25th Annual Scandinavian Atherosclerosis Conference

April 10-13, Krogerup Højskole, Humlebæk, Denmark

2019 Program

SCIENTIFIC COMMITEE	Line Bisgaard (Denmark), Daniel Ketelhurt (Sweden) Jeanine Roeters van Lennep (The Netherlands), Anne Langsted (Denmark) Stefan Stender (Denmark), Matti Jauhiainen (Finland) Jacob J. Christensen (Norway), Petri Kovanen (Finland)
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Organized by	
SCANDINAVIAN SOCIETY FOR ATHEROSCLEROSIS RESEARCH:	Christina Christoffersen (Chairperson) Mette Christoffersen (Treasurer) Tuva Dahl (Secretary) Emil D. Bartels (Webmaster) Katariina Öörni Vesa Olkkonen Vilmundur Gudnason Gunnar Sigurdsson Trine Ranheim Stefano Romeo Paolo Parini
HOMEPAGE	www.ssar.dk (Emil D. Bartels, Webmaster)

Wednesday, April 10, 2019

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 2019 NIKKILÄ MEMORIAL LECTURES	
19.35 – 19.40	Introduction of the 2019 Nikkilä Lecturer **Vesa Olkkonen** *(Finland)*
19.40 – 20.25	2019 Nikkilä Lecture: **Katariina Öörni** (*Finland*)
20.25 – 20.45	Discussion
20.45 –	Pub will be open

Thursday, April 11, 2019

08.00 – 09.00	Breakfast
SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY** Chaired by **Line Bisgaard** *(Denmark)* and **Daniel Ketelhurt** *(Sweden)*
09.00 – 09.25	Resolution of cardiovascular inflammation **Hildur Arnardottir** *(Sweden)*
09.25 – 09.30	Discussion
09.30 – 09.45	Release of extracellular traps by macrophages under chronic inflammatory conditions **Clare Hawkins** *(Denmark)*
09.45 – 10.00	Knockout of the atherosclerosis risk interval alters inflammation and causes advanced plaques in mice **Sanna Kettunen** *(Finland)*
10.00 – 10.15	Continuous TCR signaling in the atherosclerotic environment induces immunomodulatory CD8+ T-cells expressing CD39 **Janine van Duijn** *(The Netherlands)**
10.15 – 10.30	Sex-specific lipid molecular signatures in obesity-associated metabolic disorders revealed by lipidomic characterization in ob/ob mouse **Marion Korach-André** *(Sweden)*
10.30 – 11.15	Coffee, **poster walks (Session I)** and exhibitions
11.15 – 11.40	Modeling vascular inflammation in bioengineered human vessels **Jerôme Robert** *(Canada)*
11.40 – 11.45	Discussion
11.45 – 12.00	Absence of the NLRP3 inflammasome improves survival and cardiac remodeling following myocardial infarction **Mieke Louwe** *(Norway)**
12.00 – 12.15	HDL particles reprogramming circulating monocytes toward immune tolerance **Nikita Nikiforov** *(Russia)**

12.15 – 12.30	The biological function of CETP: modulation of HDL to resolve infections **Patrick Rensen** *(The Netherlands)*
SESSION II	**CARDIOVASCULAR DISEASE** Chaired by **Anne Langsted** *(Denmark)* and **Jeanine Roeters van Lennep** *(The Netherlands)*
12.30 – 12.55	The role of trained immunity in atherosclerosis **Niels Riksen** *(The Netherlands)*
12.55 – 13.00	Discussion
13.00 – 14.00	Lunch
14.00 – 15.00	**General meeting of the *Scandinavian Society for Atherosclerosis Research*** Open for all participants, decision on next year's topics and chairpersons
	Afternoon free for the Louisiana Museum of Modern Art (5 min walk), beach (5 min walk), Kronborg, the castle of Hamlet (12 min by train) or downtown Copenhagen (50 min by train)
16.30 – 17.30	**The traditional soccer match between countries** Remember to bring sports clothing and suitable footwear
17.45 – 18.45	Dinner
SESSION II	**CARDIOVASCULAR DISEASE – continued** Chaired by **Anne Langsted** *(Denmark)* and **Jeanine Roeters van Lennep** *(The Netherlands)*
18.45 – 19.10	Lipid-lowering therapies to reduce cardiovascular risk **Kausik Ray** *(United Kingdom)*
19.10 – 19.15	Discussion
19.15 – 19.30	Eligibility and Preventive Potential for New Evidence-Based Cardiovascular Drugs in Secondary Prevention: Lessons from a Contemporary General Population **Martin Bødtker Mortensen** *(Denmark)*

19.30 – 19.45	Statin use is independently associated with premature mortality, cardiovascular specific mortality and cardiovascular events in renal transplant recipients **Josephine Anderson** *(The Netherlands)**
19.45 – 20.00	High glucose concentrations and risk of microvascular and peripheral vascular disease - an observational and Mendelian randomization study **Frida Emanuelsson** *(Denmark)*
20.00 – 20.45	Coffee, **poster walks (Session II)** and exhibitions.
20.45 – 21.00	Unmet need for secondary prevention in individuals from the general population with increased lipoprotein(a): a contemporary population-based study **Christian Medon Madsen** *(Denmark)**
21.00 – 21.15	Aggregation-prone LDL in South Asians: a missing link with high prevalence of cardiovascular disease **Lauri Äikäs** *(Finland)**
21.15 – 21.30	Prevalence of genetically verified familial hypercholesterolemia among young Norwegian patients (<45 years) hospitalized with acute myocardial infarction **Kirsten Holven** *(Norway)*
21.30 – 21.45	Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population **Børge G. Nordestgaard** *(Denmark)*
21.45 –	Pub will be open

Friday, April 12, 2019

08.00 – 09.00	Breakfast.
SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT** Chaired by **Stefan Stender** *(Denmark)* and **Matti Jauhiainen** *(Finland)*
09.00 – 09.25	Finding novel regulators of LDL metabolism **Jan Albert Kuivenhoven** *(The Netherlands)*
09.25 – 09.30	Discussion
09.30 – 09.45	A disordered acidic domain in GPIHBP1 harboring a sulfated tyrosine regulates lipoprotein lipase **Kristian Kølby Kristensen** *(Denmark)**
09.45 – 10.00	Angiopoietin-like 4 regulates a diurnal rhythm in brown adipose tissue **Wietse In het Panhuis** *(The Netherlands)**
10.00 – 10.15	Elevated plasma apolipoprotein M levels impair triglyceride turnover in mice **Stefan Hajny** *(Denmark)**
10.15 – 10.30	When protein disorder provides “crystal clear” insights into intravascular triglyceride metabolism **Michael Ploug** *(Denmark)*
10.30 – 11.15	Coffee, **poster walks (Session III)** and exhibitions
11.15 – 11.40	Metabolic biomarker profiling of cardiovascular disease risk: a biobank perspective **Peter Wurtz** *(Finland)*
11.40 – 11.45	Discussion
11.45 – 12.00	One third of plasma cholesterol is in remnant lipoproteins: metabolomic profiling in 9,293 individuals **Mie Balling** *(Denmark)**
12.00 – 12.15	Remnant Cholesterol Predicts the Development of New Onset Diabetes Mellitus after Transplantation (NODAT)in Renal Transplant Recipients **Uwe Tietge** *(The Netherlands)*

12.15 – 12.30	Body mass index, triglycerides and risk of acute pancreatitis: A general population study of >117,000 individuals **Signe Elisa Johanne Hansen** *(Denmark)**
12.30 – 12.45	Secular trends in LDL-cholesterol among 18-49-year-olds in Norway **Erik Kristoffer Arnesen** *(Norway)**
12.45 – 13.45	Lunch
SESSION IV	**OTHER TOPICS** Chaired by **Jacob J. Christensen** *(Norway)* and **Petri Kovanen** *(Finland)*
13.45 – 14.10	Mechanisms behind the association between periodontitis and cardiovascular diseases **Pirkko Pussinen** *(Finland)*
14.10 – 14.15	Discussion
14.15 – 14.30	Genetically determined plasma C-Reactive Protein levels and risk of Alzheimer's disease in the general population **Sharif Hegazy** *(Denmark)**
14.30 – 14.45	Blood-brain barrier transcytosis genes and risk of cerebral vascular diseases - a prospective cohort study of 74,754 individuals **Ida Juul Rasmussen** *(Denmark)**
14.45 – 15.00	The function of ORP2 in endothelial cell polarization and angiogenic tube formation in vitro **Annika Koponen** *(Finland)**
15.00 – 15.15	Patient-Reported Outcome Measures in Familial Hypercholesterolemia (FH) Patients: Knowledge is Health **Janneke Mulder** *(The Netherlands)**
15.15 – 16.00	Coffee, **poster walks (Session IV)** and exhibitions
16.00 – 16.25	Omics and nutrition: key partners in Precision Medicine **Josê M. Ordovás** (*United States)*
16.25 – 16.30	Discussion

16.30 – 16.45	Proatherogenic Function of LDL is Prospectively Associated with Graft Failure in Renal Transplant Recipients but not LDL Cholesterol Levels **Hannah L.M. Steffen** *(The Netherlands)**
16.45 – 17.00	Genetic variation in PPP1R3B and plasma levels of glucose, lipids, and liver enzymes in the Danish general population **Anne-Sofie Seidelin** *(Denmark)**
17:00 – 17:15	A systems analysis of lipidemic heterogeneity in APOE*3Leiden.CETP mice **Bert Groen** *(The Netherlands)*
17.15 – 19.00	Time free
19.00 – 19.30	**Cocktail**
19.30 –	**Banquet and dancing**

Saturday, April 13, 2019

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

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2019 Posters

Thursday, April 11, 2019

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

SESSION I	**INFLAMMATION AND VASCULAR BIOLOGY**
No 20	Characterisation of extracellular matrix in human atherosclerotic lesions **Christine Y. Chuang** *(Denmark)*
No 21	Tropoelastin oxidation by peroxynitrous acid: a key modulator of arterial structure, function and loss of elasticity **Michael Davies** *(Denmark)*
No 24	Temperature Triggered Myokine Release **Solveig Krapf** *(Norway)*
No 27	The anti-inflammatory function of follicular fluid HDL predicts the outcome of modified natural cycle in vitro fertilization **Congzhuo Jia** *(The Netherlands)*
No 31	Characterisation of neutrophil extracellular traps and their role in atherosclerosis **Line Halberg** *(Denmark)*
No 51	The Interaction Between Mitochondrial Stress and the Innate Immune System Receptor NLRP3 **Trine Ranheim** *(Norway)*
YIA Poster walk 10.30 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No 25	Impact of myeloperoxidase-derived oxidants on vascular smooth muscle cell damage and death in atherosclerosis **Konstantina Flouda** (*Denmark*)
No 32	Role of thiocyanate in the repair of myeloperoxidase-derived thiol oxidation during chronic inflammation **Chaorui Guo** *(Denmark)*
No 63	miR-107 as an inhibitor of human adipocyte differentiation and lipid storage **Maria Ahonen** *(Finland)*
No 72	Effects of hypoxia on extracellular matrix synthesis by human coronary artery endothelial cells **Christine Y. Chuang** *(Denmark)*

SESSION II	**CARDIOVASCULAR DISEASE**
No 6	Effectivity of dietary nitrates on vascular function: does age matter? - A randomized, placebo-controlled, double-blind crossover study **Fabio Aguilar Mora** *(The Netherlands)*
No 8	Mitochondrial Genetics of Atherosclerotic Disease **Igor Sobenin** *(Russia)*
No 15	SmartDiet – A brief questionnaire for the assessment of dietary habits and lifestyle **Tone Svilaas** *(Norway)*
No 19	The role of sphingolipids in essential hypertension: a case-control study **Edith Friesema** *(The Netherlands)*
No 60	Anti-inflammatory diet in rheumatoid arthritis effects on cardiovascular risk factors **Erik Hulander** *(Sweden)*
YIA Poster walk 20.00 – 20.45	**Selected abstracts (3 min presentation + 2 min discussion)**
No 2	Does myeloperoxidase-induced modification of extracellular matrix (ECM) of the arterial wall contribute to vulnerable plaques? **Huan Cai** *(Denmark)*
No 3	Higher expression of genes related to T- and B-cell pathways in PBMCS from children with versus without familial hypercholesterolemia: a cross-sectional study **Ingunn Narverud** *(Norway)*
No 10	Lipoprotein(a) plasma levels and association to cardiovascular disease in a Stockholm County cohort – a retrospective observational cohort registry study **Karin Littmann** *(Sweden)*
No 12	PCSK9 inhibitors in high cardiovascular risk patients: an update on clinical experiences **Michelle Schreuder** (*The Netherlands)*

Friday, April 13, 2019

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SESSION III	**LIPOPROTEINS AND LIPID TRANSPORT**
No 34	Plasma levels of apolipoprotein E, APOE genotype, and all-cause and cause-specific mortality in 105,949 individuals from the general population **Katrine Laura Rasmussen** *(Denmark)*
No 35	Metabolomic signature of angiopoietin-like protein 3 deficiency under fasting and postprandial conditions **Vesa Olkkonen** *(Finland)*
No 38	Neutrophil protease 3 is present in human atherosclerotic lesions and modifies LDL and HDL particles **Katariina Öörni** *(Finland)*
No 42	Dietary saturated fats increase and plant stanol esters decrease LDL aggregation **Maija Ruuth** *(Finland)*
No 43	ANGPTL3 depletion alters lipid profile and metabolism in vitro and in vivo **Hanna Ruhanen** *(Finland)*
No 46	Is maternal lipid profile in early pregnancy associated with pregnancy complications and blood pressure in pregnancy and long-term postpartum? **Jeanine Roeters van Lennep** *(The Netherlands)*
No 71	Statin treatment increases lipoprotein(a) levels in subjects with low molecular weight apolipoprotein(a) phenotype **Monique Mulder** *(The Netherlands)*
No 73	The role of PCSK9 on the regulation of hepatic cholesterol metabolism and overall lifespan in mice **Ella Bäckebjörk** *(Sweden)*
YIA Poster walk 10.30 – 11.15	**Selected abstracts (3 min presentation + 2 min discussion)**
No 40	Identification and replication of six loci associated with gallstone disease **Helene Gellert-Kristensen** *(Denmark)*

No 41	Loss-of-function mutations in ABCA1, HDL-cholesterol, metabolomic profiles and risk of vascular disease and dementia – a cohort study of up to 100.000 individuals **Liv Tybjærg Nordestgaard** *(Denmark)*
No 45	Treatment with 2-hydroxypropyl-ß-cyclodextrin induces macrophage cholesterol efflux in vitro but does not induce lesion regression in APOE knockout mice in vivo **Yiheng Zhang** *(The Netherlands)*
No 47	Role of inflammation-related genes in macrophage cholesterol accumulation **Vasily Sukhorukov** *(Russia)*
No 55	Treat-to-target Familial Hypercholesterolemia - a prospective study of adult patients with familial hypercholesterolemia at the Lipid Clinic of Oslo, Norway **Ann Phung** *(Norway)*
SESSION IV	**OTHER TOPICS**
No 54	Pnpla3 Silencing Ameliorates NASH and Fibrosis in Pnpla3 I148M Knock-in Mice **Ester Ciociola** *(Sweden)*
No 68	Characterization of PBMC gene expression and lipoprotein subclasses among plasma TG responders and non-responders to omega-3 supplementation **Stine Marie Ulven** *(Norway)*
No 69	Dietary supplementation of inulin reduces Western diet-induced hepatic inflammation, a proxy of NASH **Fan Liu** *(The Netherlands)*
No 70	The Framingham risk score is useful to predict chronic graft failure in renal transplant recipients **Margot Poot** *(The Netherlands)*
YIA Poster walk 15.15 – 16.00	**Selected abstracts (3 min presentation + 2 min discussion)**
No 49	MBOAT7 is anchored to endomembranes by six transmembrane domains **Andrea Marco Caddeo** (*Sweden*)
No 58	Evidence of high 18F-fluorodeoxyglucose uptake in the subcutaneous adipose tissue of the dorsocervical area in young adults **Borja Martinez-Tellez** *(The Netherlands)*

No 59	Regulation of metabolism by activation of ERß using a synthetic ligand on male mice **Christina Savva** *(Sweden)*
No 62	Increased triacylglycerol - fatty acid substrate cycling in human myotubes exposed to eicosapentaenoic acid **Jenny Lund** *(Norway)*
No 65	Origin of bile acids in follicular fluid in human ovaries **Ruxandra Nagy** *(The Netherlands)*

Oral Presentations – Abstracts –

Inflammation and Vascular Biology

SESSION I

Release of extracellular traps by macrophages under chronic inflammatory conditions

Yunjia Zhang[1,2], Benjamin Rayner[1,2], Mathias Jensen[3] and Clare Hawkins[3]

[1]Heart Research Institute, 7 Eliza St, Newtown, NSW 2042, Australia; [2]Sydney Medical School, University of Sydney, NSW 2006, Australia; [3]Department of Biomedical Sciences, University of Copenhagen, Blegdamsvej 3B, Copenhagen 2200, Denmark

The production of extracellular traps (ETs) by neutrophils in response to a range of inflammatory stimuli, is now recognized to play an important role within a range of chronic disease settings, including driving lesion development in atherosclerosis. ETs are released following a novel mode of cell death called ETosis, which results in the release of a mesh of DNA, histones, myeloperoxidase (MPO) and proteolytic enzymes. In innate immunity, these structures trap and kill bacteria, but they can also promote inflammation and contribute to pathological processes, and have been strongly implicated in atherosclerosis. It is well established that pro-inflammatory stimuli, including the MPO-derived oxidant hypochlorous acid (HOCl), can trigger ET release from neutrophils. The aim of this study, was to examine whether macrophages also release ETs on exposure to inflammatory stimuli, because these cells play a critical role in the development of lesions in atherosclerosis. We show that exposure of human monocyte-derived macrophages (HMDM) to HOCl results in the extrusion of DNA and histones into the cellular supernatant, consistent with ET formation. Unlike neutrophils, ET release from HMDM occurs independently of histone citrullination, but does involve an immediate and sustained cytosolic accumulation of calcium. Polarisation of the macrophages prior to HOCl exposure revealed a greater propensity for inflammatory M1 macrophages to produce ETs, whereas alternatively-activated M2 macrophages were less susceptible to HOCl insult. M1 macrophages also produced ETs on exposure to other inflammatory stimuli, including phorbol myristate acetate (PMA), interleukin-8 (IL-8) and tumour necrosis factor a (TNFa). Taken together, these data indicate a potential role for macrophages in mediating ET formation, which may be relevant in pathological conditions, particularly atherosclerosis, which is characterised by chronic inflammation and excessive HOCl formation.

