC ablation, chimeras received 4 ng diphtheria toxin (DT)/g.bw i.p. 2x/week.
Chronic cDC depletion did not alter cholesterol levels or immune cells (T-B-NK cells, neutrophils, monocytes) in blood, spleen or lymph nodes. Analysis of depletion efficiency showed induction of cDC death in vitro but analysis in vivo demonstrated that reoccurring splenic cDC no longer express DTR. Nonetheless, total DC were decreased and qPCR analysis showed reduced Batf3 expression in spleens from DT treated chimeras. Furthermore, cDC depletion affected the size of early but not advanced plaques. Except for a decrease in macrophages, plaque composition was not altered, as no changes were observed in smooth muscle cell content, collagen, T cells or apoptosis. Strikingly, a strong reduction in pDC and NKT cells was observed in blood, spleen and LN after chronic DT treatment. Splenic expression of E2-2 and Runx2, two regulators of pDC commitment, was increased in DT treated chimeras.
Taken together, the lack of a major atherosclerosis phenotype in Zbtb46-DTR-->Ldlr-/- chimeric mice is possibly due to compensatory mechanisms to sustain the adaptive immune response.

Participates in Young Investigator Award

ER stress conditions lead to an upregulation of TFPI and is associated with an anti-apoptotic macrophage phenotype.

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Aim of the study

Endoplasmic reticulum (ER) stress is an important event during the initiation and clinical progression of several cardiovascular diseases, including atherosclerosis. In the present study we determined the role of tissue factor pathway inhibitor (TFPI) under ER stress conditions using human monocyte-derived macrophages (PBMC) and human carotid endarterectomies.

Results and conclusion

TFPI was expressed in carotid plaques from patients with internal carotid stenosis, especially the TFPIα isoform. Moreover, TFPI colocalized with the ER stress marker CHOP in the plaques. Cholesterol crystals (CC) were used in human M1 and M2-polarized macrophages *in vitro* as an inducer of ER stress. CHOP and TFPI mRNA levels were upregulated after CC treatment, especially in the M2 phenotype. The ER stress inhibitor 4-Phenylbutyric acid (PBA) reversed the CC-mediated upregulation of CHOP and TFPI. Furthermore, M2-polarized macrophages incubated with CC had higher levels of Bcl-2:Bax mRNA ratios and Bcl-2 protein expression, and these effects were reversed by TFPI knockdown and PBA treatment. Our results indicate that TFPI could promote survival of the M2-polarized macrophages under ER stress conditions. These findings may have implications for the pathogenesis of atherosclerosis.

Participates in Young Investigator Award

Poster presentations – Abstracts

Session III

Oxidation of LDL by iron at lysosomal pH leads to the formation of tryptophan radicals which is not inhibited by probucol

Feroz Ahmad and David S. Leake

LDL oxidation is inhibited by interstitial fluid, however, and large clinical trials have shown no protection by antioxidants, including probucol. We therefore proposed that LDL might be nonoxidatively modified and aggregated by enzymes, such as sphingomyelinase, in interstitial fluid, rapidly phagocytosed by macrophages and oxidised by iron inside lysosomes, which have a pH of about 4.5. Tryptophan in apoB-100 might be involved in the initiation of LDL oxidation by copper. We investigated the mechanisms of LDL oxidation by iron at lysosomal pH. LDL (50 µg LDL protein/ml) was oxidised by $FeSO_4$ or $FeCl_3$ (5 µM) at 37 °C in 150 mM NaCl/10 mM sodium acetate buffer, pH 4.5. Lipid oxidation was measured in terms of conjugated dienes at 234 nm and tryptophan oxidation by the loss of fluorescence (Ex/Em 282/331 nm).

Unexpectedly, both lipid peroxidation and loss of tryptophan fluorescence was faster with Fe^{2+} than Fe^{3+}. Interestingly, probucol did not inhibit lipid oxidation for about 100 min with Fe^{2+} at pH 4.5 and did not prevent the loss of tryptophan fluorescence. Fe^{2+} was completely consumed during 100 min of LDL oxidation at pH 4.5, as shown using bathophenanthroline and might generate superoxide radicals. Superoxide radicals would protonate at pH 4.5 to produce highly reactive and lipid-soluble hydroperoxyl radicals, which might abstract hydrogen atoms form lipids and tryptophan. As probucol would be expected to scavenge lipid radicals in the surface monolayer of LDL particles, the initial oxidation of LDL at pH 4.5 would be expected to happen in the core of LDL where probucol has limited excess. We propose the following mechanism for LDL oxidation by Fe^{2+} at lysosomal pH.

