European Lipoprotein Club

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Conference report

European Lipoprotein Club: Report of the 29th ELC Annual Conference, Tutzing, 4–7 September 2006☆

1. Introduction

The 29th meeting of the European Lipoprotein Club was held from 4 to 7 September at the Evangelische Akademie in Tutzing Germany and attended by some 120 participants from all over Europe, Israel and the United States. On Monday night, after the welcome by the ELC secretary, Marten Hofker, the state of the art lecture was presented by Larry Rudel (Winston-Salem, USA) entitled: Acyl-CoA:cholesterol-acyl-transferase (ACAT)-2 modifies LDL composition in atherosclerosis. About 9 years ago, two different ACAT enzymes were discovered and it has become clear that the two enzymes are localized in different cell types, providing separate physiologic functions. Whereas ACAT1 is localized in most tissue of the body, the expression of ACAT2 is confined to only two cell types in the body: the enterocytes and the hepatocytes. The keynote speaker has greatly contributed to the understanding of role of ACAT2 for the modification of LDL composition and for the subsequent genesis of atherosclerosis. His experience derives from studies in both non-human primates and in genetically modified rodents.

On Tuesday, the first session focused on lipid transport and was introduced by Elina Ikonen (Helsinki, Finland). Her presentation was entitled: Intracellular cholesterol transport: vesicular and non-vesicular mechanisms meet. The second session revolved around adipose tissue (AT) and obesity and was introduced by Jacqueline Capeau (Paris, France), with a presentation on diseases of adipose tissue: from lipoatrophy to obesity. On Wednesday, the third session focused on fatty liver disease. Jukka Westerbacka (Helsinki, Finland) presented an invited lecture entitled "The fatty liver: a key player in the metabolic syndrome". The fourth session on lipid signaling was introduced by Hans Aerts (Amsterdam, The Netherlands) and he presented a lecture entitled: Pharmacological inhibition of glucosylceramide synthase enhances insulin sensitivity. The fifth session on novel therapeutic targets was introduced by Marcus Stoffel (New York, USA) on the role of apoM in HDL metabolism. The final "Varia" session on Thursday, consisted exclusively of speakers invited on the basis of their submitted abstracts.

To promote participation by junior researchers, two types of awards were presented to two young scientists. The young investigator award was presented to Malin Levin (San Francisco, USA) for her presentation entitled Acyl CoA:Diacylglycerol Acyltransferase 2 (DGAT2)-mediated Triacylglycerol accumulation in glycolytic muscle promotes insulin resistance. The poster award was presented to Willeke de Haan (Leiden, The Netherlands) for her poster entitled: Torcetrapib increases HDL by inhibiting CETP activity, while atorvastatin increases HDL by reducing CETP expression. The winner of the poster prize was also given the opportunity to orally present her poster in the "Varia" session.

2. State of the art lecture: chaired by Paolo Parini (Stockholm, Sweden)

The scientific part of this year's meeting was opened by Larry Rudel (Winston-Salem, USA) by a lecture on the role of ACAT in atherosclerosis. When atherogenic diets are fed to monkeys, cholesteryl oleate accumulates in plasma LDL. The extent of this accumulation was found to be proportional to the severity of the atherosclerotic lesions in the coronary arteries. The source of the cholesteryl oleate was the liver as suggested by perfusion studies in isolated non-human primate livers. The rate of cholesteryl ester secretion from those livers was highly correlated ($r \geq 0.8$) to the extent of atherosclerotic lesion formation in the coronary artery. Since ACAT2 was found to be the enzyme in liver hepatocytes responsible for cholesteryl ester secretion into apoB-lipoproteins, studies were initiated in LDLreceptor−/− mice in which the acat2

☆ For information about the preliminary program and abstract forms, please contact Prof. Dr. Marten Hofker, secretary of the ELC. Updates and forms will be published on the website of the ELC: http://www.elc-tutzing.org.

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gene (soat2) was deleted. In ACAT2−/− mice, the secretion of cholesteryl esters by isolated perfused livers was decreased by over 90% compared to wild-type mice, and ACAT2−/− mice had less than 20% of the amount of aortic atherosclerosis. This decreased amount of atherosclerosis in ACAT2−/− mice showed none of the dietary fat-related differences seen in wild-type mice. Plasma LDL cholesterol concentrations were not significantly lower in ACAT2−/− mice, but the proportion of cholesteryl oleate in the cholesteryl esters was significantly lower. Finally, when apoB-100-only/LDLreceptor−/− mice were treated with an antisense oligonucleotide specific to ACAT2, liver ACAT2 mRNA was reduced by 80%, cholesteryl oleate concentrations in plasma were significantly decreased, and the extent of aortic atherosclerosis was halved. Therefore, in all experimental models in mice and monkeys, including those where ACAT2 was selectively targeted, reduction of hepatic cholesteryl oleate secretion was associated with a reduction of atherosclerosis. These data strongly support the conclusion that ACAT2 is the source of atherogenic cholesteryl esters that circulate in plasma LDL.

