European Lipoprotein Club

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Meeting report

European Lipoprotein Club: Report of the 27th ELC Annual Conference, Tutzing, 6–9 September 2004☆

1. Introduction

The 27th meeting of the European Lipoprotein Club was held from 6 to 9 September at the Evangelische Akademie in Tutzing Germany and attended by some 115 participants. The state of the art lecture was presented by Alan Tall (New York, USA) entitled "New pathways of cellular lipid efflux". The first session was focused on human metabolism and introduced by Barbara Fielding (Oxford, UK) on the methodological aspects of tracking human metabolism with stable isotopes. The second part of this session was introduced by Jan Albert Kuivenhoven (Amsterdam, The Netherlands) on CETP and atherosclerosis. The session on intestinal lipid metabolism was opened by Nicholas Davidson (St. Louis, USA) with a lecture bearing the intriguing title: "Genetic modulation of intestinal fat transport: unexpected tales from the crypt". The session on endothelial function was opened by Thomas Luscher (Zurich, Switzerland) with a comprehensive review on the vascular protective effects of HDL. The next session was HDL regulation, consisting of speakers selected from the submitted abstracts. The lipase session was introduced by Rudolf Zechner (Graz, Austria), who presented new insights in the regulation of adipocyte lipolysis and fatty acid release. The session on nuclear receptors/gene regulation consisted exclusively of speakers invited from the submitted abstracts, as did the traditionally last session termed "Varia".

This year, two types of awards were presented to three young scientists. The young investigator award was presented to Torsten Plosch (Groningen, The Netherlands) for his presentation entitled "Evidence for abcg5-independent hepatobiliary cholesterol transport in mice". One of the two poster awards was presented to Marisa Tschernatsch (Graz, Austria) for her presentation entitled: "Implication of HuR in the regulation of mRNA-stability of genes relevant in lipid metabolism". The second poster award was presented to Susan Coort (Maastricht, The Netherlands) for her presentation entitled: "Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese Zucker rats".

2. State of the art lecture: chaired by Marten Hofker (Maastricht, The Netherlands)

Alan Tall (New York, USA) was the first speaker of this year's meeting. He presented new findings about the role of ATP-binding cassette (ABC) transporters in HDL metabolism. *ABCA1*, being the culprit gene in Tangiers disease, has been regarded as the principle transporter in reverse cholesterol metabolism. However, he demonstrated that ABCA1 mainly works in concert with lipid poor apoA1 as a cholesterol ester (CE) acceptor. LXR can be considered as the crucial regulator of reverse cholesterol transport in macrophages. Interestingly, LXR activation mediated CE efflux from macrophages independently of ABCA1. An important LXR target gene is *ABCG1*. ABCG1 is a half-transporter and forms with ABCG4 a heterodimer, which is the complete transporter. By using siRNA technology, ABCG1/ABCG4 was down regulated in primary macrophages, leading to a marked decrease of cholesterol efflux to native HDL.

3. Session I. Human metabolism: chaired by Fredrik Karpe (Oxford and UK) Vincent Mooser (Philadelphia, USA)

Stable isotopes are being used increasingly to study the metabolism of lipids and lipoproteins. Dr. Barbara Fielding (Oxford, UK) gave an overview of technical aspects related to the use of stable isotope-labeled molecules in the study of lipid metabolism. Very high sensitivity can be achieved by gas-chromatography isotope ratio mass-spectrometry whilst conventional mass-spectrometry is less sensitive but more

☆ 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-tutzinq.org.

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versatile. Hans Dieplinger (Innsbruck, Austria) presented results from a study on the role of the kidney in apolipoprotein metabolism, with a particular focus on Lp(a). Endogenous labeling of apolipoproteins was achieved by an intravenous infusion of deuterium-labeled Valine in 12 patients on haemodialysis and 13 matched control subjects. Data were analyzed in a multi-compartmental model. The hyperlipidaemia often seen in patients was attributed to slow fractional catabolic rate of low-density lipoprotein (LDL) and IDL. Similarly, this group provided additional data to indicate that the elevation in plasma Lp(a) levels observed in patients with chronic renal failure was at least partly due to reduced clearance by the kidney.