$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$

$O_2^{\bullet -} + H^+ \leftrightarrow HO_2^{\bullet}$ (pK_a 4.8)

$HO_2^{\bullet} + TrpH \rightarrow Trp^{\bullet} + H_2O_2$

$Trp^{\bullet} + O_2 \rightarrow TrpOO^{\bullet}$

$HO_2^{\bullet} + LH \rightarrow L^{\bullet} + H_2O_2$

$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$

Effects of moderate and excess alcohol consumption on the reverse cholesterol transport in vivo

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Epidemiological studies revealed that moderate and binge alcohol consumption exerts opposite effect on cardiovascular disease. Atherosclerotic cardiovascular disease is inversely correlated with reverse cholesterol (RCT), the process promoting the removal of excess cholesterol from arterial wall.
We aim to evaluate whether moderate and binge alcohol consumption may differently impact RCT in an animal model of atherosclerosis-prone mice.
RCT was measured through a standardized, radiolabeled technique in apolipoprotein E knockout mice: placebo group (n=9) received water, mimicking the abstainers; moderate group (n=10) received 0.8g/kg alcohol/day for 28 days, mimicking a moderate alcohol consumption; binge group (n=10) received 0.8g/kg alcohol/day for 5 days, followed by the administration of 2.8g/kg alcohol/day for 2 days/week, mimicking a binge alcohol consumption.
Binge alcohol consumption caused an increase of 37.2 % in plasma total cholesterol and an increase of 44.35 % in HDL-C levels versus placebo group. Binge group also showed an increase of 30.95% in LDL-C and an increase of 23% in triglycerides compared to placebo group. Conversely, moderate consumption does not affect plasma lipoprotein profile. The removal of radioactivity from macrophages along RCT pathway was higher in the moderate group (12.2%±3.1, 15.1%± 3.7, 13.3%±2.4 in placebo, moderate and binge group respectively).
In conclusion, moderate alcohol consumption promotes the removal of cholesterol from macrophages along RCT pathway.
Conversely binge alcohol consumption exerts deleterious effects on lipoprotein profile, but it does not seem to significantly affect RCT process.

Participates in Young Investigator Award

Disturbed skin lipid composition and organization in hyperlipidemic ApoE KO mice

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Purpose. Alterations in skin lipid composition is associated with an altered lipid organization and, consequently, an impaired barrier functionality. Here, it was investigated whether hyperlipidemic apolipoprotein E knockout (ApoE KO) mice, a well-established atherosclerosis mouse model, shows changes in skin lipid composition and organization.
Results. Plasma levels of free and total cholesterol were respectively 10 and 15-fold (p<0.0001) higher in ApoE KO mice than in C57BL6 wild-type (WT) mice. Plasma triglycerides were increased 2-fold in the KO group (p<0.0001). Epidermal lipids were isolated from the back skins by a modified Bligh and Dyer method. Qualitative LC-MS lipid analysis showed similar ceramide subclasses in both experimental groups. In contrast, the variety of (unsaturated) free fatty acids (FFAs) present in WT mice is limited compared to ApoE KO mice. ApoE KO mice contain saturated (C14-C26) and unsaturated (C14:1-C26:1) FFA chains while most of these lipids were not detected in WT mice. Fourier transformed infrared analysis showed orthorhombic lateral lipid organization in the skins of both groups with slightly less dense lipid packing in the KO group. Interestingly, skin cryosections of ApoE KO mice showed an increase in the number of mast cells/monocytes. This coincided with higher mRNA levels of mast cell chymase tryptase-2, although no evident physical changes were evident in the skins of these animals upon gross examination.
Conclusions. Hyperlipidemia in ApoE KO mice alters skin lipid composition and organization associated with an increased inflammatory state. Further studies will be performed to investigate how these changes affect skin barrier function.

Evaluation of HDL cholesterol efflux capacity (CEC) after consumption of an innovative food enriched with bioactive components and functional probiotics

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Epidemiological evidence indicate that high intake of whole grain is associated with a reduced risk of cardiovascular disease. This study aimed to investigate the effect of consumption of a new functional whole grain on serum HDL-CEC, a metric of HDL functionality, recently emerged as a new biomarker for cardiovascular risk evaluation. 40 healthy volunteers were randomly assigned to two treatments: 1.Experimental pasta made with whole-wheat flour enriched in β-glucan from barley and spores of *B.coagulans GBI-30,* 2.Control pasta produced with the same technological process and with the same, but not integral, variety of wheat as the functional one. CEC measurement was performed *ex vivo* on whole plasma collected from subjects before and after three months of treatment. Individual cholesterol efflux pathways were evaluated by using widely accepted cell-based radioassays. In our study, we observe an improvement in ABCG1 CEC after treatment with the innovative pasta. Despite no change in HDL concentration, a rearrangement of their composition can occur with enhancement of their function. Additionally, ABCG1-mediated CEC of treated subjects inversely correlates with homocysteinemia, an independent risk factor for coronary disease, while a direct significant relation was found with plasmatic folic acid. Importantly, these correlations were absent in placebo group. Clinical relevance of our results refers to the indication that ABCG1 plays a fundamental role in cholesterol efflux process. Moreover, its activity is related to activation of intracellular anti-inflammatory signaling pathways.