3. Session I. Lipid transport: chaired by Athina Kalopissis (Paris, France) and Gertrud Schuster (Davis, USA)

Elina Ikonen (Helsinki, Finland) presented an overview on the convergence of vesicular and non-vesicular mechanisms in intracellular cholesterol transport. Vesicular cholesterol trafficking (the prototype of which is the LDL receptor pathway) proceeds in several distinct compartments: early or sorting endosomes, recycling endosomes, late endosomes and lysosomes. The LDL receptor dissociates from LDL in early endosomes, and recycles back to the plasma membrane in recycling endosomes. LDL is transferred to late endosomes (LE) that deliver it to lysosomes for degradation, or to the trans-Golgi for sorting and recycling. Hydrolysis of LDL cholesterol esters in LE/lysosomes yields free cholesterol. A major issue is to elucidate the mechanisms of disposal/exit of endosomal cholesterol, and routing to other organelles, such as the endoplasmic reticulum (ER) for esterification (followed by cytoplasmic storage) or the plasma membrane (efflux to extracellular acceptors). A number of proteins involved in these processes were discussed.

Failure of cholesterol exit from LE/lysosomes results in massive accumulation in NPC disease, a rare autosomal recessive neurovisceral lipid storage disorder. Mutations in either one of two LE proteins, NPC1 and NPC2, are responsible for the disease.

Non-vesicular transport between organelles and membranes involves proteins with a START (Star-related lipid transfer) domain binding cholesterol at a 1/1 ratio. Knockdown of MLN64, a LE START-domain protein, induced dispersion of late endocytic organelles to the cell periphery (mimicking the effects of actin disruption), impaired fusion of LE and delayed cargo degradation. Overexpression of wild-type MLN64 (but not of mutants defective in cholesterol binding), rescued the endosome dispersion. However, MLN64 depletion neither impaired cholesterol trafficking nor induced cholesterol accumulation in LE, arguing against a role for MLN64 in LE-cholesterol export.

The small Rab GTPases regulate intracellular membrane trafficking events at specific endocytic vesicles, i.e., Rab5, Rab7 and Rab11 target early, late and recycling endosomes, respectively. Overexpression of Rab5, Rab7 and Rab11 inhibited cholesterol esterification (a measure of cholesterol transport to the ER), Rab11 also inducing cholesterol deposition in recycling endosomes. Overexpression of Rab8 in NPC fibroblasts moved cholesterol to the cell periphery, and increased ABCA1-mediated cholesterol efflux to apoA-I. Conversely, Rab8 knockdown in normal fibroblasts induced cholesterol accumulation in LE/lysosomes, whereas efflux to apoA-I of LE-cholesterol was impaired, suggesting that Rab8 is a key component of the ABCA1 dependent cholesterol efflux pathway.

These studies open new perspectives to our understanding of the regulation of intracellular cholesterol trafficking that is essential for normal cellular metabolism.

Thomas Grewal (Sydney, Australia) presented exciting new data on the role of Annexin A6 in the export of LDL-derived cholesterol from late endosomes/prelysosomes. Ectopic expression of Annexin A6 in CHO and A431 cells induced accumulation of cholesterol in late endosomes. This is associated with a cholesterol-dependent retention of caveolin in the cis-Golgi and correlates with reduced activity of cytoplasmic phospholipase A2, which is required for cholesterol-inducible Golgi vesiculation. The resulting reduced number of caveolae at the cell surface led to decreased SR-BI dependent cholesterol efflux to HDL, suggesting a potential role of high Annexin A6 levels regulating caveolae formation and SR-BI activity in hepatocytes.

The study presented by *Alexander Laatsch* (Hamburg, Germany) aimed to elucidate a molecular link between the metabolism of chylomicron remnants (CR) and HDL through an effect of LRP1 on the recycling of apoE that mediates CR clearance. Expression of LRP1 in HEK293 cells increased recycling of CR/apoE, whereas LRP1 knockdown decreased recycling rate. Microscopy data revealed that HDL induces a separation of apoE from LRP1 vesicles. HDL associates with intracellular apoE during transient uptake into peripheral endosomes. A central role of LRP1 for hepatic clearance of CR and HDL-E formation is suggested.