Kevin Evans (Oxford, UK) presented data on the use of stable isotopes in the investigation of fatty acid metabolism in familial combined hyperlipidaemia (FCH). Adipose tissue function was investigated by arterio–venous techniques across subcutaneous abdominal adipose tissue. Carbon-13 labeled palmitic acid was incorporated into a standardized meal and traced by sequential blood sampling. It was concluded that adipose tissue function is essentially normal in patients with FCH and that the hyperlipidaemia is largely explained by overproduction of triglyceride-rich lipoproteins rather than slow catabolism. Results from a very detailed investigation of the fate of orally ingested labeled fat were presented by Alex Bickerton (Oxford, UK). Here the effect of insulin resistance was examined in subjects carefully selecting based on anthropometric measures. In this study, the major defect appeared to be in metabolic flexibility. Subjects with insulin resistance appeared to be unable to effectively switch between fat and carbohydrate as principal fuel. However, the high insulin concentrations found in subjects with insulin resistance seemed to compensate in terms of overall body combustion of fat, which appeared to be normal.

Dieter Lütjohann (Bonn, Germany) presented data on the effect of statin treatment on skeletal muscle cholesterol concentrations. This study was prompted by the well-known muscular side effects of statins. Skeletal muscle concentrations of cholesterol and ubiquinone were quantified by HPLC/mass-spectrometry in biopsy specimens. Statin treatment reduced the plasma concentrations of LDL-cholesterol and the lathosterol/cholesterol ratio (a surrogate marker for cholesterol synthesis) by 66%. A high dose of either simvastatin (80 mg per day) or atorvastatin (40 mg per day) reduced the cholesterol content of skeletal muscle by almost 40% and the ubiquinone content was also significantly reduced.

Jan Albert Kuivenhoven (Amsterdam, The Netherlands) was invited to give an outline on the role of the cholesteryl ester transfer protein (CETP) and its relationship to atherosclerosis. First, he presented data on the relationship between CETP deficiency in Japanese populations and in a family from the Netherlands and atherosclerosis. He next described the relationship between plasma CETP activity, or the common intron 1 Taq1B polymorphism and atherosclerosis. Based on a meta-analysis, he showed that the genetic variant with lower CETP activity and higher HDL cholesterol concentrations was associated with a reduced incidence of coronary heart disease (CHD). Second, he presented previously published data on the CETP inhibitors JTT-705 and torcetrapib in humans. These compounds are effective HDL-raising agents, but the effect on cardiovascular endpoints remains to be proven.

The enzymes involved in cholesterol esterification in the liver were discussed by Paolo Parini (Stockholm, Sweden). The hepatocellular distribution and gene expression of the two acyl co-enzyme A: cholesterol acyl-transferases, ACAT1 and ACAT2, were studied in human specimens. ACAT2 appears to be the major cholesterol acyl-transferase in the human liver whereas the expression of ACAT1 was confined to Kupffer cells. A promoter region in the *ACAT2* gene was identified as an important regulatory region and as a potential therapeutic target to reduce the production of VLDL particles by the liver. On a similar note, Cecilia Gälman (Stockholm, Sweden) presented data on the diurnal rhythm of bile acid synthesis in humans. The cholesterol metabolite 7alpha-hydroxy-4-cholesten-3-one (C4) is a marker of CYP7A enzymatic activity, which is the rate-limiting step in bile acid formation. The plasma concentration of C4 was monitored in healthy volunteers over a 24-hr period. It turned out that bile acid production has a marked circadian rhythm, which is distinct from that of cholesterol production and also unrelated to food intake.

Martin Hersberger (Zurich, Switzerland) described an elegant system by which blood samples could be obtained from coronary vessels undergoing revascularization using a double-balloon catheter. This technique was used to give a quantitative estimate of the amount of inflammatory markers such as IL-6 and serum amyloid A (SAA) secreted from areas of coronary arteries with a ruptured plaque. It was demonstrated that the levels of IL-6 and SAA were higher, whereas the levels of CRP were lower, at the site of plaque rupture than in the aorta, clearly indicating local production of IL-6 and SAA. Steve Humphries (London, UK) described how a genetic approach could be used to investigate the link between oxidative stress and CHD. The mitochondrial uncoupling protein 2 (UCP2) gene contains a functional promoter variant (−866G/A), which was associated with total antioxidant status (TAOS) in plasma. The TAOS measurement was verified against F2-isoprostane excretion in urine and then applied to stored plasma from the Northwick Park Heart Study. Homozygous carriers of the UCP2 A-variant (low TAOS) had almost twice the risk of CHD after adjustment for established risk factors. This was taken as evidence for a role of UCP2 in modifying oxidative stress and CHD risk in humans.