Participates in Young Investigator Award

Role of a Cytoprotective Protein on Cholesterol Efflux in Human Macrophage Foam Cells

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Deposition of cholesterol-laden macrophages in the arterial intima is a characteristic feature of atherosclerosis. Therefore, intracellular transport and efflux of cholesterol is very critical for cellular and whole body cholesterol homeostasis (Shao B.Z. et al., APS, 37, 2016).
In this study, we have studied the effect of a well-characterized cytoprotective protein (Protein X) on cholesterol efflux in primary human macrophage foam cells. Exogenously applied recombinant Protein X caused a decrease in cholesterol ester levels in macrophage foam cells compared to control. Furthermore, recombinant Protein X treatment, before and during cholesterol efflux, resulted in increased ABCA1 levels in primary human foam cells. Live cell imaging has been performed to gain further insights into the process of cholesterol efflux from foam cells. We will discuss the results obtained in the presence of recombinant Protein X in the presentation.

Participates in Young Investigator Award

Mitochondrial function in brown adipose tissue is rapidly diminished by high fat diet

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Background and aim: Mitochondria in brown adipose tissue (BAT) burn fatty acids to produce heat, thereby contributing to energy expenditure. In diet-induced obese (DIO) mice, less and dysfunctional mitochondria are present in BAT accompanied by reduced cold tolerance. The aim of the current project is to unravel the rate and mechanism by which high fat diet (HFD) induces mitochondrial dysfunction in BAT in the course of DIO development, with the eventual goal to discover novel therapeutic avenues in obesity.
Methods and results: 12-week-old C57Bl/6J mice were fed a HFD (45% of calories derived from fat) for 0, 1, 3 or 7 days (n = 10 per group). The HFD increased body fat mass at 3 and 7 days. Of note, 1 day of HFD already increased BAT weight and lipid droplet content. This was accompanied by reduced uptake of [^{3}H]oleate derived from glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles by BAT, suggesting that reduced mitochondrial activity rather than enhanced fatty acid uptake underlies the increased lipid content in BAT. Accordingly, HFD decreased mRNA expression of the mitochondrial biogenesis master regulator *Pgc1α* in BAT, an effect that could be mimicked by treatment of brown fat cells with oleate *in vitro*. Additionally, HFD increased expression of *Opa1, Mfn2* and *Fis1* in BAT, implicating alterations in mitochondrial dynamics.
Conclusion: Short term HFD rapidly reduced BAT function as reflected by reduced uptake of fatty acids by BAT and rapidly increased BAT weight, accompanied by a marked reduction in *Pgc1α* and a gene expression profile consistent with an increase in mitochondrial fusion.

Participates in Young Investigator Award

Short-term cold exposure modulates human VLDL and HDL metabolism

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Background and aim: Upon cold activation, brown adipose tissue (BAT) combusts intracellular triglycerides (TG) into heat. Mouse studies showed that BAT consequently internalizes VLDL-TG-derived fatty acids to replenish intracellular TG stores (Hoeke, Circ Res 2016). Since the effect of cold-induced BAT activation on human lipoprotein metabolism remained elusive, we now assessed the effect of short-term mild cold exposure on the lipoprotein profile in men.

Methods and results: 10 lean adolescent white Caucasian males were exposed to a personalized cooling protocol (water cooling; average 9.9°C) for 3 h. Before and after cooling, serum samples were collected for analysis of lipids and lipoprotein composition using 1H-NMR. Mild cold exposure increased TG (+18%; $p<0.05$) as well as VLDL-C (+8%; $p<0.05$) and the VLDL particle concentration (+68%; $p<0.05$), suggesting that cold exposure increases hepatic VLDL production. At the same time, cold exposure increased HDL particle concentration (+9%; $p<0.05$) as well as HDL-C (+9%; $p<0.05$), specific for small HDL, and decreased the average HDL size (-0.1 nm), which is consistent with LPL-mediated generation of VLDL surface remnants that attract cholesterol from tissues into the circulation.

Conclusions: Short-term cold exposure modulates human lipoprotein metabolism by enhancing hepatic VLDL production as well as LPL-mediated VLDL-TG processing. This is likely a consequence of enhanced uptake of TG-derived fatty acids by BAT, resulting in the generation of surface remnants as precursors of HDL that enhance cholesterol efflux into the circulation.