The HDL receptor, SR-BI, mediates selective uptake of HDL cholesteryl esters. *Herbert Stangl* (Vienna, Austria) showed that SR-BI also mediates HDL endocytosis in CHO cells that are deficient in the LDL receptor but overexpress SR-BI. After uptake, HDL particles were localized in endocytic vesicles and organelles en route to the perinuclear area. Cells internalized about 0.8% of ^{125}I-labeled HDL in 1 h

and finally almost all ^{125}I-labeled HDL was resecreted. Of note, resecreted HDL particles were enriched in cellular ^{3}H-cholesterol. Thus, HDL endocytosis is a novel mechanism facilitating cholesterol efflux.

Clara Cavelier (Zurich, Switzerland) investigated the role of cell surface F0F1 ATPase on apoA-I transcytosis through aortic endothelial cells (previously shown to lipidate apoA-I and to involve ABCA1 and caveolin-1). The β-chain of F0F1 ATPase was shown to be expressed on the cell surface. Extracellular ADP and, to a lesser extent, ATP was hydrolyzed in an apoA-I stimulated manner. Inhibition of ATP hydrolysis decreased apoA-I internalization and transport showing that F0F1 ATPase modulates apoA-I transcytosis through endothelial cells.

Ruth Frikke-Schmidt (Copenhagen, Denmark) reported from the Copenhagen City Heart Study, investigating SNPs and mutations of the ABCA1 gene in association with HDL-cholesterol (HDL-C) and ischemic heart disease (IHD). Five mutations of ABCA1 (S364C, P1065S, G1216V, N1800H and R2144X) were associated with low HDL-C levels. Heterozygosity for an ABCA1 mutation (K776N; frequency: 0.4%) conferred two- to three-fold risk of IHD that is not reflected in plasma HDL-C.

Albert Groen's (Amsterdam, The Netherlands) group studied the mechanism of the sterol transporting heterodimer Abcg5/Abcg8, mediating the transmembrane transport of cholesterol in liver and intestine. In vivo experiments in wild-type and Abcg8 knock-out mice revealed that Abcg5/Abcg8 act as a floppase. Further studies in Abcg5/Abcg8 over-expressing dog gallbladder epithelial cells showed that the floppase transfers cholesterol preferentially to bile salt micelles. Addition of low concentrations of phosphatidylcholine stimulated taurocholate mediated cholesterol uptake, whereas higher concentrations inhibited. Other cholesterol acceptors such as ApoA1, HDL and non-specific detergents did not induce Abcg5/Abcg8 mediated cholesterol secretion.

Ylva Bonde (Huddinge, Sweden) investigated the cause for the lost resistance to dietary cholesterol in hypophysectomized (Hx) rats. Their data revealed a profound (100%) increase of intestinal absorption of dietary cholesterol in Hx rats involving the Niemann Pick C1 like 1 (NPC1L1) protein. Using the fecal dual-isotope technique it was shown that thyroid hormone dampens the intestinal absorption of dietary cholesterol by so far unknown mechanisms.

Mutation of hepatocyte nuclear factor-4 (HNF4A), which are causing maturity-onset diabetes of the young 1 (MODY 1), can lead to reduced plasma triglyceride levels. *Ferdinand van 't Hooft* (Stockholm, Sweden) addressed these observations by studying the effects following knockdown of HNF4A in human Huh7 hepatoma cells using siRNA. This HNF4A knockdown resulted in marked reduction of DGAT1 expression and protein levels. siRNA-mediated knockdown of both HNF4A and DGAT1 was associated with reduced secretion of triglycerides and apoB. These data and the finding of a functional HNF4A binding site in DGAT1 promoter suggests that the effects of MODY1 gene on hepatic triglyceride secretion in mediated by DGAT1.

4. Session II. Adipose tissue/Obesity: chaired by Fredrik Karpe (Oxford, UK) and Ko Willems van Dijk (Leiden, The Netherlands)

The session on Adipose Tissue/Obesity was opened by invited speaker *Jacqueline Capeau* (Paris, France) on diseases of adipose tissue. She introduced the subject by describing the complex cellular composition of adipose tissue (i.e., also containing pre-adipocytes, macrophages and endothelial cells). In addition, AT functions as an endocrine organ, it releases adipokines that affect liver and muscle insulin sensitivity. In physiological responses, subcutaneous AT and visceral AT play distinct roles and this impacts on the role of central AT distribution in the metabolic syndrome. The remainder of the presentation concerned congenital and acquired lipodystrophies. An interesting overlap was discussed in the lipodystrophies between the genetic laminopathies and HIV-treatment related (acquired) lipodystrophy: both are characterized by mitochondrial dysfunction and accumulation of prelamin A, which may play a central role in AT dysfuntion and is involved in premature aging. One of the main conclusions was that both too much AT (as in the metabolic syndrome), as well as too little AT has a detrimental effect on insulin sensitivity of liver and muscle tissue.