Knockout of the atherosclerosis risk interval alters inflammation and causes advances plaques in mice

Sanna Kettunen, Anna-Kaisa Ruotsalainen, Nihay Laham-Karam, Seppo Ylä-Herttuala

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Introduction: Atherosclerosis, a progressive disease of narrowing arteries, is a leading cause of death in western societies. In 2007 GWA-studies identified a geneticrisk locus for coronary artery disease (CAD). The risk seems to be independent of classic CAD risk factors, such as high plasma cholesterol. The risk region doesn't contain protein-coding genes, but overlaps with a lncRNA of unknown function, ANRIL. Mouse model with deletion of homologous region has been developed, but not studied in atherogenic background before. Here we characterize the effect of CAD-risk knockout in atherosclerotic mouse, aiming to find out its function. **Methods:** We crossbred Chr4D70kb/D70kb mice with LDLR-/-/ApoB100/100 and put these "ANRIL-/-" mice and their litter mate controls on high fat diet (HFD) for 3 months & followed their weight & plasma lipids. Then we sacrificed the mice and collected their tissues. Atherosclerosis was analysed from sections of aortic root. We isolated and cultured bone marrow derived macrophages (BMDMs) from ANRIL-/- & control mice. We treated BMDMs for 16h with IFN, oxLDL & acLDL, assessed foam cell formation, and isolated RNA. We ran qPCR for the expression of inflammatory cytokines, knockout area and nearby genes. The National Animal Experiment Board in Finland approved the animal procedures. **Results & Conclusion:** There weren't difference in body weight or blood values. Analysis of atherosclerosis revealed increased plaque area in ANRIL-/- aortic roots. There was also a trend of ANRIL-/- plaques containing less macrophages, indicating more advanced phenotype. There weren't difference in foam cell formation, but MCP-1 was upregulated in IFN treated ANRIL-/- BMDMs. Control BMDMs had upregulation of the knockout exons after treatments, when compared to no-treat samples. This indicates these transcripts playing a role in inflammation. Our results demonstrate the effect of risk interval on macrophages' gene expression. We show for the first time the atherogenic phenotype of ANRIL-/- mouse, and provide evidence of ANRIL's inflammatory function.

Continuous TCR signaling in the atherosclerotic environment induces immunomodulatory CD8+ T-cells expressing CD39

J. van Duijn[1], M. van Elsas[1], N. Benne[1], M. Depuydt[1], A. Wezel[2], H. Smeets[2], I. Bot[1], W. Jiskoot[1], J. Kuiper[1] and B. Slütter[1]

[1]Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden University, the Netherlands; [2]HMC Westeinde, The Hague, the Netherlands

Aim: CD8+ T-cells can be atheroprotective in clinically relevant advanced stages of atherosclerosis, as their depletion results in less stable lesions with a more inflammatory phenotype. However, the phenotype and function of these cells in the lesional microenvironment remains to be determined. Here, we address how the atherosclerotic environment affects the functionality of CD8+ T-cells. **Methods and Results:** We compared the cytokine production of CD8+ T-cells derived from spleens and aortas of apoE-/- mice with advanced atherosclerosis by flow cytometry. CD8+ T-cells isolated from atherosclerotic lesions produced lower amounts of IFN-γ and TNF-α than their splenic counterparts. The observed dysfunctional phenotype of the lesion-derived CD8+ T-cells was associated with an increased expression of the ectonucleotidase CD39, which converts inflammatory extracellular ATP into immunomodulatory adenosine. Indeed, pharmacological inhibition of CD39 in apoE-/- mice partly restored cytokine production by CD8+ T-cells. Using a bone-marrow transplantation approach, we showed that induction of CD39 was a consequence of antigen-specific CD8+ T-cell activation via T-cell receptor signaling within the lesions. Importantly, analysis of human endarterectomy samples showed a clear microenvironment specific upregulation of CD39 on CD8+ T-cells in the plaques of human patients compared to matched CD8+ T-cells from the blood. **Conclusion:** Our results indicate that the continuous TCR signaling in the atherosclerotic plaque induces an immune regulatory CD8+ T-cell phenotype that is associated with decreased cytokine production through increased CD39 expression in both a murine atherosclerotic model and in atherosclerosis patients. This provides a new understanding of atheroprotective immune regulation by CD8+ T-cells.

Participate in YIA

Sex-specific lipid molecular signatures in obesity-associated metabolic disorders revealed by lipidomic characterization in ob/ob mouse

Marcela González-Granillo[7,8], Luisa A. Helguero[2#], Eliana Alves[3#], Amena Archer[1,5], Christina Savva[7,8], Matteo Pedrelli[1,4], Osman Ahmed[4], Xidan Li[7,8], Maria Rosário Domingues[3], Paolo Parini[4], Jan-Åke Gustafsson[1,6] and Marion Korach-André[1,7,8]

[1]Center for Innovative Medicine, Department of Biosciences and Nutrition, Huddinge, Sweden; [2]Institute for Biomedicine, Department of Medical Sciences, University of Aveiro, Portugal; [3]Organic Chemistry and Natural Products Unit, Department of Chemistry, University of Aveiro, Portugal; [4]Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, Sweden; [5]Department of Proteomics, Science for Life Laboratory, School of Biotechnology, KTH, Stockholm, Sweden; [6]Department of Biology and Biochemistry, Center for Nuclear Receptors and Cell Signalling, University of Houston, Houston, Texas, US; [7]Metabolism Unit, Center for Endocrinology, Metabolism, and Diabetes, Department of Medicine, and Molecular Nutrition Unit, S-141 86 Stockholm, Sweden; [8]Karolinska Institutet/AstraZeneca Integrated Cardio Metabolic Center, Department of Medicine, Karolinska Institutet at Karolinska University Hospital Huddinge, C2-94, S-141 86 Stockholm, Sweden.

There is substantial evidences that females and males differ in their basic metabolic physiology and in their susceptibility to develop obesity-associated metabolic diseases including insulin resistance and liver steatosis. Indeed, the response to overfeeding is sex-dependent and metabolic syndrome is more likely associated to obesity in men or postmenopausal women than in young fertile women. These changes over the lifespan or as a function of life style make these sex-differences even more complex and difficult to treat. A better understanding of the sex differences in body composition would help to anticipate these changes and may prevent the development of associated metabolic diseases. We, thus, hypothesized that obesity-induced metabolic syndrome is sex-dependent due to a sex-specific regulation of the lipid synthesis pathways in liver and white adipose depots. We aimed to characterize the lipid species and genes related to fat partitioning in female and male metabolic tissues in obesity. Liver, perigonadal visceral and inguinal adipose tissue were collected for lipidomic analysis. Males had less total body fat but lower subcutaneous on visceral fat ratio together with higher liver weight and, higher liver and serum triglyceride (TG) levels and were insulin resistant compared to females. Fatty acid (FA) and TG profiles differed between sexes in both fat pads, with longer chains FAs and TGs in males compared to females. Remarkably, hepatic phospholipid composition was sex-dependent with more abundant lipotoxic FAs in males than females. This may be a key contributor to the sexual dimorphism in response to obesity towards more metaflammation in males. Our work presents an exhaustive novel description of a sex-specific lipid signature in the pathophysiology of metabolic disorders associated with obesity. These data could settle the basis for future pharmacological treatment of obesity.

Absence of the NLRP3 inflammasome improves survival and cardiac remodeling following myocardial infarction

Mieke C. Louwe[1,2,3], Ole J. Kaasbøll[1,3], Maria B. Olsen[1,2,3], Kuan Yang[1,2,3], Linn E. Fosshaug[1,2,3], Katrine Alfsnes[1,2,3], Jonas D.S. Øgaard[1,2,3], Azita Rashidi[1,2], Elisabeth Schrumpf[1,2], Jan Magnus Aronsen[1,3], Vidar Skullberg[1,3], Espen Melum[1,2], Håvard Attramadal[1,3], Ivar Sjaastad[1,3], Pål Aukrust[1,2], Arne Yndestad[1,2,3]

[1]Oslo University Hospital, Oslo, Norway; [2]K.G. Jebsen Inflammation Research Centre, University of Oslo, Oslo, Norway; [3]Centre for Heart Failure Research, University of Oslo, Oslo, Norway

Background: Myocardial infarction (MI) causes a sterile inflammatory response through activation of the innate immune system. Upon activation of the cytosolic pattern recognition receptor NLRP3 inflammasome are formed leading to IL-1beta and IL-18 release. While the inflammatory response is a prerequisite for healing of the infarction, a dysregulated response may have harmful effects promoting maladaptive cardiac remodeling. In this study we investigated whether absence of NLRP3 affects post-MI survival and remodeling. **Methods & Results:** C57Bl/6J (WT) and NLRP3 knock-out (NLRP3 KO) mice were subjected to an MI by coronary artery ligation. WT mice had a high mortality (43%) primarily due to ventricular rupture at day 4-6. Mortality was markedly reduced in NLRP3 KO mice (17%, $p<0.05$), despite comparable infarct size and heart function 1 day post-MI. Cardiac remodeling was more beneficial in NLRP3 KO mice up to 21 days post-MI, with improved ejection fraction (+14%, $p<0.09$), decreased expression of ANP, IL-6 and collagen ($p<0.01$). Decreased neutrophil and enhanced macrophage infiltration in NLRP3 KO hearts was observed just before rupture ($p<0.05$), pointing at a primary role of NLRP3 in infarction healing. To investigate this more closely, WT and NLRP3 KO bone marrow chimeras were created and subjected to MI. Transplantation of NLRP3 KO bone marrow into WT mice greatly improved post-MI survival compared to WT controls (100% vs. 53% survival, $p<0.001$). In addition, cardiac function was improved accordingly in NLRP3 KO chimeras ($p=0.06$). In vitro experiments revealed an improved clearance of apoptotic cells by macrophages in NLRP3 KO mice ($p<0.05$), suggesting enhanced efferocytosis. **Conclusion:** NLRP3 activation in hematopoietic cells infiltrating in the myocardium disturbs infarction healing, thereby causing ventricular rupture, with a key role for macrophages.

Participate in YIA

HDL particles reprogramming circulating monocytes toward immune tolerance

Nikiforov NG[1,2,4], Wetzker R[3], Kubekina MV[2], Petukhova A[2], Sukhorukov VN[4], Sobenin IA[1], Orekhov AN[2,4]

[1]National Medical Research Center of Cardiology, Institute of Experimental Cardiology, Moscow, Russia; [2]Institute of Gene Biology, Centre of collective usage, Moscow, Russia; [3]Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Jena, Germany; [4] Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Moscow, Russia

Aim: We evaluated the ability of circulating monocytes isolated from atherosclerotic patients being activated and tried to find factors responsible. **Methods:** Clinical study was performed, which involved healthy donors (N=13) and patients with subclinical atherosclerosis (N=23). Quantitative diagnostics of atherosclerotic states was performed by ultra-sonographic measurement of intima-media thickness (IMT) of common carotid arteries as well as other characteristics were evaluated such as age, BMI, Tchol, Tg, LDLc, HDLc, etc. Monocytes were isolated from patients using magnetic CD14+ separation and had been incubating with 1 mkg/ml of LPS for 24h. Then secretion and expression level of TNF was measured using ELISA and qPCR respectively. In order to find pathways responsible for identified phenomena, monocytes were isolated from healthy individuals in a similar way and incubated with native, acetylated, oxidized LDL or HDL for 24h. Then transcriptome analysis was performed. **Results:** The secretion and expression levels of TNF were significantly increased in LPS-stimulated monocytes isolated from atherosclerotic patients comparing with healthy participants. Moreover, the strong correlation between TNF expression and IMT was observed indicating that monocytes from atherosclerotic patients have increased inflammatory state. Surprisingly, secretion and expression of TNF in LPS-stimulated had strong reverse correlation with participant's HDL cholesterol. In order to evaluate the ability of HDL to activate pathways associated with innate immune tolerance, the transcriptome analysis was performed. It turned out that HDL suppresses associated with trained innate immune PI3K-mTOR pathway and activates associated with innate immune tolerance ATM-AMPK-p53/SIRT1 pathways accompanied with decreased expression of TNF. On the other hand, modified LDLs showed diametrically opposite effect. **Conclusion:** We suggest that HDL particles can program circulating monocytes toward immune tolerance as well as modified LDLs exhibit opposite effect.

This work was supported by the RFBR (Grant No. 18-34-00997).

Participate in YIA

The biological function of CETP: modulation of HDL to resolve infections

Lisanne L. Blauw[1], Mark Trinder[2], Raymond Noordam[1], Sebastian Soidinsalo[3], Yanan Wang[1], Diana van Heemst[1], Peter Würtz[3], Ko Willems van Dijk[1], John H. Boyd[2,4], Liam R. Brunham[2,4], Patrick C.N. Rensen[1]

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Background: We previously showed that CETP is primarily produced by resting hepatic macrophages, and that bacterial lipopolysaccharide rapidly lowers CETP expression in these cells to decrease plasma CETP and raise HDL (Hepatology 2015; JAHA 2018). Since HDL-C declines during sepsis, and lower HDL-C levels are associated with worse survival, we hypothesized that LPS sensing by hepatic macrophages may elevate HDL to combat the underlying invading infection. Therefore, the aim of the present study was to evaluate the effects of CETP on HDL composition and on clinical outcomes during sepsis. **Methods & Results:** First, we used a genetic score for serum CETP concentration to estimate causal effects of CETP on 159 standardized metabolic biomarkers (NMR; Nightingale platform) in the Netherlands Epidemiology of Obesity (NEO) study, to show that higher CETP concentrations were casually associated with less large HDL (largest effect XL-HDL-C, P=6×10-22). Next, we performed targeted re-sequencing of CETP in 200 patients admitted to an emergency department with sepsis (Early Infection cohort), and identified a rare missense variant in CETP gene (rs1800777-A) that was associated with significant reductions in HDL-C levels during sepsis. We next examined the association of this genetic variant with 28-d survival and organ dysfunction. Carriers of the A allele (n=10) had decreased survival and more organ failure compared to non-carriers. We replicated this finding in cohorts of the VASST trial (n=632) and SPHICU2 trial (n=302), in which carriers of the A allele (n=35 and n=12, resp.) had significantly reduced 28-day survival. Mendelian randomization was consistent with genetically reduced HDL levels being a causal factor for decreased sepsis survival. **Conclusions:** Although CETP has been targeted to increase HDL-C and thereby lower CVD risk, our results identify CETP as a critical regulator of HDL to modulate clinical outcomes during sepsis. Therefore, studies are warranted to assess the effect of CETP inhibitors on sepsis rather than atherosclerosis.

Oral Presentations – Abstracts –

Cardiovascular Disease

SESSION II

Eligibility and Preventive Potential for New Evidence-Based Cardiovascular Drugs in Secondary Prevention: Lessons from a Contemporary General Population

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Aim: Recently, twelve randomized controlled trials (RCTs) have demonstrated the efficacy of novel therapies for mainly secondary prevention of atherosclerotic cardiovascular disease (ASCVD). We determined eligibility and preventive potential for the new preventive therapies in a contemporary general population. **Methods:** The Copenhagen General Population Study is a population-based cohort with 109,429 individuals enrolled in 2003-2015. Of those, 6292 had ischemic heart disease (IHD) and 2277 had a previous myocardial infarction (MI) at baseline. We determined drug eligibility and evidence-based potential for prevention of MI, stroke and deaths of the twelve cardiovascular drugs tested in recent major RCTs: IMPROVE-IT, PEGASUS, EMPA-REG, LEADER, SUSTAIN-6, FOURIER, CANVAS, REVEAL, CANTOS, COMPASS, ODYSSEY-OUTCOMES, and REDUCE-IT. **Results:** In all individuals and in those with IHD or MI at baseline, the eligibility for one or more new medication was 8% (n=8246), 80% (n=5036), and 99% (n=2273), respectively. Dividing the new preventive therapies into 4 drug-classes (lipid-modifying, anti-thrombotic, anti-inflammatory and anti-diabetic drugs), 41% (n=2594) and 81% (n=1834) of individuals with IHD or MI were eligible for 2 or more different drug classes simultaneously. The 5-year estimated percentage of either MI's, strokes or deaths that could be prevented for each of the new therapies varied from -1% to 5% in the general population setting, and from -3% to 33% in patients with IHD or MI at baseline. **Conclusions:** These data raises critically important questions for the cardiovascular community about access to these potentially expensive therapies, including strategies for prioritizing their use.