Participates in Young Investigator Award

Regulation of HDL-c levels via ABCA1: a role for *LRP1*

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Aim: The genetic architecture of HDL-c deficiencies is well-acknowledged through rare mutations in *ABCA1, APOA1 and LCAT*. However, recent studies in liver-specific *Lrp1* KO mice have indicated that LRP1 also modulates plasma HDL-c levels via an ABCA1-mediated pathway. So far, a role for LRP1 in human HDL metabolism has not yet been elucidated. This study focuses on the molecular mechanism underlying a low HDL-c phenotype in heterozygotes for *LRP1* mutations.
Methods: Targeted-sequencing efforts in individuals with extremely low HDL-c levels (<0.6mmol/L) identified the first 2 missense mutations in *LRP1*, c.9730G>A (p.V3244I) and c.11949G>T (p.E3983D). Both *in-vitro* (overexpression and deficient cell lines) and *ex-vivo* (patient-derived fibroblasts) approaches have been employed to evaluate their functional importance and study the molecular mechanisms regulating ABCA1 plasma membrane localization.
Results: Mutant LRP1 proteins are significantly reduced by 50% and less stable when compared to wild-type. Notably, ABCA1 cell surface localization has been shown to be significantly decreased by 60% in carrier fibroblasts and LRP1-deficient cells. Molecular evidence supports the notion that alteration of both lysosomal enzyme CathepsinD and Prosaposin levels affect ABCA1 plasma membrane presence.
Conclusion: It is shown that in humans as in mice, LRP1 affects intracellular trafficking of ABCA1 which could explain low HDL-c in our study subjects.

Participates in Young Investigator Award

Brown and beige adipocyte activity controls metabolic flux through the HDL compartment

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Brown adipose tissue (BAT) is the primary organ for heat production in small mammals in response to cold and it is also present and active in humans. Previously we demonstrated that BAT activation 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. Lipoproteins were isolated from BAT-activated wild-type and adipocyte-specific LPL knock-out mice and their composition was determined by high-resolution, full scan mass spectrometry. Metabolic turnover studies were performed 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 isolated from BAT-activated mice displayed a characteristic lipidomic fingerprint, which was associated with increased macrophage-to-feces cholesterol transport. Mechanistically, we show that lipolysis by adipocyte LPL is the driving force of HDL remodeling and increased HDL turnover. Our findings corroborate the notion that systemic metabolic flux regulated by the high metabolic activity of thermogenic adipocytes determines the atheroprotective properties of HDL.

Participates in Young Investigator Award

G protein-coupled receptor 120 signaling activates brown adipocytes

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Background: Brown adipose tissue (BAT) has been shown to contribute to total energy expenditure in human adults, which makes it an attractive target to combat obesity and related disorders. Recent studies have shown that the G protein-coupled receptor 120 (GPR120) may influence BAT activity. Both GPR120-deficient mice and humans carrying a mutation associated with decreased GPR120 signaling are predisposed to obesity. The aim of this study was to investigate whether GPR120 signaling could have beneficial metabolic effects by increasing the activity of brown adipocytes.

Methods: To elucidate the role of GPR120 in BAT, murine brown adipocytes were stimulated with the GPR120 agonist TUG-891 (10 µM) for 5 min to measure protein phosphorylation (Western blot), and for 6 h to measure gene expression (qPCR). In addition, gene expression was compared between wild-type and GPR120-deficient brown adipocytes.

Results: GPR120 is highly expressed in BAT and browning by rosiglitazone induced an increase in GPR120 expression in subcutaneous white adipose tissue (+139%; $P<0.01$). TUG-891 strongly increased phosphorylation of ERK (+479%; $P<0.05$) in brown adipocytes. In addition, TUG-891 increased gene expression of adipocyte protein 2 (Ap2; +39%; $P<0.05$), and tended to increase gene expression of adipose triglyceride lipase (Atgl; +18%; $P=0.06$). GPR120 knockout cells have a diminished gene expression of uncoupling protein 1 (Ucp1; -97%, $P<0.05$), and Atgl (-22%, $P<0.05$).

Discussion: GPR120 signaling increases protein phosphorylation and gene expression of key molecules involved in lipolysis and thermogenesis in brown adipocytes. These data suggest that activation of GPR120 enhances BAT activity, which may therefore be a promising novel therapeutic strategy to increase energy expenditure and combat the obesity epidemic.