The Angiopoietin-like protein 4 was introduced by *Sander Kersten* (Wageningen, The Netherlands). Angptl4 is induced by fasting and is expressed mainly in adipose tissue in the mouse, but also in the liver in the human. It is a target gene for PPARα mediated transcriptional regulation and mice expressing Angptl4 are severely hypertriglyceridemic upon fasting. They also have decreased white AT mass as compared to non-transgenic littermates. In vivo studies revealed a defect in LPL-mediated TG-hydrolysis and delivery of TG-derived fatty acids to AT. Thus, Angptl4 seems to affect LPL activity.

The mechanism whereby Angptl4 affects LPL activity was addressed by *Valentina Sukonina* (Umea, Sweden). The mature Angptl4 protein contains a coiled-coil domain (ccd) and a fibrinogen-like domain. Inhibition of LPL has been associated with the ccd of Angptl's and the ccd of Angptl4 (ccd-Angplt4) was cloned into an expression vector and used for further study. Using a series of elegant studies, it was shown that ccd-Angptl4 binds to active dimeric LPL and converts this to inactive monomeric LPL. Angptl4 can do this also with LPL bound to heparan sulphate proteoglycans. Since Angptl4 mRNA levels are regulated according to nutritional status and LPL mRNA levels are not, it seems likely that the Angptl4 protein is an important post-transcriptional regulator of LPL-activity in adipose tissue.

The various sources for uptake of fatty acids by adipose tissue was delineated by *Barbara Fielding* (Oxford, UK) who had used a model comprising direct measurements in human subcutaneous tissue by arterio-venous cannulation

techniques in combination with stable isotope labelled fatty acids. A previously neglected pathway, that of direct uptake of fatty acids from the free fatty acid (FFA) pool appeared to play a rather significant role with perhaps up to 30% of all fatty acids taken up by adipose tissue in the post-prandial state.

Aspects of genetic heterogeneity determining the systemic concentration of FFA was reported by *Iris Heid* (Innsbruck, Austria), who had investigated the role of ATGL. FFA concentrations were significantly associated with several SNPs in the ATGL gene in a large cohort of European ancestry from Utah. Functional characterization of the molecular variants in the ATGL gene remains to be done.

5. Session III. Fatty liver disease: chaired by Folkert Kuipers (Groningen, The Netherlands) and Florian Kronenberg (Innsbruck, Austria)

Jukka Westerbacka (Helsinki, Finland) presented an invited lecture on the fatty liver as key player in the metabolic syndrome. He discussed the causes and particularly the adverse consequences of ectopic fat accumulation in the liver (also known as hepatic steatosis). Hepatic steatosis is becoming highly common in the western society, in conjunction with the current obesity epidemic: subjects with steatosis are often insulin resistant and show other features of the metabolic syndrome. Elevated markers of liver dysfunction (e.g., ASAT, ALAT as "surrogate markers" for fatty liver) predict development of type II diabetes independent of obesity. Fatty liver could, according to Westerbacka, therefore be included in the definition of the metabolic syndrome. The fatty liver overproduces most cardiovascular risk factors, e.g., triglyceride-rich lipoproteins, glucose, coagulation factors and probably contributes to increased cardiovascular risk in metabolic syndrome and type 2 diabetes. Potential means to reduce liver fat content, i.e., weight loss, low fat diet and glitazone treatment, were discussed. Data were presented to show that expression of macrophages in adipose tissue was related to the degree of hepatic fat accumulation rather than to obesity, delineating the important cross-talk between liver and adipose tissue in development of various aspects of the metabolic syndrome. An important issue that still remains to be solved is why some people deposit large amounts of fat in their livers why others do not or, in other words, why large differences in insulin sensitivity exist between people that are equally obese. The answer to this question is eagerly awaited for.