Statin use is independently associated with premature mortality, cardiovascular specific mortality and cardiovascular events in renal transplant recipients

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Background: In the general population, statins achieve potent LDL lowering and a significant reduction of cardiovascular (CV) risk. In renal transplant recipients (RTR) statins are included in treatment guidelines, despite a lack of convincing evidence of improved clinical outcomes. This study aimed to elucidate the effect of statins on premature mortality, CV mortality and CV events in RTR. **Methods:** 622 prospectively included RTR (follow-up 5.4 years) were divided into those receiving statin therapy (n=322) and those not (n=302). Using survival analysis and predictive margins the association of use of statins with incidence of all-cause mortality (n=128) and a compound CV endpoint (ischemic CV events and mortality, n=96) was assessed. Analyses were repeated after dividing the population based on male gender (n=189 of statin users) and use of the immunosuppressant cyclosporine (n=140 of statin users). **Results:** Weibull survival regression demonstrated that use of statins significantly increased the risk of all-cause mortality (HR=1.64, p=0.007) and the CV endpoint (HR=1.88, p=0.004), even after adjusting for LDL-C and history of CV events. In RTR receiving cyclosporine the association of statins with all-cause mortality became stronger and more significant (HR=2.57, p=0.002). Both drugs are metabolized by cytochrome P450 3A4 and excreted via MRP2, making a bilateral pharmacological interaction plausible, resulting in increased blood concentrations and toxic effects of cyclosporine. Furthermore, when analysis was repeated in male RTR the association of statins with the CV endpoint was stronger and more significant, which remained in a fully adjusted model (HR=2.39, p=0.002). Predicted margins showed that use of statins decreased the life expectancy after inclusion by 20.9% (p=0.003). **Conclusion:** Statin use is independently associated with all-cause mortality as well as CV specific mortality and events in RTR. Current treatment guidelines should be critically re-evaluated.

Participate in YIA

High glucose concentrations and risk of microvascular and peripheral vascular disease – an observational and Mendelian randomization study

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Aim: To investigate the observational and causal associations between glucose levels in the nondiabetic range and risk of vascular diseases in the general population. **Methods:** First, we included 112,457 non-diabetic individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study and assessed their prospective risk of retinopathy, neuropathy, diabetic nephropathy, peripheral arterial disease (PAD), and myocardial infarction (MI) as a function of increasing glucose levels. Second, we used Mendelian randomization (MR) to assess a potential causal effect of high glucose levels on the vascular endpoints using genetic variants known to be associated with elevated glucose levels. Third, we replicated our analyses in a 2-sample MR design using glucose data from the MAGIC consortium and vascular endpoint data from the UK Biobank. **Results:** Observationally, glucose levels in the nondiabetic range and higher were associated with increased risks of retinopathy, neuropathy, diabetic nephropathy, PAD, and MI (p trend for all<0.001). In genetic, causal analyses risk ratios for a 1 mmol/L higher glucose were 2.01 (95% confidence interval: 1.18-3.41) for retinopathy, 2.15 (1.38-3.35) for neuropathy, 1.58 (1.04-2.40) for diabetic nephropathy, 1.19 (0.90-1.58) for PAD, and 1.49 (1.02-2.17) for MI. Summary level data from the MAGIC consortium and the UK Biobank gave similar results. **Conclusion:** In this general population, glucose levels in the nondiabetic range and higher were prospectively associated with a high risk of retinopathy, neuropathy, diabetic nephropathy, PAD, and MI. These associations were causal for retinopathy, neuropathy, diabetic nephropathy, and MI, but causality could not be confirmed for PAD.

Unmet need for secondary prevention in individuals from the general population with increased lipoprotein(a): a contemporary population-based study

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Aim: We tested the hypothesis that high concentrations of lipoprotein(a) are associated with high risk of recurrent cardiovascular disease (CVD) in individuals from the general population with preexisting CVD. **Methods:** From the Copenhagen General Population Study (CGPS) (2003-2015) of 58,527 individuals with measurements of Lp(a) at baseline, 2,527 aged 20-79 with a history of CVD were studied. The primary endpoint was major adverse cardiovascular event (MACE). We also studied 1,115 individuals with CVD at baseline from the Copenhagen City Heart Study (CCHS) (1991-1994) and the Copenhagen Ischemic Heart Disease Study (CIHDS) (1991-1993). **Results:** During a median follow-up of 5 years (range: 0-13, 13,974 person-years), 493 individuals (20%) experienced a MACE in the CGPS. MACE incidence rates per 1,000 person-years were 29 (95% CI: 25-34) for individuals with Lp(a)<10mg/dL (<18nmol/L), 35 (30-41) for 10-49mg/dL (18-104nmol/L), 42 (34-51) for 50-99mg/dL (105-213nmol/L), and 54 (42-70) for ≥100mg/dL(≥214nmol/L). Compared to individuals with Lp(a)<10mg/dL (<18nmol/L), the MACE incidence rate ratios were 1.21 (0.98-1.50) for 10-49mg/dL (18-104nmol/L), 1.43 (1.12-1.82) for 50-99mg/dL (105-213nmol/L), and 1.85 (1.38-2.49) for ≥100mg/dL (≥214nmol/L). Independent confirmation was obtained in individuals from the CCHS and CIHDS with MACE incidence rates per 1,000 person-years of 94 (95% CI: 84-106)) for individuals with Lp(a) <10mg/dL (<18nmol/L), 115 (103-129) for 10-49mg/dL (18-104nmol/L), 134 (115-156) for 50-99mg/dL (105-213nmol/L), and 140 (116-169) for ≥100mg/dL (≥214nmol/L). **Conclusion:** High concentrations of Lp(a) are associated with high risk of recurrent CVD in individuals from the general population with preexisting CVD. This points to a possible unmet need for secondary prevention in individuals with increased lipoprotein(a), and such individuals could be a target group for future randomized cardiovascular outcome trials.

Participate in YIA

Aggregation-prone LDL in South Asians: a missing link with high prevalence of cardiovascular disease

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People from South Asian descent are prone to develop cardiovascular disease compared with white Caucasians, which is not fully explained by classical risk factors. Recently, we demonstrated that the presence of aggregation-prone LDL in the circulation is associated with increased mortality from cardiovascular diseases, as related to enrichment of LDL particles in sphingolipids (Ruuth, Eur Heart J 2018). We therefore hypothesized that LDL may be more prone to aggregate in South Asians. To this end, we isolated LDL particles from serum of healthy young Dutch South Asian (n=12) and white Caucasian (n=12) men, and analyzed their aggregation susceptibility and lipid composition. While LDL levels were similar between both ethnicities, LDL particles from South Asians were markedly more prone to aggregate compared with white Caucasians. In line with our previous findings, aggregation-prone LDL particles were enriched in sphingomyelins. In addition, we now also observed that these aggregation-prone LDL particles had a high proportion of triacylglycerol (TAG) 56:8 within their core. TAG 56:8 likely contains two arachidonic acids (AA) and one palmitate attached to a glycerol backbone. We previously showed that compared with white Caucasians, South Asians have higher circulating AA levels, which correlated with body fat percentage (Kantae, Sci Rep 2017). Indeed, in line with our previous findings (Bakker& Boon et al, Lancet Diab Endocrinol 2014), body fat percentage was higher in the South Asians. Importantly, body fat percentage correlated positively with the susceptibility of LDL to aggregate (r=0.486, p=0.016) as well as with LDL-TAG 58:6 (r=0.609, p=0.002) and LDL-sphingomyelins (r=0.531, p=0.008). These findings suggest that the disadvantageous metabolic phenotype of South Asians could lead to the formation of aggregation-prone LDL particles already in healthy young subjects, which may contribute to their increased cardiovascular disease risk later in life.

Participate in YIA

Prevalence of genetically verified familial hypercholesterolemia among young Norwegian patients (<45 years) hospitalized with acute myocardial infarction

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Background: Patients with familial hypercholesterolemia (FH) have increased levels of LDL-cholesterol (LDL-C) and increased risk of premature cardiovascular disease (CVD) and early death. The aim of the present study was to investigate the prevalence of FH among young patients <45 years hospitalized with acute myocardial infarction (MI). **Methods:** Data were collected from medical charts of all patients <45 years admitted with acute MI to the Department of Cardiology, Oslo University Hospital Ulleval, in the period 2012-2016. Patients who had not already been genetically tested for FH were contacted and offered genetic testing if the pre-treatment or statin adjusted LDL-C level was >4.0 mmol/l. **Results:** Of 9332 patients admitted with AMI, 357 were <45 years and 328 patients were eligible for investigation. Of these, 130 had pre-treatment or statin adjusted LDL-C level >4.0 mmol/l and data from 52 patients genetically tested for FH were available. Eleven patients had genetically verified FH constituting 3.4% of the total eligible population (n=328), 8.5% of those with indications for genetic testing for FH diagnosis (n=130) and 21.2% of the actual genetically tested population (n=52). Dutch Lipid Clinic Network score for clinical FH diagnosis showed low accuracy; a definite score identified only 5 of the 11 FH patients (45%). Including a probable score identified all FH patients but also 17 of the 41 patients (41%) with a negative genetic test. **Conclusion:** The prevalence of FH in young patients with acute MI was higher than in the general population. Routinely evaluation of FH diagnosis among these patients could identify more patients and relatives with FH thereby increasing the possibility of initiating early and adequate treatment also among affected relatives.

Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population

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Aim: High remnant cholesterol concentrations are associated with high risk of ischemic heart disease, but whether this is also the case for ischemic stroke is unknown. We tested the hypothesis that high remnant cholesterol concentrations are associated with increased risk of ischemic stroke in the general population. **Methods:** 102,964 individuals from the Copenhagen General Population Study with information on remnant cholesterol at baseline in 2003-15 were included in a prospective observational association study. Individuals were followed for up to 14 years, during which time 2,488 were diagnosed with an ischemic stroke. Hazard ratios were estimated using Cox proportional hazard regression models. Results were independently confirmed in 9,548 individuals enrolled in the Copenhagen City Heart Study in 1991-94; 983 ischemic strokes developed during up to 26 years of follow-up. **Results:** Stepwise higher remnant cholesterol concentrations were associated with stepwise higher ischemic stroke risk in the Copenhagen General Population Study, with multivariable adjusted hazard ratios up to 1.99(95%confidence interval: 1.49-2.67) for individuals with remnant cholesterol concentrations ≥1.5mmol/L(58mg/dL), compared to individuals with remnant cholesterol <0.5mmol/L(19mg/dL). Results were similar in the Copenhagen City Heart Study. Cumulative incidence of ischemic stroke at age 80 in the Copenhagen General Population Study ranged from 7.3% for individuals with remnant cholesterol <0.5mmol/L(19mg/dL) to 11.5% for individuals with remnant cholesterol ≥1.5mmol/L(58mg/dL). **Conclusion:** Individuals with high remnant cholesterol concentrations had higher risk of ischemic stroke. These results indicate that randomized clinical trials with remnant cholesterol lowering in individuals with high concentrations, with the aim of preventing ischemic strokes, are needed.

Oral Presentations – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

A disordered acidic domain in GPIHBP1 harboring a sulfated tyrosine regulates lipoprotein lipase

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Lipoprotein lipase (LPL) is the rate-limiting enzyme and master regulator of intravascular lipolysis. As the gate-keeping enzyme of energy-rich FFAs to the underlying adipocytes and myocytes, LPL is tightly regulated by both intrinsic (enzyme instability) and extrinsic (ANGPTLs and Apo-lipoproteins) factors. To reach LPLs site of action in the capillary lumen LPL needs to be transported by the small endothelial protein, GPIHBP1. GPIHBP1 is a two domain protein consisting of a folded LU-domain and a negatively charged intrinsically disordered acidic region (IDR), in this study we investigated the role of the acidic domain of GPIHBP1 on its different biological functions and identified a tyrosyl-O-sulfation in the acidic domain (1). First, we found that the IDR of GPIHBP1 increases the affinity for LPL by >250 fold and that the tyrosyl-O-sulfation adds to this affinity. Second, we found that the protecting effects of GPIHBP1 against ANGPTL4 –catalyzed unfolding of LPL critically depends on its acidic IDR and that the tyrosyl-O-sulfate assist in this process ensuring that LPL activity remains focalized on the cell surface, when challenged with ANGPTL4. Third, we show that the acidic IDR of GPIHBP1 is indispensable for mobilization of LPL from a HSPG bound pool, and that the tyrosine sulfation potentiates this effect. Our results, suggests that LPL does not diffuse randomly in the subendotheilal space, but could be diffusing directionally towards the basolateral side of the capillaries. Fourth, we generated a model of GPIHBP1 based on SAXS analysis, where the acidic domain structure occupies a large space. Combined this study present an extensive characterization of the functional properties of the acidic domain of GPIHBP1, providing a detailed molecular understanding of the LPL-protective effects of GPIHBP1. This study sets the stage for molecular understanding of the dynamic regulation of LPL activity in the capillary unit, which could assist future studies on intervention strategies regulating elevated TG levels.

1: Kristensen et al. 2018, PNAS 115, E6020-E6029

Participate in YIA

Angiopoietin-like 4 regulates a diurnal rhythm in brown adipose tissue

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Introduction: Activity of brown adipose tissue (BAT) has been implicated in cardiovascular health. Recently, we identified a strong diurnal rhythm in BAT that was linked to rapid clearance of plasma lipids at wakening in mice. This rhythm coincided with the rhythm in plasma corticosterone levels. The aim of this study was to identify the consequences of reduced plasma corticosterone rhythmicity for diurnal BAT activity. We hypothesized that this interaction is mediated by regulators of lipid and lipoprotein metabolism, such as peroxisome proliferator-activated receptor y (PPARy) and angiopoietin-like 4 (ANGPTL4). **Methods & Results**: We implanted corticosterone pellets (2.5%) in wild-type C57Bl/6J mice which led to attenuated rhythmicity of plasma corticosterone after 7 days. This resulted in the loss of rhythmicity of the uptake of glycerol tri[3H]oleate-labeled triglyceride (TG)-rich lipoprotein-like particles by BAT and PPARy expression, with a consequent increase in body weight. The absence of rhythmicity in [3H]oleate uptake by BAT was also observed in Angptl4-/- mice. **Conclusion & future perspectives**: We showed that reduced plasma corticosterone rhythmicity abolishes rhythmicity of BAT activity. The expression of PPARy, a regulator of Angptl4, showed attenuated rhythmicity, suggesting a loss of diurnal expression of Angptl4. We previously showed that Angptl4, an inhibitor of LPL, is a potential mediator of diurnal BAT activity. Here we illustrate that knocking out Angptl4 abolishes BAT rhythmicity. We anticipate that Angptl4 is a potential target to promote BAT activity in cardiometabolic diseases.

***Participate in YIA**

Elevated plasma apolipoprotein M levels impair triglyceride turnover in mice

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Hypertriglyceridemia typically develops in parallel with obesity, insulin resistance, type 2 diabetes and metabolic syndrome. Lack of understanding impairs clinical diagnostics. Hydrolysis of triglycerides (TG) is mediated by Lipoprotein lipase (LPL) and thus controlling plasma TG clearance. Lipoprotein particles play an invaluable role in transporting TGs, among others. Such particles carry apolipoprotein M (ApoM), which in return, primarily binds S1P. We previously showed that apoM deficient mice display improved TG turnover and protection against diet-induced obesity. In the present study, we highlight the importance of apoM/S1P signalling in plasma TG clearance in a human apoM transgenic mouse model (apoM-TG). Markedly increased plasma apoM (11x) and S1P (4x) levels provided a valuable basis to probe for essential pathways implicated in efficient TG metabolism. Although LPL expression was not affected in our mouse model, its activity was decreased. High fat diet receiving apoM-TG animals further display a delayed weight gain in comparison to respective controls. Injection of the S1P analogue FTY720 furthermore improved TG clearance in WT animals whereas the opposite effect was observed in apoM-TG mice. It appears that moderately increased plasma S1P levels thus enhances TG clearance, whereas excessive concentrations overstimulate the S1P system, thereby impairing TG turnover. Taken together, the project features the important role of apoM/S1P signalling in maintaining a healthy lipid metabolism. Obesity and associated aftereffects lead to hypertriglyceridemia, which may be countered by pharmacological apoM/S1P targeting in the future.

Participate in YIA

When protein disorder provides "crystal clear" insights into intravascular triglyceride metabolism

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Despite the activity of lipoprotein lipase (LPL) was reported more than 75 years ago (Hahn 1943) and that its physiological importance in normal fuel redistribution is indisputable, its structure has proved particularly resilient to several decades of protein crystallization attempts. Allegedly, that frontier proved completely impenetrable due to the notorious instability of LPL activity. Our recent studies by hydrogen-deuterium exchange MS (1) showed that the catalytic domain of LPL unfold spontaneously thus providing the long sought molecular mechanism explaining why LPL activity is so unstable. That property is of high biological and medical relevance as the natural inhibitors ANGPTL4 and ANGPTL3/8 catalyze this unfolding and thus accelerates the inactivation of LPL (2). Importantly, the endothelial protein GPIHBP1, that shuttles LPL from the subendothelial spaces to its site of action in the capillary lumen, has additional important biological functions. First, GPIHBP1 stabilizes LPL against unfolding and mitigates the inhibition from ANGPTLs and second, GPIHBP1 is required for the mobilization of LPL that is tethered to heparin sulfate proteoglycans (1,2). Both these functions of GPIHBP1 is dependent of the intrinsically disordered and highly acidic region of GPIHBP1 (3). This discovery is important for the dynamics of the LPL flux through the capillary unit. Taking advantage of the stabilizing effect of GPIHBP1, we now finally succeeded in crystallizing and solving the three-dimensional structure of human LPL in complex with GPIHBP1 (4). This achievement is expected to revitalize the biochemical aspects of intravascular lipid metabolism and has already provided the molecular basis for several missense mutations in LPL known to cause chylomicronemia.