4. Session II. Intestinal lipid metabolism: chaired by Folkert Kuipers (Groningen, The Netherlands) and Mats Rudling (Stockholm, Sweden)

Nick Davidson (St. Louis, USA) presented an excellent lecture entitled “Genetic modulation of intestinal fat

transport-unexpected tales from the crypt" and indeed, a considerable part of the data presented could be categorized as "unexpected". After a general overview of the processes involved in fat absorption, including description of newly identified transporter proteins and of the chylomicron assembly cascade, a detailed account on the role of APOB mRNA editing in the process was provided. Mammalian enterocytes generate a truncated form of apoB (apoB48) through posttranslational C to U RNA editing of the nuclear transcript, which introduces a translational stop-codon (UAA) that results in the formation of a protein encoding the amino-terminal 48% of apoB100. While APOB editing in humans is restricted to the intestine, the rodent liver secretes both apoB100 and apoB48. C to U RNA editing is mediated by a multicomponent holoenzyme complex that contains a core enzyme composed of apoB-mRNA-editing-enzyme catalytic protein 1 (APOBEC-1), an RNA specific cytidine deaminase, and APOBEC-1 complementation factor (ACF) as RNA recognition subunit. The target-sequence specificity of RNA editing is tightly controlled by cis-acting sequence requirements that include and AU-rich context and sequence elements flanking the targeted base and requires an optimal stoichiometry and distribution of APOBEC-1 and ACF. To address the role of apoB100 versus apoB48 in murine lipoprotein metabolism, the Davidson laboratory made use of apobec-1−/− mice with an apoB100—only lipoprotein profile. Earlier studies in mice with a mixed background revealed no gross defects in weight gain, triglyceride absorption and plasma lipid levels. However, a more detailed analysis of apobec-1−/− mice backcrossed onto a C57BL/6 background demonstrated a number of subtle defects. It was found that inbred apobec-1−/− mice absorb triglycerides normally; yet secrete triglyceride-rich lipoproteins more slowly than their congenic controls. There was a comparable induction of apoB synthesis in response to fat feeding in both genotypes, but apoB100 was preferentially retained and more extensively degraded than apoB48. By contrast, synthesis, secretion and content of apoAIV were indistinguishable between the groups with 100% recovery, suggesting no degradation of this protein in either genotype. Newly synthesized lipoproteins from isolated enterocytes of wild-type mice revealed apoB48 in both HDL and VLDL fractions. By contrast, apobec-1−/− mice secreted apoB100 containing particles that were almost exclusively in the LDL and VLDL range with no apoB100-containing HDL. These studies establish the existence of preferential degradation of intestinal apoB100 and subtle defects in triglyceride secretion in mice, coupled with a shift to the production of larger particles. These findings suggest an important divergence in intestinal lipoprotein assembly with the different isoforms of apoB.

The unexpected part of the presentation addressed a novel role of APOBEC-1 independent of RNA editing, providing evidence that the protein is involved in binding to and stabilizing RNA targets that contain AU-rich motifs. AU-rich sequence elements (AREs) are found in the 3′ untranslated regions of many proto-oncogene and cytokine mRNAs, and act as mRNA instability determinants or as translation inhibitory elements. Data suggest that altered expression of APOBEC-1 might alter the stability or metabolism of rapidly degraded RNAs, some of which are involved in regulation and proliferation in the setting of carcinogenesis. Particularly, recent findings indicate that APOBEC-1 might regulate intestinal stem cell survival through alterations in the stability of the mRNA encoding cyclooxygenase-2 (COX-2), an mRNA with an ARE containing the canonical APOBEC-1 binding site.

All textbooks on modern medicine state that hepatobiliary cholesterol excretion is the only way to excrete substantial amount of cholesterol from the body and represents an important component of reverse cholesterol transport. Recent cholesterol balance studies have shown that, under certain experimental conditions, more neutral sterols are present in feces than can be derived from hepatobiliary excretion. Bert Groen (Amsterdam, The Netherlands) addressed the origin of non-bile derived cholesterol present in the intestinal lumen, making use of a newly developed mouse model in which intestinal segments were perfused with a bile salt/phospholipid mixture, and at the same time, bile was diverted. Radiolabeled cholesterol was intravenously administered to label the plasma cholesterol pool. It was shown that plasma derived cholesterol was secreted into the duodenum, jejunum and ileum, with the highest secretion rate in the first. Specific activity of perfusate cholesterol was similar to that of plasma but nine-fold higher than that in the intestinal wall, indicating that cholesterol present in the lumen was not simply derived from shedded cells. It could be calculated that the total flux through the three segments amounted up to 30–40% of the daily fecal sterol loss in mice. This indicates that direct secretion of cholesterol into the intestine plays an important role in reverse cholesterol transport.