Participates in Young Investigator Award

Mechanisms behind ANGPTL3-deficiency induced hypolipidemia

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Aim: Angiopoietin-like protein 3 (ANGPTL3) -deficiency causes familial combined hypolipidemia (OMIM#605019), a rare phenotype with a low VLDL, LDL and HDL levels in plasma. Absence of ANGPTL3 in plasma contributes to hypolipidemia by enhancing TG-clearance via lipoprotein lipase (LPL) but whether ANGPTL3 -deficiency would affect lipoprotein production in the liver or in the small intestine has not been addressed in detail. Plasma concentration of ANGPTL3 is linked to TG and LDL-C levels in genome wide linkage studies, however, it is unclear, whether ANGPTL3 sequence variation can predict combined low TG and low cholesterol phenotype in humans.
Methods: We investigated whether silencing of ANGPTL3 would affect lipoprotein metabolism in human enterocytes (CACO-2) and human hepatocytes (IHH). We measured secretion rates of apoB, TG, cholesterol and phospholipids in the presence of glucose, fatty acids or in combination. We analyzed plasma ANGPTL3 concentration in two subsamples of Finnish (FINRISK study) subjects with either very low or high combined TG and cholesterol levels in plasma and in subjects with rare sequence variants in ANGPTL3 gene.
Results: Silencing of ANGPTL3 affects energy substrate utilization in hepatocytes by enhancing uptake of glucose and its use as a substrate for TG-formation. Hepatic fatty acid uptake and use in TG-lipogenesis or VLDL -lipoprotein secretion did not differ between silenced or control cells. Our preliminary results suggest that no major differences in TG -secretion were detected between ANGPTL3 silenced and non-silenced enterocytes.
Conclusions: Silencing of ANGPTL3 affects majorily hepatic glucose and lipid metabolism with non-existent or milder effects on enterocyte-derived lipid metabolism.

Participates in Young Investigator Award

Poster presentations – Abstracts

Session IV

Other Topics – poster presentations

Energy balance method and the adequate analysis of lipid content in the study of body composition.

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Energy balance method (EBM) consists of the direct application of the first law of thermogenesis into the analysis of body composition changes: somatic mass variations in a mouse or human are the result of energy intake minus energy expenditure during a given time interval. In this study, we applied the concepts of EBM to their full extent by revisiting metabolic measurements of laboratory mice, while converting macronutrients in the food, elements of body composition, and energy expenditure into a single workable unit (kcal). Body composition variations in animal models were better understood after the conversion of lean and fat mass into their respective energy contents (1 kcal/g and 9.4 kcal/g, respectively), thus unveiling the true effect of diets, ambient temperatures and pharmacological interventions on seemingly small changes in body weight. This conversion specially corrects the analysis for the true contribution of lipid content of an organism to its endogenous energy availability. Additionally, the EBM allowed us to directly compare information on body composition with the data gathered by indirect calorimetry and measurements of food intake, making possible to estimate e.g. daily turnover rates of an organism's energy stocks and to predict the time relationship between interventions and variations of total body weight. Moreover, EBM seem to open a yet unexplored avenue in lipid catabolism research, where heat generated by thermogenic processes, such as brown adipose tissue thermogenesis, are understood to temporarily affect the total energy content of the body. In summary, this new set of conceptual tools may allow scientists to achieve a broader understanding of metabolic balance in the body.

Participates in Young Investigator Award

Impact of fetal oxidative stress on the development of metabolic disease in adulthood

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Oxidative stress is a major contributing factor to the development and worsening of cardio-metabolic disease. Fetal oxidative stress, e.g. induced by maternal preeclampsia, is linked to an increased incidence of components of the metabolic syndrome in adult life. However, a mechanistic explanation for this observation is lacking. Therefore, the present study aimed to determine the effects of increased oxidative stress in utero on adiposity, glucose and cholesterol metabolism in adult offspring using a novel preclinical model

We crossed either male *Ldlr* KO mice with *Ldlr* KO dams heterozygous for *Sod2* (increased in utero oxidative stress, **ox**) or male *Ldlr* KO heterozygous for *Sod2* with *Ldlr* KO dams (**con**). Importantly, only *Ldlr* KO offspring were further investigated, Sod2 +/- offspring were excluded. At 12 weeks of age mice received Western diet for 12 weeks followed by assessment of metabolic parameters.

Upon dietary challenge male **ox** offspring developed lower body weight (-12% , $p<0.05$) compared to **con**; a similar trend was seen in females. Body composition analysis indicated lower adiposity in male **ox** mice. **Ox** mice from both genders had improved glucose tolerance and insulin sensitivity at 24 weeks, which in males was significant ($p<0.05$) already before Western diet feeding. Plasma lipids, cholesterol absorption, and synthesis as well as fecal neutral sterol excretion did not differ between groups.

In summary, increased in utero oxidative stress programs male **ox** offspring for reduced obesity and better glucose tolerance upon dietary challenge in adult life, while cholesterol homeostasis was not affected in either gender. Further research is carried out to delineate the molecular mechanisms of this observation.