1) Mysling et al. 2016, eLife, doi:10.7554/eLife.12095
2) Mysling et al. 2016, eLife, doi:10.7554/eLife.20958
3) Kristensen et al. 2018, PNAS 115, E6020-E6029
4) Birrane et al. 2018, PNAS, doi: 10.1073/pnas.1817984116

One third of plasma cholesterol is in remnant lipoproteins: metabolomic profiling in 9,293 individuals

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Aim: Increased concentrations of calculated remnant cholesterol in triglyceride-rich lipoproteins are observationally and genetic, causally associated with increased risk of ischemic heart disease; however, when measured directly the fraction of plasma cholesterol present in remnant particles is unclear. We tested the hypothesis that a major fraction of plasma cholesterol is present in remnant lipoproteins in individuals in the general population. **Methods:** We examined 9,293 individuals from the Copenhagen General Population Study using nuclear magnetic resonance spectroscopy measurements of total cholesterol, free- and esterified cholesterol, triglycerides, phospholipids, particle concentration, and particle size of 14 lipoprotein subclasses with decreasing size. Six subclasses were in very low-density lipoprotein(VLDL), one in intermediate-density lipoprotein(IDL), three in low-density lipoprotein(LDL), and four subclasses were in high-density lipoprotein(HDL). Remnant lipoproteins were VLDL and IDL combined. **Results:** Mean cholesterol concentration was 1.84mmol/L(72mg/dL) for remnants, 2.01mmol/L(78mg/dL) for LDL, and 1.83mmol/L(71mg/dL) for HDL, equivalent to remnants containing 32% of plasma total cholesterol. Large LDL and IDL were the lipoprotein subclasses containing most of plasma cholesterol. The plasma concentration of remnant cholesterol increased from ~1.4mmol/L(54mg/dL) at age 20 to ~1.9mmol/L(74mg/dL) at age 60. Corresponding values for LDL cholesterol were from ~1.5mmol/L(58mg/dL) to 2.1~mmol/L(81mg/dL). **Conclusion:** Using direct measurements, one third of total cholesterol in plasma was present in remnant lipoproteins, that is, in the triglyceride-rich lipoproteins IDL and VLDL. This suggests that a major part of cardiovascular disease risk could be attributable to cholesterol present in lipoproteins larger than LDL.

Participate in YIA

Remnant Cholesterol Predicts the Development of New Onset Diabetes Mellitus after Transplantation (NODAT)in Renal Transplant Recipients

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Renal transplantation is becoming more and more common with an associated increased number of renal transplant recipients (RTR). New onset diabetes after transplantation (NODAT) is a frequent and serious complication in RTR associated with worse patient and graft outcomes, but currently no biomarker is available to identify RTR at risk. Since cholesterol loading of beta cells with apoB-containing lipoprotein remnants impairs beta cell function, the current study investigated, if baseline plasma remnant cholesterol is associated with incident NODAT.

405 diabetes free RTR with at least 1 year of functioning allograft were included in this prospective longitudinal study. During a median [interquartile range] follow up of 9.6 [6.6-10.2] years, 57 patients (14.1%) developed NODAT. Remnant cholesterol was calculated by subtracting HDL and LDL cholesterol from total cholesterol levels in the fasted state.

Kaplan-Meier analysis showed a substantially increased risk for incident NODAT with increasing gender stratified tertiles of remnants ($p<0.001$). Receiving Operating Characteristic (ROC) curves confirmed the predictive capabilities of remnant cholesterol regarding NODAT with an area under the curve of 0.722 (95% Confidence Interval [CI] [0.649-0.796] $p<0.001$). Cox regression analysis demonstrated as well that remnant cholesterol is significantly prospectively associated with NODAT incidence in age and gender adjusted models (Hazard Ratio [HR] 2.39 95% CI [1.67-3.43], $p<0.001$) as well as after adjustment for relevant confounders such as lipid parameters ($p=0.007$), kidney function ($p<0.001$) and immunosuppressive medication ($p<0.001$).

This study demonstrates that the baseline remnant cholesterol level is a strong predictor of NODAT independent of several other recognized risk factors, and thus might constitute a clinically useful biomarker to identify patients at risk.

Body mass index, triglycerides and risk of acute pancreatitis: A general population study of >117,000 individuals

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Aim: The incidence of acute pancreatitis is rising worldwide and currently no curative treatment exist. Clarifying preventable risk factors are important for reduction of morbidity and mortality from acute pancreatitis. In this study we tested the following hypotheses: 1) High BMI is associated with high risk of acute pancreatitis, and 2) the risk of acute pancreatitis associated with BMI is partly mediated by high triglycerides. **Methods:** Two prospective cohort studies, the Copenhagen City Heart Study and the Copenhagen General Population Study with a combined median follow-up of 8 years, were used as study population. A total of 118,085 individuals with BMI and triglycerides measured at baseline were included. **Results:** 118,085 individuals, 45% males, with median age of 58 years(IQR:48-68), median BMI of 26 kg/m2 (IQR:23-28) and median triglycerides of 1.4 mmol/L(IQR:1.0-2.0) were included. Higher BMI was associated with higher risk of acute pancreatitis with a multivariable adjusted hazard ratio of 1.4(95% confidence interval:1.1-1.8) for BMI of 25-29.9, 2.1(1.6-2.9) for BMI of 30-34.9 and 2.8(1.8-4.3) for BMI>35 as compared to individuals with BMI of 18.5-24.9. Triglycerides mediated 25%(95%CI:9-40,P=0.002)of the association between BMI and risk of acute pancreatitis in the age and sex adjusted model and 16%(3-29%,P=0.02) in the multivariable adjusted model. **Conclusion:** High BMI is associated with high risk of acute pancreatitis in individuals from the general population, and this association is likely partly mediated by increased triglycerides. This indicates that there could be a potential for preventing acute pancreatitis by reducing BMI and triglycerides in individuals with high values.

Participate in YIA

Secular trends in LDL-cholesterol among 18-49-yar-olds in Norway

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Background: The risk of CVD increases progressively with increasing LDL-cholesterol (LDL-C) levels. Modest secular changes, even within the normal range, could therefore be of major importance for CVD trends at the population level. Despite this, there is no nationwide system for monitoring this important risk factor in Norway, and there is scarce knowledge about trends in LDL-C levels in young adults. The aim of the present study was to estimate annual trends in serum LDL-C from 2010 through 2017. **Methods:** We obtained data from lipid measures requisitioned mainly from primary and occupational health care services from all over the country, analysed at one laboratory. LDL-C was directly measured. All laboratory lipid tests available, excluding those from institutionalized patients, were examined (n = 1.217.653). Our analysis was restricted to persons between 18-49 years of age (mean 38.2 (±8.5) years). **Results:** Mean LDL-C in 2010-17 was 3.22 mmol/l (95 % CI 3.22-3.24), with the lowest levels in 2013-14 (3.03 mmol/l, 95 % CI 3.02-3.03). LDL-C was significantly higher in 2017 (3.31 mmol/l) than in 2012-2016 ($p<0.001$). The trends were similar in men and women. The prevalence of elevated LDL-C (= 3.0 mmol/l) was 56 % (men: 62 %, women: 50 %), and ranged from 35 % in 18-24-year-olds to 65 % in 45-49-year-olds. In 2017, the prevalence was 60 %, not significantly different from 2010 ($p = 0.67$). Mean non-HDL-C levels similarly decreased from 2010 through 2015, but then increased again. In 2017, 56 % of men and 32 % of women had non-HDL-C =3.9 mmol/l. **Conclusion:** Previously observed decreases in LDL-C in the Norwegian population seem to have flattened out in recent years, and may be reversing in young and middle-aged adults. This is in line with surveys from other countries. Whether this is related to dietary or other secular trends deserves further examination.

Participate in YIA

Oral Presentations – Abstracts –

Other Topics

SESSION IV

Genetically determined plasma C-Reactive Protein levels and risk of Alzheimer's disease in the general population.

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Introduction: Alzheimer's disease and other forms of dementia are devastating neurodegenerative diseases currently affecting more than 47 million people. There are no curative treatments, and large parts of the underlying biology remain unknown. Because the study of genetics in unbiased populations of the general population often helps to elucidate biological mechanisms and causal pathways, the analysis of genes underlying plasma biomarkers are of significant interest. Inflammation is increasingly understood as a primary driver in Alzheimer's disease, and this study aims to elucidate the role of a crucial molecule in inflammation - C-Reactive Protein (CRP) - for risk of Alzheimer's disease. **Methods:** We combined epidemiological and genetic data in cohorts totaling 110,960 individuals in a Mendelian randomization approach to explore the genetic and thereby potentially the causal role of CRP for Alzheimer's disease. **Results:** In observational analyses risk of Alzheimer's disease increased stepwise as a function of stepwise lower CRP levels (P for trend 5x10-15). Weighted CRP-allele scores were associated with a stepwise decrease in plasma CRP levels (P for trend 2x10-283), and a corresponding increase in risk of Alzheimer's disease (P for trend =0.02). In instrumental variable analysis, the causal risk ratio for a genetically determined 50% reduction in plasma CRP levels was 1.43 (0.98-2.10) (F-statistics = 307). **Conclusion:** Low plasma levels of CRP robustly associated with increased risk of Alzheimer's disease, and genetically decreased CRP suggested this association to be of a potential causal nature. However, further studies are needed to support the genetic results. Biologically, these findings make sense, as lifelong lower levels of CRP may indicate a reduced capacity to remove cell debris in the brain.

Participate in YIA

Blood-brain barrier transcytosis genes and risk of cerebral vascular diseases - a prospective cohort study of 74,754 individuals

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Alzheimer's disease often co-exists with cerebral vascular diseases, clinically. Pathologically, Alzheimer's disease is characterized by accumulation of a neurotoxic, sticky peptide, amyloid-β, in the brain and cerebral vessels. An important clearance pathway of amyloid-β from the brain is via transcytosis across the blood-brain barrier into the vascular lumen. Previously we have confirmed the association of genetic variants in PICALM, BIN1, CD2AP, and RIN3 – four genes suggested to be involved in blood-brain barrier amyloid-β transcytosis pathways – and risk of Alzheimer's disease and all-cause dementia. Here we wanted to test whether the same genetic variants also associate with cerebral vascular endpoints, since the blood-brain barrier most likely is central for dementia related vascular events. Further, we wanted to test whether such associations are independent of the strong ε4 APOE risk allele.

In a prospective cohort study of 74,754 individuals from the general population we genotyped PICALM (rs10792832), BIN1 (rs6733839), CD2AP (rs10948363), and RIN3 (rs10498633), and generated a weighted and a simple allele score.

Multifactorially adjusted hazard ratios for the fourth quartile versus the first quartile of the weighted allele score were 1.71 (1.18-2.49) for vascular dementia, 1.17 (1.04-1.31) for ischemic stroke, 1.18 (0.94-1.48) for hemorrhagic stroke, and 1.12 (1.04-1.22) for all cerebrovascular disease. Hazard ratios were similar after APOE adjustment.

Genetic variants in PICALM, BIN1, CD2AP, and RIN3 are associated with increased risk of vascular dementia independent of the strong APOE ε4 allele but do not seem to be associated with stroke of any kind. These findings may suggest that clathrin-mediated endocytosis in clearance of amyloid-β across the blood-brain barrier is important for the integrity of both brain tissue and cerebral vessels.

Participate in YIA

The function of ORP2 in endothelial cell polarization and angiogenic tube formation in vitro

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Oxysterol-binding protein homologs, ORPs, constitute a family of lipid-binding proteins with documented functions in lipid transport and signaling at membrane contact sites (MCSs). ORP2, a short ORP protein, has a lipid-binding cavity with affinity for oxysterols, cholesterol and phosphatidylinositol phosphates (PIPs). ORP2 has several cellular functions related to e.g. cholesterol homeostasis, triglyceride metabolism, cell adhesion, migration and proliferation. ORP2 targets lipid droplet-endoplasmic reticulum MCSs, late endosomes (LE) and the plasma membrane (PM) where it co-localizes with lamellipodial F-actin and induces PM protrusions.
In the present work we assess the role of ORP2 in endothelial cell (EC) polarization, migration and angiogenic tube formation in vitro. We investigate the effect of ORP2 on angiogenic gene expression and identify novel interaction partners of ORP2 in ECs by pull-down and bimolecular fluorescence complementation assays. Moreover, since cholesterol trafficking is essential for angiogenic signaling, we examine the effect of ORP2 on the subcellular distribution of cholesterol in ECs by employing a fluorescent mCherry-D4H probe.
ORP2 knock-down significantly inhibited EC migration and angiogenic tube formation in vitro by decreasing several parameters of the tubular network, while its overexpession had a modest opposite effect. Overexpression of ORP2 shifted the D4H cholesterol probe towards the PM as compared to control cells. Intriguingly, a PIP-binding deficient mutant of ORP2 induced a drastic accumulation of D4H in LEs, suggesting a PIP binding-dependent function of ORP2 in the cholesterol flux between LEs and PM. Interestingly, we also found that ORP2 interacts with Rab8a, a protein involved in LE-PM cholesterol delivery and cell protrusion formation.
To conclude, ORP2 was identified as a novel controller of EC functions, with synergistic effects on EC polarization, migration and angiogenic tube formation. Our results suggest a role for ORP2 in angiogenic signaling mediated by regulation of cytoskeletal dynamics and intracellular transport of cholesterol and PIPs.

Participate in YIA

Patient-Reported Outcome Measures in Familial Hypercholesterolemia (FH) Patients: Knowledge is Health

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Aim: Familial Hypercholesterolemia (FH) is the most common hereditary cause of early-onset cardiovascular disease (CVD) and requires life-long treatment. Since most FH patients do not experience any symptoms, a patient's understanding of FH is especially important and might influence patient outcomes. Currently, no Patient-Reported Outcome Measures (PROMs) are available for FH. Therefore, this study aims to (1) identify PROMs for FH patients and (2) investigate the influence of patient knowledge on PROMs. **Methods:** This study presents the first analysis (March 2017-October 2018) of prospectively collected data in a cohort of FH patients attending Erasmus MC. A multidisciplinary group of FH experts in collaboration with a sounding board of FH patients (n=166) developed a set of PROMs, including a knowledge questionnaire on heritability, lifestyle, and lipid parameters. Patients could score full or half points resulting in final scores from 0-10 (no knowledge-perfect knowledge). Two groups were created based on knowledge scores: (1) Insufficient knowledge (0-7), and (2) Sufficient knowledge (7.5-10). **Results:** In total, 429 (81.4%) FH patients completed the questionnaires. The insufficient knowledge group showed significant ($p<0.05$) differences compared to the sufficient knowledge group regarding BMI (26.33 kg/m2 vs. 25.28 kg/m2), quality of life (EQ-5D-5L index values) (.85 vs. .89), and current smoking (14% vs. 7%). Additionally, more patients in the insufficient knowledge group indicated to experience side-effects from medication (62% vs. 49%, $p<0.05$). Self-efficacy scores regarding the domains diet, exercise and weight were significantly better in the sufficient knowledge group (respectively $p=0.01$, $p<0.01$, $p=0.02$). **Conclusions:** This outcome set for FH patients, including PROMs, shows that insufficient knowledge of FH is negatively related to health outcomes. Improving patients' knowledge of FH might lead to better health.

Participate in YIA

Proatherogenic Function of LDL is Prospectively Associated with Graft Failure in Renal Transplant Recipients but not LDL Cholesterol Levels

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**These authors contributed equally to the work.*

Atherosclerosis has a major impact on poor outcomes in renal transplant recipients (RTR), since (i) cardiovascular mortality is 4 to 6-fold increased and (ii) chronic graft failure is mainly caused by de novo atherosclerosis formation in the graft. Binding of pro-atherogenic lipoproteins to proteoglycans (PG) is an early key event in atherosclerotic lesion formation. The present study determined, if lipoprotein-PG binding predicts all-cause mortality, cardiovascular-specific mortality and graft failure in RTR. RTR (n=589) with a functioning graft for at least one year were prospectively included at a single center university hospital. Susceptibility of lipoprotein binding to PG was assessed using baseline plasma. During a median follow-up of 9.5 years, 183 (31%) RTR died (52% from confirmed cardiovascular causes) and chronic graft failure occurred in 73 (13%) patients. Lipoprotein binding to PG was significantly higher in patients who subsequently developed graft failure compared to those with a surviving graft (1.46 ± 0.49 nmol bound cholesterol/mmol LDL-C versus 1.68 ± 0.93 nmol bound cholesterol/mmol LDL-C, p=0.001). Cox regression demonstrated a significant association of a higher susceptibility of lipoprotein-PG binding with graft failure in a gender and age adjusted model (hazard ratio=1.45, 95% confidence interval=1.14 to 1.85, p=0.002) but not with overall or cardiovascular-specific mortality. In contrast, circulating levels of LDL-cholesterol as classical biomarker for atherosclerosis showed no association with mortality or graft failure in RTR. The functional biomarker of LDL binding to arterial PG can predict chronic atherosclerosis-driven graft failure in RTR, but not LDL-cholesterol as quantitative parameter. For (cardiovascular) mortality neither quantity of LDL-cholesterol nor LDL function are useful predictors.

Participate in YIA

Genetic variation in PPP1R3B and plasma levels of glucose, lipids, and liver enzymes in the Danish general population

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Aim: A common variant near the gene PPP1R3B (rs4841132) likely associates with increased hepatic glycogen content in humans. The aim of this study was to assess the broader phenotypic effects of rs4841132. **Methods:** We tested the association of rs4841132 with plasma levels of glucose, lipids, lipoproteins, and liver enzymes, and with risk of ischemic heart disease (IHD) and liver disease in 106,971 participants from the Danish general population. **Results:** The minor A-allele of rs4841132 was associated with up to 0.17 mmol/L higher plasma levels of glucose among participants that had not eaten for at least four hours (P=0.005). Paradoxically, the A-allele tended to associate with lower glucose levels among those that had eaten within two hours (P-interaction between rs4841132 and time since last meal on plasma glucose= 6×10E-5). Moreover, the A-allele was associated with up to 0.18 mmol/L lower plasma levels of total cholesterol, 0.08 mmol/L lower LDL-C, and 0.08 mmol/L lower HDL-C, and with up to 2.1 U/L higher plasma alanine aminotransferase and 6.1 U/L higher plasma alkaline phosphatase (all P<0.001). The A-allele was associated with 15% increased risk of liver disease (P=0.031), but it did not influence the risk of IHD. **Conclusion:** PPP1R3B rs4841132 exhibits distinct pleiotropic effects on plasma glucose, lipids, and markers of liver damage. The A-allele of rs4841132 is likely associated with an increased uptake of glucose in the liver in the postprandial phase.