Thierry Claudel (Groningen, The Netherlands) addressed the role of the bile salt-activated Farnesoid X Receptor (FXR, NR1H4) in control of the formation of triglyceride (TG)-rich lipoproteins by liver and intestine to elucidate the mechanism(s) underlying hypertriglyceridemia in FXR-deficient mice. Similar VLDL-TG production rates were measured in control and Fxr−/− mice after injection of Triton WR1339, but the diameter of nascent VLDL particles of Fxr−/− mice was increased compared to those of controls (79 nm versus 64 nm). Surprisingly, production of chylomicrons by the intestine after an oral dose of olive oil containing [^{3}H]triolein and [^{14}C]oleate was impaired in Fxr−/− mice and chylomicron diameter was reduced (160 nm versus 257 nm). Plasma appearance of ^{3}H and ^{14}C-labeled TG was clearly delayed in Fxr−/− mice. Hepatic and intestinal expressions of genes involved in the assembly of TG-rich lipoproteins were similar in both groups. In contrast, hepatic expression of ApoC2 and Lpl was reduced in the knockouts. In addition, in vivo lipolysis measurements employing [^{3}H]TG labeled emulsion particles and albumin-complexed [^{14}C]palmitate revealed impaired lipolysis and reduced uptake of TG-derived fatty acids in muscle and adipose tissue in Fxr−/− mice. This study shows that disruption of the *Fxr* gene differentially affects

formation of TG-rich lipoproteins by liver and intestine. Hypertriglyceridemia in Fxr−/− mice results from impaired processing and clearance of TG-rich lipoproteins.

Finally, Mats Rudling (Stockholm, Sweden) showed results from a study in which the bile acid and cholesterol synthesis was determined using plasma markers in 125 Chilean women with and without asymptomatic gallstones together with plasma lipids. In addition, 40 Chilean Mapuche Indians, having the highest prevalence for gallstone disease in the world, were studied. It was found that bile acid synthesis was increased in subjects with gallstone disease and that bile acid synthesis was high in the Mapuche Indians regardless if gallstones were present or not. The results suggest that an increased synthesis of bile acids precedes the formation of gallstones, suggesting that the primary event in gallstone disease may not be within the liver but in the gut leading to an increased faecal loss of bile acids. The cause may be related to environmental factors (diet) and/or genetic factors.

5. Session III. Endothelial function: chaired by Mats Rudling (Stockholm, Sweden)

In the session on endothelial function Thomas Luscher (Zurich, Switzerland) started the session where he highlighted different aspects on the atheroprotective effects of HDL. He showed that hypercholesterolemia reduces the acetylcholine-induced blood flow. This effect of cholesterol can be rapidly counteracted by the infusion of intravenous recombinant HDL. Although the mechanism of action is not yet understood, this experiment illustrates the therapeutic potential of increasing HDL in patients at risk. He further stressed this potential by pointing out previous results where the infusion of Apo Al Milano to hypercholesterolemic rabbits was shown to reduce plaque volume. In an on-going study they are now evaluating whether the pharmacologic inhibition of the cholesterol ester transfer protein, CETP, will result in plaque regression in humans. Although CETP inhibiting drugs clearly increase HDL, it is still not known whether these drugs actually do increase reverse cholesterol transport. Nevertheless, one might benefit from other beneficial effects that HDL might exert such as the anti-oxidative effects that have been reported. It will be most interesting to learn more in the near future whether the pharmacologic elevation of HDL will actually reduce atherosclerotic plaques in man.

Danielo Norata (Milan, Italy) showed data from an investigation where effects on endothelial cells of triglyceride-rich lipoproteins, TGRL, from hyper-and normotriglyceridemic patients were compared. It was shown that TGRL from hypertriglyceridemic subjects resulted in a higher induction of factors such as CD40, TLR-4, MCP-I and certain adhesion molecules as compared to TGRL from normolipidemics, indicating that there may be a role of TGRL from hypertriglyceridemic subjects in endothelial dysfunction. Data on HDL effects were presented by Monica Gomaraschi (Milan, Italy) who showed that HDL could reduce the TNF-alpha mediated increase in IL-6 upon incubation of endothelial cells from the human umbilical vein. Reconstituted HDL was equally effective as was native HDL. A study on a large number of individuals also showed that plasma II-6 was inversely related to the HDL-cholesterol level indicating that one beneficial effect of HDL may be due to HDL effects on II-6 production.