Participates in Young Investigator Award

Influence of mothers´- knowledge, attitude and behavior on diet and physical activity of their children: a cross-sectional study from Nepal

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INTRODUCTION: Non-communicable diseases account for half of all deaths in Nepal; 25% is attributed to cardiovascular diseases. The aims of this study were to explore knowledge, attitude and behavior of mothers regarding diet and physical activity of their children.
METHODS: In a semi-urban area outside Kathmandu we interviewed mothers with children aged 2-7 years and explored socio-demographic characteristics as well as knowledge, attitude, and behavior regarding their own as well as their childrens' diet and physical activity.
RESULTS: Mean age was 28.9±4.5 years (n=962). Mothers with higher education and income had higher knowledge, attitude, and behavior scores ($p<0.001$). Housewives and farmers had low knowledge ($p<0.001$). They, along with laborers, exhibited lower attitude ($p<0.001$) and behavior scores ($p<0.001$). Children's diet score increased with mothers' level of education ($p<0.001$) and income ($p=0.041$). Their physical activity declined with increasing level of their mothers' education ($p<0.001$) and income ($p<0.001$). Children's overall behavior correlated poorly with mothers' knowledge ($r2=0.009$, $p=0.003$), attitude ($r2=0.012$, $p=0.001$), and behavior ($r2=0.007$, $p=0.008$). **CONCLUSIONS:** Poor correlation of mothers' knowledge and attitude with children's behavior suggests that improving mothers' knowledge or attitude is not sufficient to improve childrens' dietary and physical activity habits.

Using genetics to explore whether the cholesterol-lowering drug ezetimibe may cause an increased risk of cancer

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Aim: Results from randomized trials have raised concern that the cholesterol-lowering drug ezetimibe might increase the risk of cancer. We tested the hypothesis that genetic variation in *NPC1L1*, mimicking treatment with ezetimibe, was associated with an increased risk of cancer.
Methods: We included 67,257 individuals from the general population. Of these, 8,333 developed cancer and 2,057 died of cancer from 1968 to 2011. To mimic the effect of ezetimibe, we calculated a genotype score (from <2.0 to ≥5.0) of four *NPC1L1* variants based on the LDL cholesterol-lowering (NPC1L1-inhibitory) effect of each variant, and previously shown to associate with a reduction in risk of ischemic vascular disease (positive control). Finally, we tested associations between genotype score and risk of any cancer, death from any cancer, death after a cancer diagnosis, and risk of 27 cancer types.
Results: Cumulative incidence by age of any cancer or cancer death was not associated with genotype score (P-trend: 0.42 and 0.98). Hazard ratios for genotype scores ≥5.0 versus <2.0 were 1.04(0.92-1.19) for any cancer, 1.11(0.85-1.45) for cancer death, 0.92(0.73-1.14) for death after a cancer diagnosis (P-trend: 0.42, 0.81, 0.98) and 0.82(0.71-0.95)(P-trend=0.002) for ischemic vascular disease. The genotype score did not associate with risk of individual cancers (P-values≥0.15).
Conclusions: Lifelong, genetic inhibition of *NPC1L1*, mimicking treatment with ezetimibe, does not associate with risk of cancer.

Participates in Young Investigator Award

Evaluation of novel apoA-I two-site immunoassays

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It is believed that the heterogeneity of HDLs has an important role in the development of coronary artery disease (CAD). We have developed immunoassays for detection of possibly CAD specific HDL subspecies utilizing apoA-I antibodies showing difference in binding to HDL from CAD patients with MI and healthy individuals.
Three different two-site immunoassays were developed for measurement of apoA-I/HDL using scFv antibodies. The assays were evaluated with samples from individuals diagnosed after symptoms suggestive of acute coronary syndrome. Admission and leave samples from 99 individuals with STEMI or MI earlier, at sample collection or during the 540 days follow up and admission samples from 101 individuals of same age who had no MI diagnosis were analyzed (leave sample was analyzed if available and hospital stay was over 48 h). The number of freeze and thaw cycles of the samples was not known. Kaplan-Meier analysis was used to investigate the association between measured apoA-I/HDL value , survival rate and STEMI rate during follow up and compared to HDL-C.
During follow-up 34 (16.9 %) patients had STEMI and 29 patients (14,4%) died. The number of deaths in assay admission tertiles were: HDL-C 12 , 9 and 4; 110 assay 14, 7 and 8; 109 assay 15, 6 and 8; 22 assay 13, 7 and 9. Incidence of death broken down by assay leave tertiles was: HDL-C 10, 10 and 8; 110 assay 14, 8 and 6; 109 assay 11, 11 and 6; 22 assay 8, 10 and 10.
The novel apoA-I/HDL immunoassays seemed to predict survival better than HDL-C in the lowest tertile. For a firm conclusion a larger number of samples should be evaluated.