Kristiaan Wouters (Maastricht, The Netherlands) reported on investigations on the pathogenesis of non-alcoholic steatohepatitis (NASH), which was, until recently, thought to be characterized by steatosis followed by inflammation. However, in a recent study it was observed that APOE2 knock-in mice developed NASH under a high-fat diet during a process in which inflammation developed concurrently with steatosis. In the present study, the authors used an LDLreceptor−/− mouse model and observed, similarly as in the APOE2 knock-in mouse model, that inflammation as indicated by macrophage infiltration appeared to be an early event. Hyperlipidemic mice were more sensitive to diet-induced hepatic inflammation than control mice and the high-fat feeding induced the expression of many inflammatory genes but no lipid genes in LDLreceptor−/− mice. It was concluded that early inflammation may be caused by uptake of plasma cholesterol-rich lipoproteins by Kupffer cells.

Marten Hofker (Maastricht, The Netherlands) described their findings on the role of macrophages in the development of steatosis and steatohepatitis. Since the role of tumor necrosis factor (TNF) in the pathogenesis of the disease is still controversial, the authors used bone marrow transplantation to study TNF-receptor-1 (TNFR1)-deficient liver macrophages, which are unresponsive to TNF. They observed no effects of TNFR1 deficiency on either lipid levels or steatosis. In a next step, they investigated whether a hyperstimulation of TNF signaling itself affects steatosis by using a knock-in model of a TNFR1 non-sheddible mutation, which lacks the negative feedback mechanism. This constellation resulted in an increased inflammation in liver, reduced plasma lipid levels and reduced hepatic accumulation of neutral lipids. Although these findings are difficult to explain at present, the data are in line with similar experimental models such as the activation or inactivation of NF-κB in the hepatocytes, which results in an decrease and increase, respectively, of triglyceride content in the liver.

Thomas Lundåsen (Huddinge, Sweden) reported on the effects of inhibition of the ileal bile acid transporter (IBAT) on plasma glucose and triglyceride concentrations in ob/ob mice. Administration of a potent inhibitor resulted in a 30% reduction of plasma glucose and in a 50% reduction of plasma insulin and triglycerides. A reduction of mRNA of FGF15 and SHP of the distal ileum was observed due to the loss of circulating bile acids. Also the hepatic mRNA for SREBP1c as well as ACC and FAS was decreased indicating an impaired fatty acid and triglyceride synthesis in treated animals. Furthermore, an 80% increase in the hepatic GK mRNA levels and a 50% reduction of G6Phosphatase mRNA levels was observed which could not be ascribed to an increased insulin receptor pathway signaling. One of the most important findings was an increased hepatic FGF21 mRNA level, which might have contributed to the beneficial effects of IBAT inhibition.

6. Session IV. Lipid signaling: chaired by Arnold von Eckardstein (Zurich, Switzerland) and Laura Calabresi (Milan, Italy)

The lipid signaling session was opened with an invited lecture given by *Hans Aerts* (Amsterdam, The Netherlands). After an overview on glycosphingolipid metabolism, he reported extensively on the treatment of patients with glucocerebrosidase deficiency (Gaucher's disease). In addition to enzyme substitution therapies, patients underwent treat-

ment with inhibitors of glucosylceramide synthase (e.g., N-Butyldeoxynojirimycin: NB-DNJ, miglustat, "Zavesca"), which is a rate-limiting enzyme in the synthesis of glycosphingolipids. Surprisingly, these compounds did not only help to improve non-neurological symptoms of Gaucher patients. In insulin resistant mouse models like the ob/ob mouse they have also been found to improve insulin sensitivity.

Sasa Frank (Graz, Austria) and colleagues analyzed the effects of HDL pre-treated with endothelial lipase (EL) on the gene expression patterns of endothelial cells. Owing to its phospholipase and lysophospholipase activities, EL produces a wide spectrum of potentially bioactive lipids. In response to EL-treated HDL, endothelial cells were found to down- or up-regulate 400 genes more than three-fold. By using pharmacological inhibitors multiple signaling pathways were found to up-regulate target genes such as VCAM-1, E-selectin, interleukin 6, RANTES or COX2 and cytosolic phospholipase A2.

Sophie Lestavel (Lille, France) discussed the role of LXR ligands in modulating cholesterol movements in human cultured monocyte-derived macrophages. The incubation of macrophages with two different LXR agonists resulted in: (i) increased selective cholesteryl ester (CE) influx from HDL, not associated with foam cell formation; (ii) increased CE hydrolysis; (iii) increased cholesterol efflux to HDL. Changes in cholesterol uptake and efflux is not mediated by changes in SR-BI, since both mRNA and protein were not changed after treatment with LXR agonists. Instead, caveolin expression and localization were modified by treatment. Moreover, the addition of heparin totally abrogated the effects of LXR agonists, thus suggesting an involvement of LXR target proteins interacting with proteoglycans, such as apoE and LPL.