Finally, Christian Rudolph (Regensburg, Germany) showed current results from their search for ABCA1 interacting proteins. Five candidate PDZ proteins have been identified that interact with the carboxyterminal peptide of ABCA1, as does the recently described PDZ protein β2-syntrophin. Expression of the β2-syntropin PDZ-domain in polarized CaCo-2 cells reduced choline-phospholipid efflux. However, measurements of Apo Al and HDL cholesterol in β2-synthopin deficient mice revealed normal levels indicating that PDZ proteins may not alter lipid efflux.

6. Session IV. HDL regulation: chaired by Laura Calabresi (Milan, Italy) and Matti Jauhiainen (Helsinki, Finland)

The session on HDL regulation was started by Joerg Heeren (Hamburg, Germany) who presented novel data on the function of the hepatic ABCA1 transmembrane protein in the regulation of plasma HDL levels in a mouse model. ABCA1 mediates efflux of cellular cholesterol to lipid-poor apolipoprotein A1 and therefore it is important in reverse cholesterol transport. Macrophage ABCA1, unexpectedly, has only a minimal effect on plasma HDL levels and therefore the aim here was to investigate whether hepatic ABCA1 affects HDL levels. The strategy used was to determine the effect of downregulation of ABCA1 specifically in the liver using siRNA-technology. RNA interference technology has been successfully used to down-regulate endogenous gene expression. Different plasmid-based siRNA vectors were generated against sequences of the murine *ABCA1* gene and their functionality to down-regulate co-transfected ABCA1 construct was assessed in human embryonal kidney cells using RT-PCR, immuno-fluorescense and by Western blot analysis. The most efficient plasmid was used to construct a recombinant adenovirus (Ad.antiABCA1) for targeting specifically the liver. Ad.antiABCA1 was injected into tail vein of C57BL/6 mice and an adenovirus construct with eGFP was used as a control. Expression of ABCA1 was followed for up to 7 days and by Western analysis of liver membrane preparations a significant reduction of ABCA1 was observed in all Ad.antiABCA1 infected animals. Among the lipoprotein classes, specifically HDL levels were reduced by approximately 50%. Serum apoE levels were also reduced. This elegant study, using adenovirus-mediated delivery of siRNA (antiABCA1) specifically to the liver clearly suggests an important role for the hepatic ABCA1 in the regulation of plasma HDL levels.

The second talk in the HDL regulation session was given by Hannelore Samyn (Rotterdam, The Netherlands). She had

studied the interplay of plasma phospholipid transfer protein (PLTP) and ABCA1 in mice lacking ABCA1. ABCA1 is important in the efflux of cholesterol and phospholipids to apoAI, the first lipidation step of this protein. PLTP in turn facilitates phospholipid transfer between lipoproteins and affects HDL size and composition. In ABCA1 deficient mice, heterozygous ABCA1+/− mice and wild type ABCA1+/+ mice, PLTP activity in serum was 23%, 67% and 100%, respectively. After cross-breeding of human PLTP overexpressing transgenic mice (PLTPTg) with ABCA1 deficient mice, serum PLTP activity was significantly reduced by 64% (ABCA1+/−) and 85% (ABCA1−/−) compared to human PLTP transgenic (ABCA1+/+) mice. Total serum PLTP mass, as determined by the ELISA method, was also reduced and followed the decline in PLTP activity. A significant reduction of total serum cholesterol was also observed, with a 37% decrease in PLTP-Tg/ABCA1+/− mice and a 75% reduction in PLTP-Tg/ABCA1−/− animals as compared to the human PLTP-Tg/ABCA1+/+ mice. These results suggest that PLTP activity (and its serum PLTP concentration) is dependent on functional ABCA1. Detailed studies are underway in the mouse models to understand the physiological relevancy of the interplay between these proteins.

Miriam Lee-Rueckert (Helsinki, Finland) discussed the effect of degradation of HDL by different physiological proteases on the ability to promote cellular cholesterol efflux. A number of physiologic proteases are able to degrade apoAI. Among them chymase, a protease of mast cells, resulted in the cutting of apoAI and degradation of pre β-HDL, and compromised the ability of HDL to promote cholesterol efflux via ABCA1. In the presented study, the authors showed that also cathepsins F and S can degrade preβ-HDL, which resulted in a 50% reduction of the ability of HDL to promote cholesterol efflux from cholesterol loaded macrophages. Cathepsins are also able to degrade lipid-free apoAI, particularly cathepsin S, and this degradation resulted in a partial or complete loss of the apolipoprotein-mediated cholesterol efflux. All together, these results suggest that a number of proteases secreted by cells present in the atherosclerotic plaques can degrade HDL components, and in particular lipid-free and lipid poor apoAI, which results in a reduced ability to promote efficient cholesterol efflux.