MBOAT7-TMC4 rs641738 Variant increases the risk of NAFLD in individuals of European descent

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Background: The rs641738 C>T variant in the M*embrane bound O-acyltransferase domain containing 7-Transmembrane Channel-Like 4* (*MBOAT7-TMC4*) locus increases the risk for cirrhosis in alcohol abusers. However, the impact of this variant on hepatic fat accumulation and on liver damage related to nonalcoholic fatty liver disease (NAFLD) is unknown.
Aim: The two strongest genetic determinants of NAFLD also increase the risk of alcoholic cirrhosis. Therefore, the aim of this work was to test whether the *MBOAT7-TMC4* is a susceptibility locus for the development and progression of NAFLD.
Methods: The rs641738 variant has been genotyped in 3854 individuals from a multiethnic population-based study, namely the Dallas Heart Study (DHS), and in 1149 individuals of European descent from the Liver Biopsy cross-sectional Cohort.
Results: We found that the rs641738 variant associates with increased hepatic fat content and more severe liver damage and fibrosis in individuals of European descent. MBOAT7, but not TMC4 is highly expressed in the liver. The *MBOAT7* rs641738 T allele is associated with lower protein expression in the liver.
Conclusions: In conclusion, we showed an association between rs641738 in the *MBOAT7* locus, and the development and severity of NAFLD in individuals of European descent. This association is mediated by a reduction in the hepatic protein expression of MBOAT7.

Participates in Young Investigator Award

Beta-cyclodextrin increases fecal sterol excretion and reverse cholesterol transport independent of the intestinal microbiota

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Non-digestible oligosaccharides are used in prebiotic formulations for their perceived health benefits, among these lowering of blood cholesterol levels. However, the mechanisms of action are incompletely understood.
The present study aimed to characterize the impact of ß-cyclodextrin (ßCD), a cyclic oligosaccharide, on cholesterol metabolism and reverse cholesterol transport (RCT) in mice dependent on the intestinal microbiota.
Feeding chow with 10% ßCD to conventional C57BL/6 wild-type mice significantly decreased plasma cholesterol levels by 40% ($p<0.05$), while fecal neutral sterol excretion increased 3-fold ($p<0.01$). Hepatic cholesterol levels and biliary cholesterol secretion were unaltered. Plasma bile acid levels and mass fecal excretion were unchanged, but the bile acid pool was more hydophobic in the ßCD group ($p<0.05$). These changes in cholesterol metabolism also translated into an increase in macrophage-to-feces RCT in ßCD-fed mice ($p<0.05$). In addition, to explore the dependency on the intestinal microbiota, ßCD was given to germ-free C57BL/6 mice. The key effects of ßCD could be replicated in germ-free animals, namely lowering of plasma cholesterol (40%, $p<0.05$) and increases in fecal neutral sterol excretion (7.5-fold, $p<0.01$) and RCT ($p<0.05$).
In summary, this study demonstrates that ßCD lowers plasma cholesterol levels and increases fecal cholesterol excretion from a RCT-relevant pool independent of the presence of an intestinal microbiota. The cholesterol-lowering effect of dietary ßCD is thus expected to translate into cardiovascular health benefits.

Impact of ezetimibe on lipid profiles in cardiac transplant recipients receiving statin: a meta-analysis

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Background: Heart transplantation is a standard treatment for end-stage failure of the organ. However, there are episodes of acute rejection of the transplanted graft due to accelerated coronary artery vasculopathy. Atherosclerosis, caused by hyperlipidemia is one of the factors determining the damage. Statin therapy is applied in the early period after heart transplantation to reduce the risk of vasculopathy. Some patients may develop a drug-resistant dyslipidemia, thus, for several years, studies on the effectiveness of ezetimibe and statins combined therapy are performed.
Methods: We searched MEDLINE and EMBASE from 2006 to 2016. The analysis included studies lasted 6 months that evaluated the effect of combination therapy of ezetimibe and statins on lipid profile in patients after heart transplantation. The studies qualified for the meta-analysis were assessed qualitatively. The dose of ezetimibe was 10 mg/day. Fixed-effects model meta-analyses were performed using MedCalc software in version 13.7.
Results: Ezetimibe/statins reduce total cholesterol (SMD -1.169 mg/dL; 95% Cl: -1.450 to -0.888; p<0.001), LDL cholesterol (SMD -1.006 mg / dL; 95% Cl: -1.282 to -0.731; p<0.001) and triglyceride compared to statins (SMD -0.336 mg/dL; 95% Cl: -0.598 to -0.0748; p=0.012). No significant change in HDL cholesterol levels was observed (SMD -0.119 mg / dL; 95% Cl: -0.511 to 0.273; p=0.549). It was found homogeneity of the analyzed data.
Conclusions: Ezetimibe enhances the effect of statins in lowering lipid levels, which in turn reduces the risk of vasculopathy in a transplanted heart.