Giuseppe Norata (Milan, Italy) discussed the role of pentraxin 3, an acute phase protein, in atherogenesis and its modulation by HDL. Pentraxin 3 is present in atherosclerotic plaques and its expression is significantly higher in plaques from asymptomatic patients compared with symptomatic cardiovascular patients. Moreover, pentraxin 3 plasma concentration correlates inversely and significantly with carotid intima-media thickness (IMT) in the general population. Mice lacking the pentraxin 3 when crossed with apoE KO mice develop increased aortic atherosclerosis. HDL can modulate pentraxin 3 production by endothelial cells. When HUVEC were incubated with HDL (200 μg/ml), the expression of pentraxin 3 was increased by three times. Moreover, mice overexpressing apoA-I, which present with very high plasma HDL cholesterol levels, have increased pentraxin 3 expression in the aortas.

7. Session V. Novel therapeutic targets: chaired by Paolo Parini (Stockholm, Sweden) and Joerg Heeren (Hamburg, Germany)

In this session, the invited speaker *Markus Stoffel* (New York, USA) described the functional role of apolipoprotein M (apoM) for pre-beta HDL formation and cholesterol efflux. ApoM has been initially considered as a lipid marker with levels found to positively correlate with obesity, leptin levels and inversely with MODY syndrome. Dr. Stoffel showed the accumulation of large, cholesterol-rich HDL particles and the lack of pre-beta HDL formation in apoM deficient mice. Cholesterol efflux from macrophages to apoM-deficient HDL was markedly reduced compared to normal HDL. Vice versa, adenovirus-mediated overexpression of apoM in wild-type mice increases the amount of pre-beta HDL and furthermore protected against the development of atherosclerosis in LDL receptor knock-out mice fed a cholesterol-enriched diet. In the second part, new insights in the regulation of apoM expression by the forkhead transcription factor Foxa2 were presented. Foxa2 is insulin-regulated and controls genes including apoM, which are relevant for glucose and lipid metabolism. Insulin induces the phosphorylation of Foxa2 via Akt kinase thereby preventing the transport into the nucleus, which in turn inhibits the activation of gene transcription. This is especially important in severe insulin resistance since in this case impaired Foxa2 activation lead to hepatic lipid accumulation and decreased apoM secretion.

Mats Rudling (Stockholm, Sweden) described in his presentation that the intestinal fibroblast growth factor 19 (FGF19) is involved in the regulation of bile acid synthesis in humans. It has been shown in mice that the mouse orthologue FGF15 suppresses bile acid synthesis. In the current study, FGF19 levels were measured in humans and were dramatically reduced after treatment with bile acid-binding resins. This was associated with a concomitant increase of the synthesis of bile acids as determined by the plasma marker C4. Further, treatment with chenodeoxycholic acid increased serum FGF19 levels concomitantly with a reduced bile acid synthesis. Interestingly, serum FGF19 was shown to have a diurnal rhythm that was abolished upon fasting. Serum FGF19 levels were peaking when bile acid synthesis was leveling off suggesting that the diurnal changes in bile acid synthesis are in part controlled by the intestine via FGF19. The results suggest that the trans-intestinal flux of bile acids in the enterocyte induces the production and secretion of FGF19 that in turn suppresses the hepatic synthesis of bile acids.

The hypocholesterolemic effects of fatty acid bile acid conjugates (FABACs) in mice and their potential mechanisms were presented by *Alicia Leikin-Frenkel* (Tel Aviv, Israel). The synthetic FABACs exert different metabolic effects such as preventing diet induced fatty liver and inducing cholesterol efflux. In this study, FABACs were shown to dose-dependently decrease plasma cholesterol levels. To investigate the underlying mechanisms the expression and activity of key enzymes regulating cholesterol metabolism were measured. Dr. Leikin-Frenkel showed that FABACs (a) reduce the activity of HMG-CoA reductase, (b) down-regulate the expression of HMG-CoA reductase and LDL receptor, (c) induce bile acid synthesis by up-regulating the activity and expression of CYP7A and (d) increase fecal sterol excretion suggesting that FABACs modulate the activity of

transcription factors which are involved in the regulation of lipid and bile acid metabolism.