Xavier Rousset (France, Paris) presented data showing that specific HDL subpopulations could be catabolized by the kidney as holo-particles. The studies have been conducted in mice injected with different HDL preparations doubly labeled with fluorescence in the protein and phospholipid components. Particles differing in size (7.0–12.0 nm in diameter), or in protein composition (containing either apoAI only or apoAI and apoAII) were prepared by FPLC from control or apoAII transgenic mice. Only the smallest HDL particles, with diameter below 7.0 nm, were internalized by the kidney, independently of the apolipoprotein composition.

Tamara Pagler (Vienna, Austria) showed the ability of the scavenger receptor SR-BI to take up HDL as holo-particles. The binding, uptake, and intracellular fate of HDL holo-particles in SR-BI over-expressing CHO cells were analyzed applying a novel ultrasensitive epifluorescence microscope, a technique, which enables to closely observe cellular events. Specific binding of the HDL particles to the cells and their subsequent internalization were followed. Two types of movement were observed: diffusion at the cell surface and a direct, straight, and fast movement from the cell membrane towards the perinuclear region. Co-staining with a Golgi marker, GFP-GPI, showed the movement of HDL to the region where the Golgi-apparatus is located but no actual co-localisation was observed. Through biochemical and microscopic experiments, it was also shown that HDL is trapped inside the cells and is accumulated. Co-localization of HDL and LDL in the same acidic compartment was also observed. Using a lysosomal marker, Lysotracker an interaction of HDL particles with acidic compartments, could be observed for a short period of time.

7. Session V. Lipases: chaired by Ko Willems van Dijk (Leiden, The Netherlands)

The section on lipases was opened by keynote speaker Rudolf Zechner (Graz, Austria). He presented an overview of the phenotype of hormone sensitive lipase (HSL) knockout mouse as introduction to the search for additional lipases in adipose tissue. One of the unexplained observations in HSL knockout mice is the accumulation of diacylglycerols (DG) in white and brown adipose tissue (WAT and BAT). If HSL were the only and true triacylglycerol (TG) hydrolase, TG and not DG should accumulate in the tissues of these mice. Their search for additional lipases started *in silico* and uncovered several candidates, one of which was characterized by an appropriate expression pattern and specific protein domains found in most lipases, including the signatures of a catalytically active site and a lipid-binding domain. The protein was termed adipocyte triglyceride lipase (ATGL) and is expressed from day 4 on in the differentiation of cells to WAT. Overexpression of the ATGL protein in vitro resulted in the accumulation of DG, whereas HSL overexpression did not lead to the accumulation of DG. Thus, ATGL seems to be an important WAT/BAT triglyceride lipase, and could be responsible for driving the so-called futile fatty acid cycle. Interestingly, ATGL is subject to phosphorylation, but not by protein kinase A (whereas HSL is a PKA substrate). Thus, our presumed comprehension of insulin mediated lipase activity in WAT has been based on an incomplete picture, but has now gained an additional and very interesting new participant in the enzyme ATGL.

Studies on the regulation of lipoprotein lipase (LPL) activity were presented by Thomas Olivecrona (Umea, Sweden). They have studied the rapid down regulation of adipose tissue LPL upon fasting. This cannot be explained by transcriptional or translational mechanisms since the respective half-life of the mRNA and protein are 17 h and 2 h, considerably more than the response time to fasting. Since the transcription in-

hibitor actinomycin D has similar effects on LPL activity as fasting it was concluded that transcription of a specific gene resulted in the conversion of active LPL to inactive LPL. Data were now presented that demonstrated that a similar type of regulation modulates LPL activity in cardiac and skeletal muscle upon fasting.

Catalytically active LPL is a homodimer of 55 kDa subunits. However, the dominant form in the blood is an inactive monomer. Studies on the conversion between catalytically active and inactive LPL were presented by Gunilla Olivecrona (Umea, Sweden). Since subunits of the LPL dimer rapidly exchange partners, it can be postulated that monomeric LPL is not necessarily irreversibly inactive. In addition, monomeric LPL can slowly regain some activity under assay conditions. In delineating the refolding conditions, it was found that Ca^{2+} is a crucial component for the reactivation of LPL. The rate limiting and Ca^{2+} dependent step in the regeneration of active LPL is the formation of a correctly folded dimerization-competent LPL momoner by proline isomerization.