Participate in YIA

A systems analysis of lipidemic heterogeneity in APOE*3Leiden.CETP mice

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Within the human population, considerable variability exists between individuals in their susceptibility to develop obesity and dyslipidemia. In humans this is thought to be caused by both genetic and environmental variation. APOE*3-Leiden.CETP mice, an inbred mouse model that develops metabolic syndrome upon feeding high-fat high-cholesterol diet (HFCD), also shows large inter-individual variation in obesity and dyslipidemia despite the lack of genetic and environmental variation. In this study, we used computational modeling to investigate underlying mechanisms explaining the phenotypic variation. APOE*3-Leiden.CETP mice were fed a HFCD for 6-months. Apart from the variability a striking phenomenon was a biphasic response in both plasma triglyceride (TG) and cholesterol. Data were fitted to a computational model of whole body glucose and lipid metabolism making use of a method called Adaptations in Parameter Trajectories (ADAPT). ADAPT integrates longitudinal data, and predicts how the parameters of the model must change through time in order to comply to the model constraints. To simplify presentation of the results mice were classified with either high (responders) or low (non-responders) plasma TG. ADAPT analysis suggested decreased cholesterol absorption, higher energy expenditure and increased fecal fatty acid excretion in non-responders. Experimental validation demonstrated that non-responders were indeed characterized by increased fecal fatty acid excretion. The amount of fatty acids excreted strongly correlated with bile acid (BA) excretion, suggesting that variation in BA homeostasis may drive the phenotypic variation. in the mice. To validate the role of BA, APOE*3Leiden.CETP mice were treated with an FXR agonist to reduce fecal BA excretion. FXR agonist treatment for 2 wks decreased both plasma TG and cholesterol by 70% indicating that indeed BA strongly control the Metabolic syndrome phenotype in the APOE*3Leiden.CETP mice.

Posters – Abstracts –

Inflammation and Vascular Biology

SESSION I

Characterisation of extracellular matrix in human atherosclerotic lesions

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

Tropoelastin oxidation by peroxynitrous acid: a key modulator of arterial structure, function and loss of elasticity

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Background: Elastin is an abundant extracellular matrix protein in major arteries and is responsible for their elasticity. Monomeric tropoelastin (TE), is processed during elastogenesis to form mature elastic fibers. Peroxynitrous acid (ONOOH), a potent oxidizing and nitrating agent, is formed in the inflamed artery wall from superoxide radicals and nitric oxide. **Hypothesis:** That ONOOH modifies elastin to give dysfunctional TE with decreased elasticity and altered function, and this contributes to atherosclerotic lesion development. **Methods:** Protein changes were assessed using immunoblotting, UPLC, and mass spectrometry. Structure and function were assessed by turbidity, dynamic light scattering, electron microscopy and molecular dynamics. Human lesions were examined by immunofluorescence. **Results:** TE is highly sensitive to damage due to its unusual amino acid composition. ONOOH exposure gives extensive oligomerization (dityrosine cross-links) and nitration of Tyr residues to give 3-nitroTyr in a dose-dependent manner. Quantification indicates up to two modified Tyr per protein molecule. Peptide mapping shows 3-nitroTyr formation occurs at most Tyr sites but the extent of modification varies markedly, with >50% modification (label-free quantification) at several sites. Both inter- and intra-molecular cross-links were detected by MS. ONOOH exposure enhances the rate and extent of protein self-assembly but yields distorted materials (seen by SEM), consistent with the modifications and cross-links. Human lesion analysis showed colocalization of 3-nitroTyr with elastin epitopes, consistent with in vivo modification. **Conclusions:** Exposure of tropoelastin to ONOOH induces marked chemical, structural and functional changes and altered matrix assembly. Such damage appears to accumulate in human arterial tissue during the development of atherosclerosis.

Temperature Triggered Myokine Release

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Aims: Physical activity is known to increase release of myokines, the muscles own cytokines, which are involved in cross talk with other tissues. Myokines are thought to be released due to contractions; however, physical activity also increases the muscle temperature. We wanted to explore the effect of cold temperature and reheatment on the release of myokines from muscle cells, and what involvement they have in inflammation and metabolic disease. **Methods:** Myoblasts where cultured and differentiated into myotubes from satellite cells isolated from biopsies of musculus vastus lateralis. Cell lysate and media was collected after 16 h incubation at either 15°C or 37°C followed by 0, 3, 6, or 24 h reheating at 37°C. Gene expression was examined by qPCR and the myokine content was measured in both cell lysate and media. **Results:** There were large donor variations in gene expression, myokine content in lysate and in media. However, there was a clear increase in the gene expression of IL-6, IL-8 and MCP-1; reaching a peak after 3 h of reheating. These are all cytokines with known inflammatory properties; however, their function as myokines and the processes involved in their transcription and release are still to be elucidated. **Conslusion:** Cold exposure is known to be associated with several diseases, including circulatory disorders and musculoskeletal disorders. Myotubes acutely express several inflammatory myokines after cold exposure or reheatment after cold exposure compared to myotubes grown under normal conditions. We hypothesize that the reheatment mimic an exercise-induced anti-inflammatory response.

The anti-inflammatory function of follicular fluid HDL predicts the outcome of modified natural cycle in vitro fertilization

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Background and aim: The evolutionary role of HDL is likely not the protection against atherosclerotic cardiovascular disease. Rather, a modulation of other fundamental processes can be hypothesized such as wound healing, infection or reproduction. Inflammation in the ovary is associated with worse fertility outcomes. HDL has potent anti-inflammatory activities and HDL is the main lipoprotein subclass found in follicular fluid (FF), the natural environment of the developing oocyte. Therefore, we evaluated the predictive value of the anti-inflammatory capacity of FF HDL for embryo quality in modified natural cycle in vitro fertilization (MNC-IVF). **Methods:** FF from 329 MNC-IVF procedures and 19 matched plasma samples were analyzed. The anti-inflammatory function of HDL was determined as the capacity of FF/plasma HDL to inhibit vascular cell adhesion molecule-1 (VCAM-1) mRNA expression induced by tumor necrosis factor-α (TNF-α) in endothelial cells in vitro. The correlation between HDL anti-inflammatory capacity and IVF outcomes was assessed by multilevel general estimating equations. **Results:** There was no association between the anti-inflammatory function of FF HDL and that of matched plasma HDL indicating an independent regulation of the inflammatory state in the microenvironment of the ovary. However, a higher FF HDL anti-inflammatory function was related to a higher chance of achieving top quality embryos, after adjustment for possible confounders (OR=1.01, P = 0.031). **Conclusion:** This study indicates that the anti-inflammatory capacity of FF HDL could be clinically useful as predictor for embryo quality during MNC-IVF.

Characterisation of neutrophil extracellular traps and their role in atherosclerosis

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Atherosclerosis is a cardiovascular condition where plaques build up in the artery walls due to a disturbed lipid metabolism and chronic inflammation. This leads to a narrowing of the vessels that can result in strokes and heart attacks. Neutrophils can release extracellular traps (NETs), which are spindles of DNA complexed with histones, myeloperoxidase (MPO) and elastase. NETs have important anti-bactericidal properties, but they are also increasingly implicated in chronic inflammatory pathologies. New evidence supports a role of NETs in thrombosis and lesion development in atherosclerosis. However, the pathways responsible for these damaging reactions are not known. The enzyme MPO is release by neutrophils and known to bind to DNA. MPO produces the potent oxidant hypochlorous acid (HOCl), which also contributes to atherosclerosis by causing oxidative tissue damage. The aim of this study was to characterize the NETs to determine whether these structures were modified by oxidation, which may help to drive lesion formation. The human PLB-985 cell line was differentiated into neutrophils and exposed to different stimuli to release NETs. Analysis by fluorescent microscopy and immuno-histochemistry revealed these structures contained both DNA, and citrullinated histones, consistent with NET formation. Initial studies to characterize oxidative modifications to the components of NETs were carried out using a commercial preparation of histones exposed to HOCl. Exposure of the histones to HOCl resulted in the modification of Lys and the formation of unstable chloramines, which decomposed over 24 h. In addition, evidence was obtained for the oxidation of Met, loss of Arg, and increase protein carbonyl products, consistent with oxidative modification of the histones. Experiments are in progress to assess whether these (and other) modifications are present on the NETs released by neutrophils.

The Interaction Between Mitochondrial Stress and the Innate Immune System Receptor NLRP3

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Recent evidence suggests that mitochondria are the main source of inflammasome-activating short-lived reactive oxygen species (ROS), and as such may constitute the signal-integrating organelle for NLRP3 inflammasome activation. Adult mouse cardiomyocytes (CM) were isolated from normal C57Bl6 (WT) or mice deficient in NLRP3. CM were incubated with the mitochondrial uncoupler (carbonyl cyanide m-chlorophenyl-hydrazone; CCCP) for 24 h. Cell viability was visualized by Annexin V (apoptosis) and propidium iodide (necrosis) staining. CM were also loaded with tetramethyl-rhodamine methyl ester (TMRM) and 20 min later subjected to ROS injury evoked by laser confocal microscopy. The time to membrane depolarization was measured and compared with control cells. Mitochondria were isolated from heart tissue after ex vivo ischemia reperfusion (Langendorff technique) and oxygen consumption by high-resolution respirometry was measured. NLRP3 protein was visualized using antibody staining and confocal microscopy. Adult NLRP3−/− CM had reduced CCCP-induced cell death as compared to WT CM, as evident by decreased number of Annexin V and propidium iodide positive cells, Moreover, NLRP3-/- cells had slower mitochondrial membrane depolarization evaluated in TMRM loaded CM during exposure to reactive ROS induced by laser confocal microscopy, indicating protection of mitochondrial function. WT mitochondria from heart had reduced oxygen consumption after ischemia reperfusion compared to NLRP3−/− mitochondria demonstrating mitochondrial respiration impairment. Perinuclear NLRP3 protein was detected by laser confocal microscopy in the CM. NLRP3 in adult CM may play a role in regulating mitochondrial and cellular viability and our results are compatible with the notion that ROS generated by mitochondria having reduced membrane potential can lead to NLRP3 inflammasome activation in CM and further to cardiac dysfunction.

YIA Poster Walk – Abstracts

Inflammation and Vascular Biology

SESSION I

Impact of myeloperoxidase-derived oxidants on vascular smooth muscle cell damage and death in atherosclerosis

Konstantina Flouda, Michael Jonathan Davies and Clare Louise Hawkins

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Atherosclerosis is a lipid-driven, inflammatory disease, which is characterized by the presence of atherosclerotic plaques consisting of lipids, immune cells and debris in the arterial wall. Myeloperoxidase (MPO), an enzyme released by activated immune cells, is elevated in atherosclerotic plaques. MPO produces hypochlorous acid (HOCl), an oxidant linked to vascular cell dysfunction and death. In the presence of thiocyanate (SCN-), MPO forms hypothiocyanous acid (HOSCN), a milder oxidant which reacts in a highly selective manner with thiols (R-SH), which may be protective in chronic inflammatory conditions. Vascular smooth muscle cells (VSMC) are essential in maintaining blood vessel structure and function. However, chronic inflammation can promote VSMC proliferation, migration and extracellular matrix production, which accelerates atherosclerosis. This project examines the reactivity of the MPO-derived oxidants HOCl and HOSCN, with Human Coronary Artery Smooth Muscle Cells (HCASMC), and the ability of SCN- to prevent oxidative damage. Treatment with HOCl and HOSCN decreased the viability and metabolic activity of HCASMC and caused irreversible toxicity, which was more extensive with HOCl. The HOCl-induced decrease in metabolic activity and cell death was prevented by addition of increasing SCN- concentrations. Cellular thiols are key targets for MPO-derived oxidants, and were significantly decreased on treatment of HCASMC with HOCl and HOSCN. Under some conditions, the cells were able to repair this oxidation, consistent with reversible thiol oxidation. HOCl also altered the expression of some genes associated with oxidative stress and inflammation, which again, was influenced by the presence of SCN-. Together, these data are consistent with SCN-consuming HOCl, to form HOSCN, which is less damaging to VSMC. This may have implications for preventing the VSMC damage observed under chronic inflammatory conditions in atherosclerosis.

Participate in YIA

Role of thiocyanate in the repair of myeloperoxidase-derived thiol oxidation during chronic inflammation

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Myeloperoxidase is a vital component of the human immune system, producing the potent oxidant hypochlorous acid (HOCl), to kill invading pathogens. However, an overproduction of HOCl causes damage to host cells, which promotes lesion development in atherosclerosis. Thiocyanate (SCN-) is the favoured substrate for MPO and a competitive inhibitor of HOCl formation. This forms hypothiocyanous acid (HOSCN), a selective oxidant that targets thiols, resulting in the formation of reversible oxidation products. This has led to the hypothesis that supplementation with SCN- may have therapeutic value to reduce oxidation during chronic inflammation. In this study, we compare the reactivity of HOCl and HOSCN with macrophages, and examine whether the addition of SCN- can alter the extent and nature of cellular damage. Exposure of the J774A.1 murine macrophage cell line to HOCl resulted in cell death, loss of cellular thiols, activation of pro-inflammatory and stress-related signaling pathways, leading to the altered expression of oxidation-related proteins, and chemokines / cytokines. HOSCN treatment also resulted in extensive thiol oxidation and cell death, though in this case, reversible thiol oxidation and less cell death than HOCl were observed. HOSCN could also increase gene expression, including some chemokines / cytokines, which were not altered by HOCl. Addition of SCN- decreased the extent of HOCl-induced cell death, altered the nature of thiol oxidation from non-reversible to reversible and changed the pattern of HOCl-induced gene expression. Interestingly, exposure of the J774A.1 murine macrophage cell line to SCN- in the absence of HOCl also resulted in altered pro-inflammatory gene expression. These data support a potential role for SCN- to reduce oxidative damage during chronic inflammation, and partly explain in vivo observations, whereby supplementation with SCN- decreases lesion development in murine atherosclerosis.

Participate in YIA

miR-107 as an inhibitor of human adipocyte differentiation and lipid storage

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MicroRNA-107 (miR-107) is a known regulator of insulin signaling and is connected to obesity. However, its mechanistic role in human adipocytes has remained inconclusive. Human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes' differentiation and lipid accumulation is inhibited by overexpression of miR-107 via CDK6 and Notch signaling. CDK6 is a validated target of miR-107 and was attenuated upon miR-107 overexpression. Inhibition of CDK6 has been shown to reduce signaling receptor Notch3 that regulates adipocyte differentiation. Our results show overexpression of miR-107 leading to a downregulation of Notch3 and its downstream target Hes1. Furthermore, in mature adipocytes miR-107 impairs glucose uptake and triglyceride synthesis. To conclude, miR-107 may inhibit adipocyte differentiation and thus in later obesity, increase ectopic fat accumulation and insulin resistance. Here, the functional roles of miR-107 in pre- and mature adipocytes are elucidated.

Participate in YIA

Effects of hypoxia on extracellular matrix synthesis by human coronary artery endothelial cells

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Background and Aim: Increased metabolic demand for O_2, and greater diffusion distances from the arterial lumen can result in localised hypoxia (<1% O_2) in atherosclerotic lesions. This is hypothesised to result in altered expression of extracellular matrix (ECM) molecules produced by arterial wall cells, and hence an altered composition of the vascular ECM in atherosclerotic lesions. This may contribute to lesion development. **Methods:** Human coronary artery endothelial cells (HCAECs) were cultured under 1% or 20% O_2. Gene expression of ECM species, cytokines and cell activation markers was examined by rt-qPCR. Protein levels were detected by ELISA and immunoblotting. Cell proliferation and adhesion were quantified using MTS and Calcein-AM respectively. ICAM-1, VCAM-1 and reactive oxidant production were studied by flow cytometry. **Results:** HCAECs showed increased mRNA expression of HIF1□, VEGF and VEGFR when cultured at 1% O_2 for 24 h. Increased oxidant production was detected in HCAECs exposed to 7 days of hypoxia, despite no significant increases in ICAM-1 gene (qPCR) and protein expression. Expression of collagen IV was decreased significantly, while versican expression was increased significantly in HCAECs exposed to 1% versus 20% O_2. Increased expression of versican was confirmed by ELISA. HCAECs showed lower adhesive ability to ECM in response to 1% O_2, while cell proliferation was increased in exposed to hypoxia. **Discussion:** These data show that hypoxia alters the ECM generated by endothelial cells. The increased levels of versican may exacerbate the progression of atherosclerosis, as this protein is a well-established binding site for lipoproteins, and hence may contribute to lipoprotein retention in lesions. The concurrent increase in oxidant production may exacerbate lipoprotein modification and the accumulation of lipid-laden cells in growing lesions.