Andreas Wehinger (Innsbruck, Austria) analyzed in his study the effect of aspirin treatment on the expression of SR-BI in human carotid atherosclerotic plaques. First, he presented data from mice, showing that low concentrations of aspirin in the drinking water activated the protein expression of SR-BI whereas high aspirin concentrations decreased SR-BI levels in isolated resident murine macrophages. The mRNA levels were unchanged, indicating that aspirin-mediated changes in SR-BI expression involve post-transcriptional processes. Second, to study SR-BI expression in a pathophysiological situation in vivo, mRNA and protein levels isolated from human carotid lesions from untreated and aspirin-treated patients were determined. While no changes in mRNA levels were found, a significant increase in SR-BI protein expression was detected in aspirin-treated patients suggesting that aspirin might induce cholesterol efflux from lipid-loaded macrophages via SR-BI in vivo.

Réjane Paumelle (Lille, France) gave new insights into molecular mechanisms dealing with the pleiotropic anti-inflammatory effects of statins. She presented data showing that statins inhibited lipopolysaccharide (LPS)-induced inflammatory response genes in wild-type macrophages but not in macrophages deficient for PPARα. LPS activates protein kinase C (PKC) which leads to the phosphorylation of PPARα and consequently to the inactivation of PPARα transrepression activity. Statins inhibited the PKC-mediated phosphorylation of PPARα and therefore enhance the transrepression activity of PPARα. Altogether the presented data indicate that the acute anti-inflammatory effects of simvastatin occur via PPARα transrepression activity.

The second part of the session was opened with an overview on the cholesteryl ester transfer protein inhibitors (CETPi) by *Cyrille Murgeais* (Basel, Switzerland). It is well accepted that there is an unmet medical need for safe drugs that can efficiently raise HDL cholesterol levels. The CETPi's JTT-705 and torcetrapib are currently in late phases of clinical trials and are the most advanced class of HDL raising compounds. CETPi as monotherapy, or in combinations with statins, has been proved to increased HDL cholesterol. Whether these beneficial effects on plasma lipids of CETPi will translate into a benefit on cardiovascular events still remain to be proven.

Fanny Lalloyer (Lille, France) presented how PPARα improves pancreatic adaptation to insulin resistance in obese mice and reduces lipotoxicity in human islets. Her studies aimed to analyse the impact of PPARα-deficiency on glucose homeostasis. Genetically depleted PPARα mice were generated in ob/ob background. Deficiency of PPARα resulted in a severe and age-dependent hyperglycemia and developed pancreatic beta-cell dysfunction characterized by reduced mean islet area and decreased insulin secretion in response to glucose. In primary human pancreatic islets, PPARα agonists prevented the fatty acid-induced impairment of the glucose stimulated insulin-secretion. The data all together suggests that PPARα may improve the response of pancreatic beta-cells to pathological condition.

Markus Neumeier (Regensburg, Germany) analyzed how high molecular weight-adiponectin (HMW-Apm) may ameliorate dyslipidemia by downregulating apolipoprotein B and E release in human hepatiocytes. Low circulating levels of HMW-Apm have been linked to dyslipidaemia and HMW-Apm was shown to reduce hepatic ApoB and ApoE release whereas ABCA1 protein, activity and ApoA-I are not altered. This indicates that HMW-Apm may ameliorate dyslipidaemia by reducing the hepatic release of ApoB and ApoE, whereas ABCA1 function and ApoA-I secretion are not influenced. The lower abundance of ApoB may be explained by a reduced expression of hepatic nuclear factor 4-α (HNF4-α) in HMW-Apm treated hepatocytes.

The session was concluded with a contribution from *Malin Levin* (San Francisco, USA) who presented how acyl CoA:diacylglycerol acyltransferase 2 (DGAT2) mediated lipid accumulation in glycolytic muscle promotes insulin resistance. Oxidative muscle normally stores more triglycerides and is more insulin sensitive than glycolytic muscle. Thus, accumulation of triglycerides in glycolytic muscle may promote insulin resistance. Transgenic mice overexpressing DGAT2 in glycolytic muscle were generated by using the muscle creatine kinase (MCK) promoter. MCK-DGAT2 mice had increased lipid accumulation and impairments in insulin signaling and in insulin-stimulated glucose uptake in glycolytic muscle and exhibited impaired glucose tolerance. These findings suggested that lipid deposition in glycolytic muscle may contribute to insulin resistance.

8. Session VI. Varia: chaired by Ken Lindstedt (Helsinki, Finland) and Marten Hofker (Maastricht, The Netherlands)

Oral contributions at the varia session included, as has become a tradition, a diversity of topics and molecules involved in lipid metabolism and atherosclerosis. The latter included apoM, Boca, the LRP, apoA-IMilano, sPLA2, PLTP, Megalin and the LXR. *Christina Christoffersen* (Copenhagen, Denmark) demonstrated that ApoM, a member of the lipocalin family, is predominantly associated with a subpopulation of HDL particles in human plasma. About 5% of the HDL fraction contained apoM (HDLapoM+). Furthermore, the HDLapoM+ particles were found to be heterogeneous in size and protein composition and to be α-migrating. The HDLapoM+ subpopulation was also found to protect LDL against oxidation and to stimulate cholesterol efflux more efficiently than HDLapoM−, suggesting that it may have antiatherogenic properties.