Little is known of the regulatory pathways involved in LPL synthesis, translocation and turnover. In the search for LPL-binding proteins, Monika Riederer (Graz, Austria) presented the results of a yeast two-hybrid screen. They identified elongation factor (EF) Iα, a previously characterized protein that plays a role in protein translation as LPL-interacting partner. Association of LPL and EF-Iα was confirmed using BIACORE analysis and co-immunoprecipitation. Since EF-Iα is also part of the intracellular proteasome, it was hypothesized that the ubiquitin–proteasome system might be involved in the regulation of LPL activity. Additional evidence for this very interesting notion was obtained using specific inhibitors of the ubiquitin–proteasome pathway.

Diederik van Deursen presented an extensive analysis of the promotor of the hepatic lipase (HL) gene. Using promotor deletion analysis and overexpression of the transcription factor upstream regulatory factor (USF), it was demonstrated that both specific upstream E-boxes and the TATA-lnr region were targets for USF. Interestingly, both statins and overexpression of sterol-regulatory-element binding protein (SREBP) completely abolished the upregulation observed with USF. The mechanism behind the SREBP-mediated interference could be either direct competition for DNA binding, or competition for the binding of cofactors.

8. Session VI. Nuclear receptors / gene regulation: chaired by Gertrud Schuster (Davies, USA)

The first speaker of this session, Bart Staels (Lille, France), presented the latest results on the cross-talk between the cAMP-activated protein kinase (PKC) signaling pathway and the peroxisome proliferator-activated receptor alpha (PPARα, NR1C1). PPARα is established as a global regulator of energy homeostasis, but also of inflammation and immunity; the latter effects are attributed to the transrepressional activities of various factors, including Nuclear Factor-κB (NF-κB) or activators of transcription (STATs). Certain fatty acids, eicosanoids and their derivatives can bind PPARα and influence the gene expression of regulated genes. It was shown previously that mitogen-activated protein kinase (MAPK) and PKC could modulate the transcriptional efficiency of fatty acids. The presented data demonstrated that a dominant negative form of PKC could decrease the ligand induced activity of PPARα on its own expression as well as that of carnitine palmitoyltransferase I (CPT-I). On the other side, PKC inhibition potentiated the ligand induced repressional effects of PPARα on fibrinogen-β transcription. Supplementary studies revealed several potential phosphorylation sites for PKC in PPARs. It appears that the classical group of PKCs regulates PPARα by direct interaction. Inhibition of PKCs blocks the capability of hepatic cell extracts to phosphorylate PPARα. PKC activity is essential for optimal binding of PPARα on the response elements of its target genes. The presented data imply that the PKC signaling pathway regulates a molecular switch between transactivation and transrepression activity of PPARα.

The next two presentations focused on potential new functions of the liver X receptors (LXRs). The two isoforms, LXRα (NR1H3) and LXRβ (NR2H2), mainly activated by oxysterols, are considered to have pivotal functions in reverse cholesterol transport. This includes cholesterol efflux from peripheral cells through the induction of ABCA1 and apolipoprotein E (APOE) gene expression, with the macrophages being one of the key cell types.

Elena Rigamonti (Lille, France) investigated if LXR can influence intracellular cholesterol trafficking upstream of ABCA1 mediated cholesterol efflux. In human macrophages, activation of LXR can increase the mobilization of free cholesterol to the plasma membrane, as revealed by cholesterol oxidase accessibility analysis and filipin staining. This was associated with elevated mRNA levels of NPC1 and NPC2, two proteins found in the late endosome and to be implicated with Niemann-Pick C (NPC) disease, as well as the cholesterol carrier protein MLN64 (StAR homologue protein). LXR activation could block Tumor Necrosis Factor alpha (TNFα)-induced esterification of cholesterol and reduce ACAT1 activity independently of altered *ACAT1* gene expression. In addition, co-treatment with progesterone, which can induce accumulation of lysosomal cholesterol due to its inhibitory effect of post-lysosomal cholesterol trafficking, restrained ABCA1 facilitated cholesterol efflux mediated by LXR activation. They concluded that LXR could control intracellular cholesterol trafficking in macrophages by enhancing cholesterol transport to the plasma membrane, where it is more acceptable for efflux.