Participate in YIA

Posters – Abstracts –

Cardiovascular Disease

SESSION II

Effectivity of dietary nitrates on vascular function: does age matter? - A randomized, placebo-controlled, double-blind crossover study -

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Background: Under special conditions such as hypoxia, supplementation of dietary nitrates has shown promising results in reducing incidence of cardiovascular disease in a number of different populations such as elderly, healthy individuals, hypertensive population among others. However, it is currently unknown whether the efficacy of dietary nitrates varies with age. We aim to determine whether age modifies the efficacy of dietary sources of nitrates on vascular function. **Methods:** A total of Twenty-five participants were recruited and stratified in two groups (18-35 years and >55 years) for a double-blind, randomized, placebo-controlled, crossover trial. Participants were randomly assigned to receive a single dose n=25 (70 ml) of either standard beetroot juice n=25(control) or nitrate-depleted beetroot juice (placebo). Blood pressure was measured before ingestion of the juice and every 20 minutes following juice consumption, for a three-hour period. Microvascular endothelial function assessments were performed at baseline and three hours after juice consumption and urine NOx was measured on three different occasions **Results:** we conduct a matched 2x8 ANOVA to study the interaction effect between vascular function/treatment and a Mann-Whitney t-test to determine the effectiveness of the treatment in comparison to the placebo on each group. Under resting condition, dietary nitrates have no significant effectivity difference in the cardiovascular function regardless the age p=>0.5. However, there is a higher interaction effect between dietary nitrates and systolic blood pressure of 2% (p= 0.01) in the elderly population. Finally, we encounter periods in which the elderly population presents a recoil effect in the systolic blood pressure. **Conclusion:** The efficacy of acute ingestion of nitrates on vascular function is not affected by age. Also, the observed recoil effect might not to be alarming, but it should be studied further as it may lead to a better understatement of NOx management during aging.

Mitochondrial Genetics of Atherosclerotic Disease

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Background: The results of epidemiological studies suggest that genetic factors may explain up to 15–20% variability of atherosclerotic diseases. However, all known nuclear genome polymorphisms provide explanations for approximately 5% cases of clinical manifestations of atherosclerosis. In recent years, considerable attention has been paid to the role of mitochondrial DNA (mtDNA) damage in the pathogenesis of atherosclerosis. The hypothetical mechanism of atherogenic effect of mtDNA mutations may be due to the enhanced production of reactive oxygen species, an increase in oxidative stress, the development of mitochondrial dysfunction and inflammatory reaction, and cell death.
Aim: The set of several consecutive studies of basic and clinical design was focused on the evidence of association of mtDNA mutations with atherosclerosis. **Results:** At least 10 mitochondrial mutations in 8 genes encoding the 12S subunit of ribosomal RNA, leucine t-RNA, cytochrome B, and 1, 2, 5, and 6 NADH dehydrogenase subunits were significantly associated with atherosclerotic lesions. The associations of these mutations with the extent of subclinical carotid atherosclerosis assessed by carotid intima-media thickness have been revealed. Our recent studies based on mtDNA next generation sequencing have demonstrated significant correlation of mtDNA mutations with CHD and myocardial infarction. The most common proatherogenic and antiatherogenic haplotypes of mtDNA mutations were identified. A panel of several mtDNA variants associated with atherosclerosis was obtained. Our current research was aimed to the studies of precise mechanisms whereby mtDNA mutations can lead to atherosclerosis development at the cellular level; the methodological approach was based on creation of cytoplasmic hybrids, that reproduce the pathogenic mitochondrial genotype, and the most recent non-disclosed results are to be reported. **Conclusion:** Our current knowledge allows to consider mutations occurring in the mitochondrial genome as the mechanistic biomarkers of both cardiovascular and metabolic diseases, although the similarity of clinical phenotypes and the generality of risk factors do not mean the similarity of the genetic background.

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SmartDiet – A brief questionnaire for the assessment of dietary habits and lifestyle

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Low-density lipoprotein cholesterol (LDL-C) is a target for lipid-lowering therapy in cardiovascular disease. Dietary intervention to reduce the intake of saturated fat is linked to reduced LDL-C levels. Recent data emphasize that some individuals are highly responsive to this dietary change. This underlines the importance of information on dietary habits to achieve treatment goals in cardiovascular prevention. Most methods evaluating a diet are too time-consuming for routine clinical practice. We will present a brief food questionnaire “SmartDietTM” developed at the Lipid Clinic, University Hospital of Oslo, and share our experiences with its use in clinical practice and research. SmartDiet is a 15–item self-administered questionnaire developed for a simple assessment for immediate feed-back in clinical practice, of foods depicting the usual diet of an individual regarding fat, fiber, fruit, and vegetables. Three response categories are specified for the item in question, each including a group of foods. These categories provide the basis for a rating system, resulting in a sum dietary score. Additional queries about lifestyle are included. The reproducibility and validity studies of the questionnaire comparing the dietary scores and dietary data obtained from a 7–day weighed food record in 101 persons gave correlation coefficients of respectively 0.95 and 0.73. SmartDiet has been in daily use at our Lipid Clinic since 2002, in more than 50,000 patient consultations, and also in research. The patients spend approximately 10 minutes answering the questionnaire in the waiting room, and health personnel get essential data for dietary advice through a quick glance at the questionnaire. We experience the questionnaire as an effective tool for identifying individuals who can markedly benefit from dietary change and health education. In addition, the questionnaire has been useful in the long-term monitoring of dietary intervention and in research.

The role of sphingolipids in essential hypertension: a case-control study

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Hypertension is a major risk factor for cardiovascular disease. Sphingolipids, especially ceramides (CER) and sphingosine-1-phosphate (S1P), have been implicated in essential hypertension. These sphingolipids are carried in plasma by different lipoproteins. Therefore, we compared sphingolipid levels in plasma and lipoproteins between hypertensive patients and normotensive controls. We included 19 hypertensive patients (SBP 140 mmHg and/or DBP 90 mmHg) and 19 age- and sex-matched normotensive controls. Lipid-lowering therapy users were excluded. EDTA-plasma was subjected to density gradient ultracentrifugation to isolate lipoproteins. Cholesterol and triglycerideswere determined using standard laboratory methods and sphingolipids by HPLC-ESI-MSMS in full plasma and in isolated lipoproteins. Data is expressed in mean ± SEM. In contrast to plasma cholesterol, plasma triglyceride levels were higher in patients than controls (1.66±0.14 vs. 0.91±0.09 mM, p<0.001). Total plasma ceramides were higher in patients than controls (14540±950 vs. 12091±651 nM, p=0.040), while S1P levels did not differ (1786±109 vs. 2090±134 nM, p=0.088). Most ceramide species were higher in patients than controls (CER-C14:0: 59±3 vs. 48±2 nM, p=0.008; CER-C18:0: 131±9 vs. 86±4 nM, p<0.001; CER-C20:0: 569±47 vs. 378±20 nM, p=0.001; CER-C22:0: 4178±358 vs. 3074±162 nM, p=0.008; and CER-C24:1: 2022±100 vs. 1564±114 nM, p=0.005). Analysis of sphingolipids in lipoproteins is pending. In line with previous work, the ceramide-S1P ratio is higher in patients with essential hypertension, supporting a role of sphingolipids in increased cardiovascular risk. Determination of sphingolipid distribution over different lipoproteins may improve cardiovascular risk assessment in these patients.

Anti-inflammatory diet in rheumatoid arthritis effects on cardiovascular risk factors

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Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease that affects 0.5-1% of the population and is characterized by systemic inflammation and joint damage. The inflammation leads to joint destruction, pain and fatigue but also an increased risk for cardiovascular diseases (CVD). **Aim:** The aim of this study was to test if a proposed anti-inflammatory diet compared to a control diet, will improve cardiovascular risk factors in RA-patients with stable disease activity. **Methods:** In total, 50 patients with RA and moderate disease activity were randomized to begin with either a portfolio diet based on several food items with suggested anti-inflammatory effects or a control diet resembling the average Swedish diet. The dietary periods lasted over 10 weeks with a 3-month wash-out period in between. A home-food delivery chain delivered food items weekly. The diets were labelled “fiber” and ”protein” diet respectively, in an attempt to maintain a single-blinded design. Both groups continued with habitual pharmacological treatment. Body mass index (BMI), total cholesterol, TG, HDL, LDL, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), blood pressure and body compositions were measured at the beginning and end of each dietary period. **Results:** TG and LDL was significantly lower after the anti-inflammatory diet period compared with after the control diet ($p = 0.02$ and $p = 0.017$). Likewise, there was a trend to towards lower BMI ($p = 0.077$) and body fat-mass ($p = 0.058$). Fat free mass increased within both groups. CRP increased significantly only within the control group ($p = 0.020$), with no significant differences between groups. **Conclusions:** Blood lipids were improved by the anti-inflammatory portfolio diet compared to the control diet, indicating a potential to modify cardiovascular risk factors in patients with RA.

YIA Poster Walk – Abstracts –

Cardiovascular Disease

SESSION II

Does myeloperoxidase-induced modification of extracellular matrix (ECM) of the arterial wall contribute to vulnerable plaques?

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Objective: This study examines how the myeloperoxidase (MPO)-derived inflammatory oxidant HOCl modifies ECM synthesised by human coronary artery smooth muscle cells (HCASMCs) and its possible link with vulnerable plaques. **Methods:** ECM laid down by HCASMCs, and subsequently decellularized, was prepared and exposed to MPO system (0-200 μM H_2O_2/200 mM Cl^-/20 nM MPO) with colocalization of MPO and ECM protein (fibronectin) detected by confocal microscopy. ECM damage was investigated using antibodies against specific ECM proteins via ELISAs, immunoblotting and immunofluorescence assay. **Results:** MPO is shown to bind preferentially to the matrix glycoprotein fibronectin with colocalization detected by confocal microscopy. Matrix-bound MPO initiates HOCl generation and ECM damage, with this detected (via ELISA and immunoblotting) as a loss of antibody reactivity against the cell-binding fragment (CBF) of fibronectin, laminins and type IV collagen. Interestingly, exposure of ECM to the MPO system with a low (~10 μM) concentration of H_2O_2 also generates ECM with an increased antibody reactivity (detected via ELISA and immunofluorescence assay) against HOCl-generated epitopes on matrix proteins. **Conclusion:** MPO shows high affinity for ECM synthesized by human coronary artery smooth muscle cells. Matrix-bound MPO generates the powerful inflammatory oxidant HOCl, that induces modification of ECM proteins, including fibronectin, laminins, and type IV collagen. The extent and nature of the damage is modulated by the initial H_2O_2 concentration. These experiments reveal the effects of MPO-generated HOCl on ECM of the artery wall, and explain how oxidative modifications to ECM accumulate with pathophysiological H_2O_2 concentrations in vivo. This damage is proposed to contribute to the formation of vulnerable plaques during the development of atherosclerosis.

Participate in YIA

Higher expression of genes related to T-and B-cell pathways in PBMCS from children with versus without familial hypercholesterolemia: a cross-sectional study

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Background and Aims: Subjects with familial hypercholesterolemia (FH) display high total and low-density lipoprotein (LDL)-cholesterol which may lead to accelerated atherosclerosis and premature coronary heart disease (CHD). Previously, we have shown higher intima-media thickness and systemic inflammation in FH versus control children. We aimed to perform a comprehensive immune gene expression analysis in peripheral blood mononuclear cells (PBMCs) from children with and without FH to understand more of the immunological pathways involved in early atherosclerosis. **Methods:** Expression analysis of immunological genes in PBMCs from non-statin treated children with (n=30) and without (n=21) FH was performed with nCounter GX Human Immunology Kit v2 on the nCounter ® analysis system (Nanostring Technologies). Genes were thereafter categorized into pathways based on their function. **Results:** Of 594 targets, 93 genes were differentially expressed between children with and without FH (FDR < 5%). In FH versus control children, the main pathways related to these genes were (higher expressed genes/genes changed): antigen presentation (1/7); apoptosis (3/7); B-cells (5/6); cell growth, proliferation and differentiation (5/7); interleukins (5/9); T-cells (18/19); toll like receptors (2/5); TNF super family (6/8). Adjusting for LDL-cholesterol mostly attenuated or neutralized the significant differences. **Conclusions:** Children with FH had higher expression level of genes related to B-cell and T-cell pathways in PBMCs compared with control children, indicating a role of these cells in early atherosclerosis. Also, LDL-cholesterol seems to be the main driver of the differences observed in these immunological genes between FH and control children.

Participate in YIA

Lipoprotein(a) plasma levels and association to cardiovascular disease in a Stockholm County cohort – a retrospective observational cohort registry study

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Aim: Lipoprotein(a) (Lp(a)) is a strong independent genetic risk factor for cardiovascular disease (CVD). At Lp(a) levels above 50 mg/dL, increased relative risk (1.46) for myocardial infarction (MI) and (1.95) for aortic stenosis (AS) has been reported (Afshar et.al. 2016). Whether reduction of Lp(a) leads to decreased risk is debated. Afshar et.al. estimated that 1 in 14 MI and 1 in 7 AS could be prevented by lowering Lp(a) to < 50 mg/dL. This study aims to define distribution of Lp(a) in a Stockholm County cohort, investigate its association to CVD, other diagnosis and risk factors. **Methods:** From the database at Karolinska University Laboratory (KUL), all Lp(a) results were retrieved. Laboratory data from each subject are combined with data from Swedish National quality registers on CVD (Swedeheart, Swedvasc, Riks-stroke) and National Board of Health and Welfare registers (death, diagnosis, prescribed drug). Lp(a) was measured by two assays that report concentration in mass or molar. **Results:** From the KUL-database, 23664 subjects (male 48%, female 52%, median 56(0-99) years) were identified. We observed a skewed distribution, median Lp(a) 16 mg/dL or 20 nmol/L, 80:th percentile at 53 mg/dL or 119 nmol/L (Figure 1-2). **Conclusions:** Initial analyses of routine measured Lp(a) in subjects referred to hospitals or general practitioners show similar distribution and 80:th percentile value as previously described in general population studies. Analysis of data from registers are ongoing. Results from our study will contribute to further understanding of Lp(a), its association to CVD, other diagnosis and to whether patients with elevated Lp(a) should be managed.

Participate in YIA

PCSK9 inhibitors in high cardiovascular risk patients: an update on clinical experiences

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Aim: In multiple large randomized placebo-controlled trials, PCSK9 inhibitors showed reductions of LDL-C levels up to 60% and also a decrease in cardiovascular outcomes. However, data about the efficacy and safety outside clinical trials is still scarce. The purpose of the study is to give an update on efficacy and side effects of PCSK9 inhibitors in patients in clinical practice. **Method:** Registry of all consecutive patients who started with a PCSK9 inhibitor at a lipid clinic of a university hospital. **Results:** We analyzed 262 patients (214 Familial Hypercholesterolemia [FH], 47 non-FH high cardiovascular risk patients), 44% women, with a mean age of 60 ± 11 years, 51.1% alirocumab. PCSK9 inhibitor treatment showed an additional reduction of 56.7% ± 22% in mean LDL-c levels after 2-3 weeks. Overall, LDL-c change ranged from a decrease of 3.09-92%. Patients with statin intolerance (n = 108, 41%) had less LDL-c decrease compared with patients on statin therapy (52.1% ± 23% and 61.8% ± 19%, p <0.001). Side effects of PCSK9 inhibitors occurred in 98 patients (37.4%). Flu-like symptoms (n = 23) and abdominal discomfort (n=13) were most frequently experiences. Sixteen patients (6.1%) discontinued treatment, 13 because of side effects and 3 because of unknown reason. **Conclusion:** Our experience of PCSK9 inhibition in a clinical setting showed comparable reduction in LDL-c levels but a higher percentage and broader range of side effects compared to clinical trials.

Participate in YIA

Posters – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Plasma levels of apolipoprotein E, APOE genotype, and all-cause and cause-specific mortality in 105,949 individuals from the general population.

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Aim: To determine whether plasma apoE levels are associated with all-cause and cause-specific mortality. **Methods:** Using a prospective cohort design with 105,949 individuals from the general population, we tested the association between plasma apoE at study enrollment and death during follow-up, and whether this was independent of APOE genotype. **Results:** We confirmed the well-known association between APOE genotypes and mortality. For all-cause, cardiovascular and cancer mortality high levels of apoE were associated with increased risk, for dementia-associated mortality low levels of apoE were associated with increased risk. For the highest versus the fifth septile of apoE, multifactorially adjusted hazard ratios (HRs) including APOE genotype adjustment were 1.20 (95% confidence interval (CI):1.12-1.28) for all-cause mortality, 1.28(1.13-1.44) for cardiovascular mortality and 1.18 (1.05-1.32) for cancer mortality. For the lowest versus the fifth septile the HR was 1.44(1.01-2.05) for dementia-associated mortality. Results were similar in APOE ε33 carriers separately. **Conclusion:** We found that high plasma levels of apoE associated with all-cause, cardiovascular and cancer mortality, while low apoE levels were associated with dementia-associated mortality. Both extreme high and low plasma levels of apoE were associated with cause-specific mortality, suggesting that plasma apoE levels in the middle of the population distribution seem to be favorable.

Metabolomic signature of angiopoietin-like protein 3 deficiency under fasting and postprandial conditions

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Aim: Loss-of-function variants in the angiopoietin-like 3 gene (ANGPTL3) have been associated with low levels of all plasma lipoproteins and decreased coronary artery disease risk. In the present study we aimed to determine detailed serum metabolite profiles of genetic ANGPTL3 deficiency in human subjects under fasting and during postprandial state. **Methods:** We studied individuals carrying the S17X loss-of-function (LOF) mutation in ANGPTL3 (6 homozygous and 32 heterozygous carriers) and 38 non-carriers. Nuclear magnetic resonance metabolomics was employed to quantify 225 circulating metabolic measures. We compared the metabolite differences between LOF carriers and non-carriers in the fasting state and after a high fat meal. **Results:** Under fasting, ANGPTL3 deficiency was characterized by a similar extent of reduction in LDL cholesterol (0.74 SD-units lower concentration per LOF allele [95%CI 0.42–1.06]) as observed for many triglyceride-rich lipoprotein (TRL) measures, including VLDL cholesterol (0.75 [0.45–1.05]). Within most lipoprotein subclasses, the absolute levels of cholesterol were decreased more than triglycerides, resulting in a reduced relative proportion of cholesterol in TRLs and their remnants. Further, β-hydroxybutyrate was elevated (0.55 [0.21–0.89]) in the ANGPTL3 LOF carriers. Homozygous ANGPTL3 LOF carriers showed essentially no postprandial increase in TRLs and fatty acids, without any evidence for adverse compensatory metabolic effects. **Conclusion:** ANGPTL3 deficiency results, in addition to overall triglyceride and LDL cholesterol lowering effects, in a reduction of the proportion of cholesterol within TRLs and their remnants. Further, ANGPTL3 LOF carriers display elevated ketone body production, suggesting enhanced hepatic fatty acid β-oxidation. Our observations further reinforce the clinically relevant idea of employing ANGPTL3 as a therapeutic target for decreasing cardiovascular risk.