Sabine Christian (Vienna, Austria) showed that Boca, a chaperone of the endoplasmatic reticulum, is ubiquitously expressed in the chicken model and is essential for the trafficking of β-propeller/EGF modules of LDLR family members, such as LR11/SorLA and LR8. The restricted

ovulator (R/O) chickens have an unpaired cysteine in the C-terminal EGF module, which disrupts the interaction with Boca, leading to a disturbed membrane localization of LR8. These results support a role for Boca in proper folding of LDLR family members in a variety of systems.

Lihui Hu (Leiden, The Netherlands) demonstrated that LRP deficiency in macrophages resulted in increased level of atherosclerosis, which was independent of plasma cholesterol. The content of macrophages and smooth muscle cells was similar in the atherosclerotic plaques of LRP− and LRP+ mice, whereas LRP− mice contained less CD3+ T cells. In addition, atherosclerotic plaques of macrophage LRP− mice were shown to contain more collagen, suggesting that macrophage LRP may affect matrix remodeling in the lesions.

Riikka Vikstedt (Helsinki, Finland) showed that macrophage PLTP-deficiency influenced serum lipid levels, lipoprotein distribution and apoA-I concentration, and decreased atherosclerotic lesion formation in LDLr−/− mice. Thus, macrophage PLTP seemed to be an important contributor to plasma PLTP activity and to have a pro-atherogenic role in LDLr−/− mice. However, Vikstedt concluded that further studies in mice with different genetic backgrounds are needed to confirm the pro-/anti-atherogenic role for macrophage PLTP.

Monica Gomaraschi (Milan, Italy) presented data that plasma levels of sCAMs and forearm arterial compliance during reactive hyperemia of apoA-IMilano carriers were comparable to those of control subjects, indicating that apoA-IMilano carriers despite severe hypoalphalipoproteinemia do not display features of endothelial dysfunction. In fact, Gomaraschi concluded that HDL from apoA-IMilano carriers are more efficient than control HDL in affecting endothelial function in vitro, as demonstrated by their superior ability to inhibit TNFα-induced VCAM-1 expression and to increase the expression of endothelial NO synthase (eNOS) in cultured HUVECs.

Uwe Tietge (Groningen, The Netherlands) discovered a new role for type IIA Secretory Phospholipase A2 (sPLA2) in end-stage renal disease (ESRD). He observed that ESRD patients had dramatically increased plasma levels of catalytically active sPLA2. This resulted in severe endothelial dysfunction as the elevated sPLA2 increased ROS production via functional coupling with COX-2. As endothelial dysfunction is an additional pro-atherogenic property of sPLA2 these findings possibly explain the role of sPLA2 as a causative risk factor contributing to the excessive CVD risk in uremic patients and indicate that sPLA2 might be a potential novel target for pharmacological intervention.

Thomas Willnow (Berlin, Germany) showed very interesting data on the role of Megalin. This receptor belongs to the LDL-receptor gene family and emerged as a crucial receptor mediating the uptake of sex hormone binding globulin (SHBG), and the hormones that are bound to it, including androgens and estrogens. Importantly, in the absence of Megalin, these hormones failed to elicit specific effects on target cells in vivo. Thus, loss of Megalin in reproductive tissues causes impaired opening of the vagina cavity in female and testicular maldescent in male mice. These effects were phenocopies of models treated with androgen and estrogen receptor antagonists. In conclusion, Megalin provides a tissue-specific pathway for delivery of steroids that plays a crucial role in specific aspect of steroid hormone action.

Monique Mulder (Maastricht, The Netherlands) presented an important mechanism how the traffic of cholesterol from astrocytes to neurons is regulated. Astrocytes express the LXR, while the basal LXR levels in neuronal cells were 10–20 lower. It was found that the brain specific 24(S)-hydroxycholesterol, produced by neurons, serves as an LXR ligand to induce apoE in the astrocyte. Other proteins involved in cholesterol efflux, i.e., ABCA1, ABCG1 as well as apoE and apoAI mediated cholesterol efflux were up-regulated in astrocytes as well. These data were further supported by using synthetic LXR agonists, which were able to stimulate cholesterol efflux from astrocytes, while neuronal cells were not responsive.

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