Enzymatic 7-Ketocholesterol degtadation-A new strategy for the treatment of Atherosclerosis

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Background
7-ketocholesterol (7KC), an oxidized derivative of cholesterol, has been implicated in a variety of chronic diseases including atherosclerosis, Alzheimer's disease, Parkinson's disease, cancer and age-related macular degeneration. It is formed by the autooxidation of cholesterol and especially cholesterol-fatty acid esters found in lipoprotein deposits, its elevated concentrations are associated with disruption of cellular homeostasis, decreased cell viability, and increased cell death. Enzymatic cleavage of 7-KC can serve as a key solution for the cure of a number of chronic diseases directly associated with its accumulation.
Methods
Isolation of potential 7KC degraders was done from a diverse environmental samples. Molecular identification was done and HPLC analysis was carried out.
Results
Alcanivorax jadensis IP4 (accession number KP309836), isolated from sea water and sediment sample, Streptomyces auratus IP2 (accession number KP309837),Serratiamarcescens IP3 (accession number KP309838) isolated from soil, and ThermobifidafuscaIP1 (accession number KM677184), isolated from manure piles was found to effectively degrade 7-KC. All the isolates were capable of utilizing 7KC as the sole organic substrate, resulting in its mineralisation.
Further characterization of microbial genes and ultimately the enzymes involved in 7KC catabolism can lead to the development of a single potential therapeutic enzyme preparation to target number of above mentioned chronic diseases.

Anti-apoptotic effect of Apolipoprotein M (ApoM) associated Sphinsosine 1-phosphate (S1P).

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Several studies have pointed out the important contribution of endothelial cell apoptosis to atherosclerosis pathophysiology. High density Lipoproteins (HDL) attenuate endothelial cell apoptosis induced by different stimuli such as oxidation, inflammation or growth factor deprivation. Importantly, both apolipoprotein and lipid components contribute to the cytoprotective effects of HDL. The bioactive lysophospholipid Sphingosine 1-phosphate (S1P) accounts for the lipid contribution. In HDL, SP1 is bound to Apolipoprotein M (ApoM), a Lipocalin only presents in around 5% of the HDL particles. The goal of this study is to characterize ApoM bound S1P in endothelial apoptosis protection and the signaling pathways implicated in it. For that purpose, we cultured HUVECs in serum and growth factor deprivation medium with and without HDL+ApoM or HDL-ApoM. Cell viability remarkably decreased in serum / growth factor deprivation and apoptosis increased in a time-dependent manner. The addition of HDL+ApoM or purified recombinant ApoM bound to S1P protected HUVECs from serum / growth factor deprivation cell-death, whereas HDL-ApoM did not improve cell viability. S1P signals through a complex network of five G protein-coupled receptors and multiple downstream targets. However, HUVECs only express receptors 1 and 3 (S1P1 and S1P3). Interestingly, we found that cooperation between both receptors is required for HDL/ApoM/S1P anti-apoptotic effect. Furthermore, the activation of AKT and ERK is also necessary to achieve the anti-apoptotic effect of the HDL/ApoM/S1P complex.
Taken together, our results indicate that ApoM and S1P are key elements of the anti-apoptotic activity of HDL and promote optimal endothelial function.

Participates in Young Investigator Award

Relation between 20210G>A polymorphism within Factor II gene and arterial ischemic stroke

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Background: Previous data showed that 20210G>A polymorphism in *FII* gene is related to increased level of prothrombin and in turn it may lead to procoagulant state. Due to heterogeneous and multifactorial character of cerebrovascular diseases, results of the studies performed on different groups of patients with arterial ischemic stroke (AIS) are often contradictory. We performed meta-analysis of available data addressing the association between FII 20210G>A polymorphism and AIS, both in adults and in children.

Methods: We searched Pubmed using appropriate keywords. We included to a study 11 case-control studies with a total number of 1368 AIS patients
(398 paediatric; 970 adults) and 1929 controls (978 children; 951 adults). Statistical analyses were conducted using MedCalc software. We used random or fixed models to calculate pooled ORs.

Results: The pooled analysis showed that carrier-state of 20210A allele is associated to AIS in adults (random model p=0.001,OR=2.56 95%CI 1.49-4.39). In case of paediatric stroke higher prevalence of polymorphic variant of *FII* in patients compared to controls was observed although the result was on border of significance (fixed model p=0.061,OR=1.85 95%CI 0.97-3.53).

Conclusions: The *FII* 20210G>A polymorphism may be considered as risk factor for ischemic stroke in adults and in children.

List of Participants

2016

22nd ANNUAL SCANDINAVIAN ATHEROSCLEROSIS CONFERENCE

April 13th-16th, 2016 at Krogerup Højskole, Krogerupvej 13, DK-3050 Humlebæk, Denmark

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