Neutrophil protease 3 is present in human atherosclerotic lesions and modifies LDL and HDL particles

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Background and Aims: Protease 3 (PR3), a major neutrophil protease present in neutrophil azurophilic granule, is a multifunctional enzyme involved in the regulation of pro-inflammatory processes but its role in atherosclerosis remains unexplored. Here, we aimed to investigate whether PR3 is present in human atherosclerotic lesion and how it affects structure and functions of LDL and HDL. **Methods**: Normal and atherosclerotic coronary segments were collected from autopsy subjects and immunostained for PR3. LDL or HDL was incubated with PR3, after which its structural and functional properties were analysed. **Results:** Positive staining of PR3 was mainly detected in intima and adventitia of advanced atherosclerotic lesions. However, PR3 was neither detected in normal coronary arteries nor in coronary arteries characterized with atherosclerotic fatty streaks. Treatment of LDL with PR3 led to an extensive degradation of apoB-100, resulting in aggregation of LDL particles with enhanced binding to human aortic proteoglycans. In cultured human monocyte-derived macrophages uptake of the PR3-modified LDL particles was highly elevated, resulting in the macrophage transformation into foam cells. Moreover, incubation of HDL with PR3 resulted in proteolysis of major apolipoproteins (apoA-I, apoA-II and apoE) in the particles. Treatment of apoA-I or HDL with PR3 impairs their ability to promote cholesterol efflux from human macrophage foam cells. **Conclusions:** The present data demonstrate that PR3 is a novel protease found in human advanced atherosclerotic lesions and indicate that PR3 may have a role in the development of atherosclerosis by modifying both LDL and HDL particles.

Dietary saturated fats increase and plant stanol esters decrease LDL aggregation

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Aim: We recently showed that LDL aggregation susceptibility predicts future cardiovascular deaths and depends on LDL surface lipid composition (Ruuth M, et al., Eur Heart J 2018). Now we examined whether consumption of plant stanol ester enriched spread or overfeeding saturated fats, unsaturated fats, or sugars affect LDL aggregation susceptibility. **Methods:** In the plant stanol ester study, subjects (age 50.8 ± 1 y, BMI 25.2 ± 0.4 kg/m2), in plant stanol ester (staest) group (n=46) consumed cholesterol-lowering rapeseed oil-based spread enriched with staest (3.0 g of plant stanols/d) or in control group (n=46) the same spread without staest for 6 months. In overfeeding study participants consumed 1000 extra kcal/day for 3 weeks (age 48 ±2 y, BMI 31 ± 1 kg/m2). Extra calories consisted of saturated fats (n=13), mono- and polyunsaturated fats (n=11), or simple sugars (n=12). Plasma samples were collected at baseline and at the end of the studies, LDL particles were isolated, and LDL aggregation was induced with sphingomyelinase and detected by dynamic light scattering. **Results:** Consumption of staest decreased LDL aggregation susceptibility ($p<0.001$) compared with with baseline, while control spread did not affect LDL aggregation susceptibility. Overfeeding saturated fats increased LDL aggregation susceptibility ($p<0.005$), but overfeeding unsaturated fats or simple sugars had no effect on LDL aggregation susceptibility. **Conclusions:** Excess consumption of saturated fats increases while consumption of plant stanol ester enriched spread decreases LDL aggregation susceptibility. Thus, these dietary changes appear to influence, in addition to LDL levels, also LDL quality, and potentially the future risk of cardiovascular disease.

ANGPTL3 depletion alters lipid profile and metabolism in vitro and in vivo

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ANGPTL3 is a known inhibitor of lipoprotein lipase. Human carriers of loss-of-function variants of ANGPTL3 have reduced levels of all major lipoprotein classes, which makes ANGPTL3 a promising target for novel treatment strategies for cardiovascular disease. However, the intracellular function of ANGPTL3 in hepatocytes and the detailed lipid composition of plasma lipoproteins of ANGPTL3 deficient subjects are still unknown. We studied the effect of stable ANGPTL3 knock-down on the lipid profile, metabolism and transcriptome of human hepatocytes and characterized the plasma lipoprotein lipidomes of ANGPTL3 deficient subjects. We observed a significant decrease in relative levels of monounsaturated fatty acids and increased levels of polyunsaturated fatty acids (PUFAs) in the total lipids of ANGPTL3 knock-down cells compared to controls. Similar changes were detected in the major membrane phospholipid classes and cholesterol esters. The total level of cholesterol esters was significantly reduced in the ANGPTL3 knock-down cells and this change was further confirmed by de novo lipogenesis assay revealing reduced synthesis of cholesterol esters. RNA sequencing demonstrated gene expression changes in several pathways related to hepatic lipid metabolism, fibrosis and inflammation. SOAT1 (ACAT1) was significantly down-regulated, and also several mRNAs in the eicosanoid synthesis pathways were altered. The plasma lipoprotein lipidome signature reflected the cell model data with relative PUFA levels increased in several lipid classes in the lipoproteins derived from ANGPTL3 deficient subjects. ANGPTL3 depletion affects relative PUFA levels both in vitro and in vivo. We suggest that the functions of hepatocellular ANGPTL3 involve PUFA metabolism, cholesterol ester synthesis and VLDL secretion. More insight into these functions is needed when hepatic ANGPTL3 is targeted by antisense oligonucleotide therapy.

Is maternal lipid profile in early pregnancy associated with pregnancy complications and blood pressure in pregnancy and long-term postpartum?

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Background: An atherogenic lipid profile is a risk factor for the initiation and progression of atherosclerosis ultimately leading to cardiovascular disease. Women with a history of a hypertensive disorder of pregnancy are at increased risk of developing chronic hypertension and CVD later in life. However currently it is unclear if dyslipidemia during pregnancy contributes to these risks. The aim of this study was to determine the associations between maternal lipid profile in early pregnancy, a hypertensive disorder of pregnancy and blood pressure throughout pregnancy, and six and nine years after pregnancy. **Methods:** We included 5692 women from the Generation R Study; an ongoing populationbased prospective birth cohort. 218 (4.1%) women developed gestational hypertension and 139 (2.6%) women developed preeclampsia. A maternal lipid profile consisting of total-cholesterol, triglycerides and HDL-c, LDL-c, remnant cholesterol and non-HDL-c was determined in early pregnancy (median 13.4 weeks of gestation). Systolic and diastolic blood pressure were measured in early, mid- and late pregnancy, and six and nine years after pregnancy. **Results:** Women with preeclampsia had higher levels of triglycerides and remnant cholesterol in early pregnancy compared to those with a normotensive pregnancy. No difference in lipid profile was found for women with gestational hypertension. Total-cholesterol, LDL-c, non-HDL-c and especially triglycerides and remnant cholesterol were positively associated with blood pressure in pregnancy, and six and nine years after pregnancy. These associations remained significant after taking BMI and glucose into account. **Conclusions:** An atherogenic lipid profile in early pregnancy reflecting impaired triglyceriderich lipoprotein metabolism is independently associated with preeclampsia and blood pressure throughout pregnancy but also long-term postpartum. Lipid levels in early pregnancy may help to identify women at risk for future hypertension.

Statin treatment increases lipoprotein(a) levels in subjects with low molecular weight apolipoprotein(a) phenotype

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Objective: To evaluate the effect of statin treatment initiation on lipoprotein(a) [Lp(a)] levels in patients with dyslipidemia, and the interactions with the apolipoprotein(a) [apo(a)] phenotype, LPA single nucleotide polymorphisms (SNPs) and change in LDL cholesterol. **Methods:** The study population consisted of patients with dyslipidemia, predominantly familial hypercholesterolemia, who first initiated statin treatment (initiation group; n=39) or were already on stable statin treatment for at least 4 months (control group; n=42). Plasma Lp(a) levels were determined with a particle-enhanced immunoturbidimetric assay before and at least 2 months after start of statin treatment in individuals of the initiation group, and at two time points with an interval of at least 2 months in the control group. High and low molecular weight (HMW and LMW, respectively) apo(a) phenotype was determined by immunoblotting, and the common LPA SNPs rs10455872, rs3798220 and rs41272110 by Taqman assay. Data were analysed using linear mixed modelling, controlling for time between measurements. **Results:** Plasma Lp(a) levels did not increase significantly in the initiation group (20.5 (IQR 10.9-80.7) to 23.3 (10.8-71.8) mg/dL; p=0.09) nor in the control group (30.9 (IQR 9.2-147.0) to 31.7 (IQR 10.9-164.0) mg/dL; p=0.61). In carriers of LMW apo(a) phenotypes, Lp(a) levels increased significantly from 66.4 (IQR 23.5-148.3) to 97.4 (IQR 24.9-160.4) mg/dL (p=0.026) in the initiation group, but not in the control group and not in patients characterized by the HMW apo(a) phenotype. Interactions with neither common LPA SNPs nor with change in LDL cholesterol were significant. **Conclusion:** Statins affect Lp(a) levels differently in patients with dyslipidemia depending on the apo(a) phenotype. Statins increase Lp(a) levels exclusively in carriers of the LMW apo(a) phenotype.

The role of PSCK9 on the regulation of hepatic cholesterol metabolism and overall lifespan in mice

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During ageing, hepatic lipid modifications occur. Previous studies in humans and rodents have shown an aged-dependent increase in plasma cholesterol levels and a decline in membrane expression of low-density lipoprotein receptors (LDLRs) that may contribute to the accelerating atherosclerosis that occurs with ageing. In conjunction with the observed aged-dependent increases in LDL-C, recent studies have shown a simultaneously aged-depended increase in expression of hepatic Proprotein Convertase Subtilisin Kexin 9 (PCSK9). Secreted into the plasma from the liver, PCSK9 binds the LDL receptor at the surface of hepatocytes, directs it to lysosomal degradation, which in turn results in a reduction of LDLRs and thus LDL-cholesterol clearance. Since the discovery of the PCSK9 gene, a variety of gain-of-function and loss-of-function mutations has been identified that is associated with high and low LDL-C levels, respectively. More recently, monoclonal antibody treatment with PCSK9 inhibitors has been shown to effectively lower LDL-C and the risk of cardiovascular events. At present, there are no strong indications that inhibition of PCSK9 could be harmful. Therefore, there are reasons to assume that a lack of PCSK9 might affect lifespan. Despite that PCSK9-knock out mice have been available for more than 10 years, no studies have reported on the consequences of a life-long lack of PCSK9 in this species. The aim of this study is to investigate the potential links between PCSK9 and lipid metabolism during ageing of PCSK9-knock-out mice. Plasma, tissue and organs have been collected from a total of 180 mice at different time points for molecular, cellular, histological, and pathological analyses. Analyses of plasma and liver tissue have recently been initiated and preliminary data of mRNA levels and plasma PCSK9, cholesterol and triglycerides will soon be obtained. The study will reveal the possible importance of PCSK9 in normal ageing, both regarding its involvement in changes in lipid metabolism and its potential to promote lifespan.

YIA Poster Walk – Abstracts –

Lipoproteins and Lipid Transport

SESSION III

Identification and replication of six loci associated with gallstone disease

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Aim: Cholesterol plays a key role in gallstone disease. Unraveling the genetic underpinnings of gallstones may lead to new insights into cholesterol metabolism. We aimed to identify new genetic associations with gallstone disease using publicly available data from the UK Biobank and two large Danish cohorts. **Methods:** We extracted genetic associations with gallstone disease from the Global Biobank Engine (GBE), an online browser of genomewide associations in UK Biobank participants (14,940 cases and 322,268 controls). Significant associations (P<5x10-8) were retested in two Copenhagen cohorts (comprising 1,522 cases and 18,266 controls). In the Copenhagen cohorts, we also tested whether a genetic risk score associated with gallstone disease, and whether individual gallstone loci were associated with plasma levels of lipids, lipoproteins, and liver enzymes. **Results:** We identified 19 loci to be associated with gallstone disease in the GBE. Of these, 12 were replicated in the Copenhagen cohorts, including six previously unknown (in HNF4A, FUT2, SERPINA1, JMJD1C, AC074212.3, and SLC10A2), and six known loci (in ABCG8, SULT2A1, CYP7A1, TM4SF4, ABCB4, and TTC39B). Five of the new gallstone associations are protein-altering variants, and three (HNF4A p.Thr139Ile, SERPINA1 p.Glu366Lys, and SLC10A2 p.Pro290Ser) conferred per-allele odds ratios for gallstone disease of 1.30-1.36. Individuals with a genetic risk score in the top 1% had an approximately 5-fold increased risk of gallstones compared to those in the bottom 10%. Of the 19 lithogenic loci, 11 and 10 exhibited distinct patterns of association with plasma levels of lipids and liver enzymes, respectively. **Conclusion:** We identified six new susceptibility loci for gallstone disease.

Participate in YIA

Loss-of-function mutations in ABCA1, HDL-cholesterol, metabolomic profiles and risk of vascular disease and dementia – a cohort study of up to 100,000 individuals

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Background and Aim: The adenosine triphosphate-binding cassette transporter A1 (ABCA1) is a major cholesterol transporter highly expressed in liver and brain. ABCA1 mediates cholesterol and phospholipid efflux to lipid-poor apolipoproteins, and is essential for the biogenesis of high-density lipoprotein (HDL) cholesterol in the circulation and HDL-like particles in the brain. We therefore investigated the impact of two well-known HDL-deficiency loss-of-function mutations in ABCA1 on lipid levels, metabolomic profiles, and on risk of vascular disease and dementia. **Methods:** The association between ABCA1 N1800H/P85L and plasma lipid levels, metabolomic profiles, and risk of vascular disease and dementia were tested in 13,000-100,000 individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study. **Results:** N1800H/P85L heterozygous carriers versus noncarriers had lower HDL cholesterol levels (p=0.0001), lower cholesterol/total lipid ratio (P=0.02), and higher phospholipid/total lipid ratio in small HDL particles (p=0.02). For heterozygous carriers versus noncarriers, multifactorially adjusted hazard ratios (HR) were 0.27 (confidence interval (CI) 0.10-0.72) for ischemic heart disease (IHD), 1.95 (1.27-3.00) for cerebrovascular disease, and 1.60 (0.88-2.90) for dementia. Corresponding HRs for the N1800H mutation alone were 0.54 (0.29-1.00), 1.39 (0.90-2.13), and 1.40 (0.77-2.52). **Conclusions:** Loss-of-function mutations in ABCA1 were associated with low plasma levels of HDL cholesterol, altered metabolomic profile, and with lower risk of IHD, but with higher risk of cerebrovascular disease and dementia. These differential findings in circulation and brain are in accordance with current knowledge of lipid biology in the two compartments.

Participate in YIA

Treatment with 2-hydroxypropyl-β-cyclodextrin induces macrophage cholesterol efflux in vitro but does not induce lesion regression in APOE knockout mice in vivo.

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Background: Macrophage cholesterol efflux is considered a therapeutic target to reduce atherosclerosis susceptibility and induce atherosclerotic lesion regression. 2-hydroxypropyl-β-cyclodextrin (CD) has been suggested to remove cholesterol from cells. In this study, we investigated the effect of CD treatment on macrophage cholesterol efflux in vitro and the ability of established lesions to regress in response to lipid lowering. **Material and methods:** Peritoneal macrophages were loaded with [3H]cholesterol or oxLDL to respectively study cholesterol efflux and foam cell formation in vitro. Western-type diet-fed APOE knockout mice were used to study lesion regression. **Results:** CD treatment dose-dependently stimulated cholesterol efflux with the highest concentration of 20 mM CD inducing 41±2% of cholesterol efflux (P<0.001). Consistently, Oil red O staining showed less lipid accumulation in oxLDL-exposed macrophages after CD treatment. A reduction in the activation of the nuclear oxysterol receptor liver X receptor was observed after CD treatment, as judged from the lowering of ABCA1 gene expression (-57%; P<0.001) and the similar trend in ABCG1 expression levels (-38%; P=0.17). Switching APOE knockout mice from a Western-type to a chow diet was associated with an increase in lesion size (360±50 x 103 µm2 versus 240±50 x 103 µm2; P<0.05) in the context of effective plasma lipid lowering (total cholesterol: 4±2 mg/ml versus 12±3 mg/ml; P<0.001). Furthermore, atherosclerotic lesions were enriched in collagen (68±8% versus 52±8%; P<0.05). Strikingly, CD treatment did not significantly impact the extent of lipid lowering or lesion progression. **Conclusion:** CD treatment induces macrophage cholesterol efflux in vitro but does not induce lesion regression in vivo.

Participate in YIA

Role of inflammation-related genes in macrophage cholesterol accumulation

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Background and Aims: In this study, we applied a transcriptome analysis and bioinformatics approach to determine genes that change their expression in monocyte-derived human macrophages exposed to modified and native LDL. Furthermore, we evaluated involvement of revealed genes in cellular cholesterol accumulation mediated by modified LDL. **Methods:** Monocyte-derived human macrophages were cultured with native human low-density lipoprotein (LDL) as well as modified (acetylated, oxidized, desialylated) LDL for 24 hours, and then RNA-seq libraries were prepared using a NEBNext Ultra RNA library kit. Libraries were sequenced on an Illumina HiSeq 1500. The capacity of LDL to mediate cellular cholesterol accumulation was evaluated in monocyte-derived human macrophages with genes knocked-downed by siRNA. **Results:** The transcriptome from macrophages incubated with native LDL was compared with the transcriptome from macrophages incubated with modified LDLs. As a result, four genes related to cholesterol accumulation were identified, notably EIF2AK3 (PERK), TIGIT (VSTM3), CXCL8 and ANXA1. All of these genes appeared to be inflammation-related. These genes were evaluated by their capacity to affect cholesterol accumulation mediated by modified LDL. Knock-down of these genes prevented cholesterol accumulation in primary human macrophages. In the case of ANXA1, knock-down decreased cellular cholesterol concentration in cells cultured with modified LDL versus cells cultured without LDL. **Conclusion:** We have identified that inflammation-related genes: EIF2AK3 (PERK), TIGIT (VSTM3), CXCL8 and ANXA1 involved in cellular cholesterol accumulation. This work was supported by Russian Foundation for Basic Research (Grant #18-34-00997).

Participate in YIA

Treat-to-target Familial Hypercholesterolemia - a prospective study of adult patients with familial hypercholesterolemia at the Lipid Clinic of Oslo, Norway

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Background: The Treat-to-Target Familial Hypercholesterolemia-project was initiated in 2006 as a quality assessment study of the treatment given to FH-patients at the Lipid Clinic (LC) in Oslo. **Objective:** To prospectively study the effect of traditional treatment as intensive as possible using high dose potent statins, ezetimibe, resins and PCSK9-inhibitors during 8-12 years. **Methods:** In 2006, 357 heterozygous FH patients attended V1. Visit 2 was conducted with 332 patients after one year. We here present the results from V3 collected over the period of 2014-18 for 279 patients. **Results:** Mean age at V3 was 53.1 years, half was male. Genetically verified FH was documented in 86.4%. The mean (95%CI) first known elevated cholesterol level was 9.8mmol/L (9.5, 10.1), and was measured at a mean age of 27.9 years. At V3, 28% were treated for hypertension, and 10% for diabetes. BMI, weight and waist circumference (WC) increased with 1.1kg/m2, 3kg and 4cm, respectively. At V1, the mean total cholesterol (TC) on treatment was 5.7 mmol/L (5.5, 5.8), and LDL-C was 4.1 mmol/L (3.7, 4.6). After median 10(8.1, 12,8) years at the LC, the achieved mean TC and LDL-C was 4.9 (4.7, 5.1) and 3 mmol/L (2.9, 3.2), respectively. 29.7% have had CVD, with a debut at mean age 47.6(45.2, 50.1) years. At V3 metabolic syndrome (MetS) was diagnosed among 39.8% of those with CVD, versus 13.3% with no CVD. 15.5% of those in secondary prevention achieved LDL-C <1.8 mmol/L. In primary prevention, 28.1% reached LDL <2.5mmol/l. Overall, 50.2% used double medication, whereof 96.4% used the combination statin-ezetimibe. **Conclusion:** Lipid values improved from V1 to V3. During the 10 years follow-up, the prevalence of MetS increased from 9% to 21.1%. Only 22.9% of the population reached their lipid targets. 34.2% suffered from side effect of the medication as judged by the treating physician.

Participate in YIA

Posters – Abstracts –

Other Topics

SESSION IV

Pnpla3 Silencing Ameliorates NASH and Fibrosis in Pnpla3 I148M Knock-in Mice

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Among the causes of nonalcoholic fatty liver disease (NAFLD) and its progression to nonalcoholic steatohepatitis (NASH) the genetic component has a pivotal role. The rs738409 polymorphism in the patatin-like phospholipase domain-containing 3 (PNPLA3) gene, resulting in the 148M protein, exerts the largest effect. We hypothesized that suppressing the expression of the PNPLA3 148M mutant protein would have a beneficial effect on the entire spectrum of NAFLD. We examined the effects of liver-targeted GalNAc3-conjugated antisense oligonucleotide (ASO)-mediated silencing of Pnpla3 in a knock-in mouse model in which we introduced the human PNPLA3 I148M mutation. ASO-mediated silencing of Pnpla3 reduced the moderate liver steatosis (p=0.038) in homozygous Pnpla3 148M/M knock-in mutant mice but not in wild-type littermates fed a steatogenic high-sucrose diet. In mice fed a NASH-inducing diet to induce marked liver steatosis, inflammation and fibrosis, Pnpla3 ASO treatment reduced plasma ALT levels and liver triglyceride contents in both Pnpla3 mutant knock-in and wild-type mice. Thus, in severe steatosis, silencing of Pnpla3 reduced liver steatosis score and NAFLD activity score independent of the Pnpla3 genotype, while reductions in liver inflammation score (p=0.018) and fibrosis stage (p=0.031) were observed only in the Pnpla3 knock-in 148M/M mutant mice. These responses were accompanied by reduced liver levels of Mcp1 (p=0.026) and Timp2 (p=0.007) specifically in the mutant knock-in mice. This may reduce levels of chemokine attracting inflammatory cells and increase the collagenolytic activity during tissue regeneration. This study provides the first evidence that a Pnpla3 ASO therapy can improve all features of NAFLD including liver fibrosis and suppressing the expression of a strong innate genetic risk factor, Pnpla3 148M, may open up for a precision medicine approach in NASH.

Characterization of PBMC gene expression and lipoprotein subclasses among plasma TG responders and non-responders to omega-3 supplementation

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Background: There are large inter-individual differences of plasma triglyceride (TG) response to omega-3 fatty acid supplementation. The aim of this exploratory study was investigate baseline differences and differences in the change in peripheral blood mononuclear cell (PBMC) gene expression and lipoprotein subclass TG levels between TG responders and non-responders to omega-3 fatty acid supplementation to understand further the variability in TG response. **Methods:** In a previous randomized controlled trial, healthy normotriglyceridemic subjects (n = 35, 71 % women) received 1.6 g EPA + DHA/day for 7 weeks. TG-responders were defined as subjects having a TG reduction $\geqslant$ 20 % and non-responders having a TG change between -20% and +20%. PBMC gene expression was measured using microarray and lipoprotein subclasses were measured using nuclear magnetic resonance spectroscopy. **Results:** Eight subjects were defined as responders (median reduction of 37%) and 16 subjects were defined as non-responders (median change of 0%). At baseline, responders had higher TG levels in two of four HDL subclasses and 909 gene transcripts ($p \leqslant 0.05$) were differentially expressed. During the intervention, the plasma TG reduction among responders was reflected in TG reductions in four of six different VLDL subclasses and three of four different HDL subclasses. Compared to non-responders, the expression of 454 transcripts was differentially altered in responders ($p \leqslant 0.05$). Responders had altered signaling pathways related to development and immune function, and two of the top 10 enriched pathways were related to lysophosphatidic acid signaling. **Conclusion:** TG responders and non-responders to omega-3 supplementation have different lipoprotein subclass and PBMC gene expression profiles at baseline and after omega-3 supplementation.

Dietary supplementation of inulin reduces Western diet-induced hepatic inflammation, a proxy of NASH

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Background: Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome, which affects millions of people worldwide, and is predicted to become the next global epidemic. Non-alcoholic steatohepatitis (NASH), the most extreme form of NAFLD, involves steatosis combined with inflammation, and can progress into fibrosis and cirrhosis. However, at present there is still no effective therapy for the treatment of NASH. Inulin is a dietary fiber, recognized for its positive effect on metabolic control and intestinal barrier function. However, little is known about the potential effects of inulin on hepatic inflammation. Therefore, the aim of the present study was to investigate the effect of dietary inulin on lipid metabolism and hepatic inflammation in a mouse model of NASH. **Methods and Results:** LDLR-/- mice fed Western-type diet with inulin supplementation (10% w/w) for a period of 7 days showed lower body weight gain (-6%) and increased feces output (+54%, $p<0.01$). Plasma triglyceride and cholesterol levels remained comparable in both groups. Both, liver triglyceride (-48%, $p<0.01$) and cholesterol levels (-25%, $p<0.01$) were significantly decreased in the inulin group. Importantly, hepatic inflammatory gene expression (MCP-1, TNF-α, CD 68) in the inulin groups was significantly reduced ($p<0.01$) indicating decreased hepatic inflammation. Furthermore, hepatic mRNA expression of SREBP1c and SREBP 2 were also significantly reduced ($p<0.01$) suggesting that inulin also reduced fatty acid and cholesterol synthesis. **Conclusion:** This study demonstrates that dietary inulin reduces hepatic triglyceride accumulation and proinflammatory gene expression in a mouse model of NASH. These effects are expected to translate into metabolic health benefits, conceivably also providing a dietary strategy against the increasing incidence of NAFLD/NASH in humans.

The Framingham risk score is useful to predict chronic graft failure in renal transplant recipients

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Background and objectives: Predicting chronic kidney graft failure is an unmet clinical need. Chronic graft failure is mostly due to transplant vasculopathy, representing de novo atherosclerosis formation in the transplanted kidney. We therefore determined whether an established atherosclerotic cardiovascular disease prediction module, namely the 10-year Framingham risk score (FRS), is useful in predicting chronic graft failure in renal transplant recipients (RTR). **Method:** In this prospective longitudinal study 600 well-characterized RTR with a functioning renal graft for at least one year, were followed for 10 years. The FRS was calculated and RTR were stratified accordingly into low (<10%), medium (10-19%), and high risk (>20%). Then the association with death-censored graft failure (n=81), defined as re-transplantation or return to dialysis, was computed. **Results:** Baseline characteristics were compared between groups. Cox regression showed that each one percent increase of the FRS significantly increased the risk of graft failure by 5% (hazard ratio [HR]: 1.05, P<0.001). The association remained significant after adjustment for potential confounders, including eGFR (HR: 1.03, P=0.034). Multi-variate cox regression of the time-varying covariate of FRS demonstrated that with every year the effect of the FRS association graft failure diminishes 1% (HR: 0.99, P=0.01) in the fully adjusted model. **Conclusion:** The FRS has clinical potential to predict chronic graft failure in RTR. Therapeutic interventions targeted to reduce the FRS are expected to extend graft survival.

YIA Poster Walk – Abstracts –

Other Topics

SESSION IV

MBOAT7 is anchored to endomembranes by six transmembrane domains

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The MBOAT7 is a susceptibility locus for non-alcoholic fatty liver disease (NAFLD) and mental retardation. The MBOAT7 gene encodes for the MBOAT7 protein (also known as LPIAT1), which is involved in the acyl chain remodeling of phospholipids via the Lands' cycle. Although it has been shown that MBOAT7 is a membrane-associated protein, the MBOAT7 topology and secondary structure is not known. To solve the topology of MBOAT7, we performed: a) in silico analysis using 22 computational methods; b) in vitro analysis on living cells, transfected with full length and truncated forms of GFP-tagged MBOAT7, using the fluorescence protease protection (FPP) assay. The in silico analysis predicted MBOAT7 as a transmembrane protein with a number of transmembrane domains ranging between 5 and 12. The in vitro experiments identified MBOAT7 as a multispanning transmembrane protein with six transmembrane domains. Furthermore, based on this model, the predicted catalytic dyad of the protein, composed of the conserved asparagine in position 321 (Asn-321) and the preserved histidine in position 356 (His-356), has a luminal localization. These data are consistent with the role of MBOAT7 in remodeling the acyl chain composition of endomembranes and can help the drug development process for the treatment of NAFLD.

Participate in YIA

Evidence of high ^{18}F-fluorodeoxyglucose uptake in the subcutaneous adipose tissue of the dorsocervical area in young adults

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Background: Rodents have brown adipose tissue (BAT) depots with high metabolic activity in the interscapular region (iBAT), and iBAT activation in rodents attenuates atherosclerosis development (Berbée, Nat Commun 2015; Bartelt, Nat Commun 2017). In human adults, the available evidence of iBAT only comes from corpses, and therefore, the metabolic activity of iBAT in human adults remained to be elucidated. Therefore, the aim of the present study was to demonstrate whether young adults have high 18F-fluorodeoxyglucose (18F-FDG) uptake in the subcutaneous adipose tissue (SAT) of the dorsocervical area. **Material & Methods:** A total of 133 young adults (age 22±2 years; BMI: 25±5 kg/m2) were included in the present study. We performed a shivering threshold test for every participant. Later, we performed 2 hours of personalized cold exposure, just before performing a positron emission tomography/computed tomography scan. **Results:** We show that 23 out of 133 participants had 18F-FDG uptake in the dorsocervical area that achieved the criteria to be considered BAT, mainly in women (96%, n=22 out of 23). In the whole sample, the glucose uptake in the SAT of the dorsocervical area positively correlated with the volume and activity of BAT located in the supraclavicular area (β=0.344; R2=0.118; p<0.001 and β=0.293; R2=0.086; p=0.001), whereas we did not observe any association between the glucose uptake in the SAT of the triceps with supraclavicular BAT (all P >0.6). **Conclusion:** We showed that the 18F-FDG uptake of the SAT of the dorsocervical area in humans is higher in comparison to other SAT area. Future studies are warranted to confirm the iBAT signature of this tissue.

Participate in YIA

Regulation of metabolism by activation of ERβ using a synthetic ligand on male mice

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Background: Obesity is an pandemic problem affecting more people each year and becoming a burden to worlds economy. However, metabolic complications in obesity is sex-dependent and the role of estrogens in these regulations is still unclear. Estrogen receptors (ERs), which include Estrogen receptor alpha (ERα) and Estrogen receptor beta (ERβ), mediate estrogen signalling and are key regulators of metabolic functions. While the role of ERα in energy metabolism is well described, the action of ERβ in metabolism homeostasis is still dubious. **Aim:** To Investigate the metabolic function of ERβ activation in male mice during obesity, using a ERβ specific synthetic ligand. **Methods:** Wild type male C57BL/6 mice were fed with control diet (CD) or high fat diet (HFD) for 6 weeks before being treated with DIP. Lipid metabolism was investigated in vivo using magnetic resonance imaging (MRI) and spectroscopy (MRS) and metabolic cages were conducted to investigate the metabolic status of the animals. Using molecular techniques such as qPCR, Western blot we investigated the molecular machinery in response to ERβ. **Results:** HFD mice gained weight and fat mass as compared to CD but lost weight when treated with DIP. Surprisingly, total fat content increased in DIP-treated animals but due to induction of subcutaneous fat content. Liver lipid content was unchanged but fatty acids composition was reversed towards more unsaturated lipids as compared to HFD baseline. **Conclusion:** ERβ could be a potential target to treat obesity and associated metabolic disorders avoiding the side effects of ERα activation.

Participate in YIA

Increased triacylglycerol – fatty acid substrate cycling in human myotubes exposed to eicosapentaenoic acid

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Aims: It has previously been shown that pretreatment of differentiated human skeletal muscle cells (myotubes) with eicosapentaenoic acid (EPA) promoted increased uptake of fatty acids and increased triacylglycerol accumulation, compared to pretreatment with oleic acid (OA) and palmitic acid (PA). The aim of the present study was to examine whether EPA could affect substrate cycling in human myotubes. **Methods:** Myoblasts where cultured and differentiated into myotubes from satellite cells isolated from biopsies of musculus vastus lateralis. Fatty acid metabolism was studied using a mixture of fatty acids, i.e. radiolabelled OA as tracer (14C-OA) together with EPA or PA. **Results:** Co-incubation of myotubes with EPA increased cell accumulation and incomplete fatty acid oxidation of OA compared to co-incubation with PA. Lipid distribution showed higher incorporation of OA into all cellular lipids, and higher lipolysis and fatty acid re-esterification rate were also found after co-incubation with EPA relative to PA. Basal respiration, proton leak and maximal respiration were significantly increased in cells exposed to EPA compared to PA. Microarray and Gene Ontology (GO) enrichment analysis showed that EPA, related to PA, significantly changed i.e. the GO terms “Neutral lipid metabolic process” and “Regulation of lipid storage”. **Conclusion:** Incubation of human myotubes with EPA, compared to PA, increased processes of fatty acid turnover and oxidation suggesting that EPA may activate futile cycling of fatty acids in human myotubes. Increased TAG - FA cycling may be involved in the potentially favourable effects of long-chain polyunsaturated n-3 fatty acids on skeletal muscle and whole-body energy metabolism.

Participate in YIA

Origin of bile acids in follicular fluid in human ovaries

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Background: Follicular fluid, the natural environment of oocyte development, is rich in cholesterol that is primarily used for the synthesis of steroid hormones. Bile acids (BA), which are important cholesterol derivatives, are also present in FF and have been linked to fertility and embryo development. However, information on the source of ovarian BA is lacking. Therefore, we aimed to study local ovarian synthesis and BA transport from blood into FF. **Methods:** Total BA levels were determined in matching FF and blood samples from women who underwent in vitro fertilization. In vitro BA production by human mural (MGC) and cumulus granulosa cells (CGC) was measured by mass spectrometry. Gene expression and protein production were quantified in human MGC and CGC and in human ovarian tissue by quantitative PCR and Western blot /immunohistochemistry, respectively. **Results:** There was a significant correlation between the levels of BA in blood and FF (rs=0.186, P=0.027). However, levels of FF BA were almost double those in blood (10.10 [8.38-11.93] µmol/l versus 5.89 [4.15-7.88] µmol/l, P<0.001), indicating that, in addition to passive diffusion, other origins of ovarian BA likely exist. The key BA enzymes CYP7A1 and Cyp8B1 were absent or barely detectable in MGC and CGC, and there was no evidence of BA production in vitro in isolated GC. Therefore, local ovarian BA production is highly unlikely. However, we identified for the first time substantial expression of the common BA importers (NTCP, ASBT) as well as a BA exporter (MRP3) in GC of human ovaries. **Conclusion:** These results indicate that passive and active transport of BA from blood into FF constitute the main sources of ovarian BA.

Participate in YIA

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