The crucial role of LXR in the regulation of reverse cholesterol was initially investigated in macrophages and hepatocytes. As discussed by Kirsten Robertson (Stockholm, Sweden) an additional essential function of LXR, in particular LXRβ, in maintaining cellular cholesterol homeostasis was found in the Sertoli cells. LXRβ appears to be the dominant transcript in the testis with highest expression in the

Sertoli and Leydig cells. In 2.5 months old LXRα−/−β−/− mice, small lipid droplets were seen within the basal compartment of the Sertoli cells. But the testis remained structurally intact and spermatogenesis progressed normally. At that age, no morphological changes were seen in single LXR KO mice. These lipid-droplets increased in size with age in LXRα−/−β−/− mice. By the age of 20 months, their testes are highly degenerated with only remnants of the tubular structures. The epididymides were completely devoid of maturing sperm, resulting in their highly reduced fertility. The testis revealed sustained elevated mRNA levels of the receptors for low density (LDLR) and very low density (VLDL) lipoprotein particles, by unchanged expression of sterol regulatory element binding protein-2 (SREBP-2). As the cellular uptake of cholesterol from the lipoprotein particles is considered as the major source of cholesterol for the Sertoli cells, a central role of LXR in sensing cholesterol as well as in preventing cholesterol uptake, presumably via an SREBP-2 pathway, might be suggested.

The last step of reverse cholesterol transport is the excretion of excess cholesterol after its conversion into bile acids or within the bile acid pool. Cholesterol-7α-hydroxylase (Cyp7a) encodes the rate-limiting enzyme in the classical pathway of bile acid synthesis. The regulation of its transcription is currently an attractive target for new cholesterol lowering drugs. Bile acids can inhibit its gene expression via FXR due the transcriptional induction of the repressor small heterodimerisation partner (SHP, NR0B2).

Maurizio Crestani (Milano, Italy) evaluated the concept that bile acids, known as crucial endogenous regulators of this gene, can rapidly reduce Cyp7a expression via an FXR/SHP independent mechanism. Bile acids facilitate the transport of histone acetylase 7 (HDAC7) from the cytoplasm to the nucleus. There it promotes the recruitment of SMRTα/N-CoR (silencing mediator of retinoic acid and thyroid hormone receptor alpha/nuclear receptor corepressor) and HDAC3 to form a repressor complex, which subsequently can inhibit the transcriptional activation of Cyp7a1. Administration of valproic acid (VPA), an inhibitor of HDAC, can induce the expression of Cyp7a, presumably by antagonizing the negative feedback loop initiated of endogenous bile acids. Subsequently, wild type and LDLR−/− mice exhibit, when treated with VPA lower circulating plasma cholesterol, mainly that of LDL-cholesterol. Crestani suggested that these results point to a new perspective for the identification of novel drugs for the treatment of hypercholesterolemia.

9. Session VII. Varia: chaired by Marten Hofker (Maastricht, The Netherlands) and Matti Jauhiainen (Helsinki, Finland)

The varia session represented a series of broad topics, including presentations focussed on atherogenesis, bile acid metabolism, and lipid metabolism in brain, bone and in insects.

Tarek Bajari (Vienna, Austria) analyzed the process of lipoprotein uptake by the ovarian follicles of the chicken. In particular, the role of the extracellular matrix was studied by focusing on the role of perlecan. Strikingly, perlecan was absent from the layer between the theca interna and granulosa cells, but is located in the extracellular matrix of the theca externa. Domain II of perlecan, containing 4 LDL-receptor binding repeats, bound to LDL and VLDL. Hence, a role of perlecan in the plasma-to-oocyte transport of lipoproteins was proposed.

Stijn Ghesquire (Maastricht, The Netherlands) studied the effect of high level macrophage specific expression of human phospholipase A2 (sPLA2) on atherogenesis in an LDLR knockout mouse model. For these studies, a transgenic mouse was generated carrying a full-length human *sPLA2* gene driven by the myeloid specific CD11b promoter. The lipoprotein profile is not affected in these mice, and also no other indications of systemic effects were observed. Strikingly, a marked increase in lesion size (2.3×) was observed in the sPLA2 mice, characterized by a pronounced macrophage accumulation and an increase in collagen content.

Paula Jansen (Maastricht, The Netherlands) investigated the relation between serum and brain phytosterol levels. Mice deficient for the plant sterol transporter ATP binding cassette transporter G5 (ABCG5) or G8 (ABCG8) will develop sitosterolemia and have highly elevated plasma phytosterol levels. It was found that brain-phytosterol levels were also markedly (about 10-fold) increased in these mice. Brain cholesterol levels were lowered by 10%. Although apoE-knockout mice also have higher serum levels of phytosterols, they did not show an increase in brain-phytosterol levels.

Torsten Plösch (Groningen, The Netherlands) was the final speaker competing for the young investigator award, and turned out to be this year's winner. He focused on the role of ABCG5 in hepatobiliary cholesterol secretion. ABCG5 and ABCG8 form a heterodimer, which is the putative transporter for cholesterol and plant sterols. Mice lacking ABCG5 show a marked decrease in total sterol and phospholipid output. Upon challenge with hydrophobic (TDCA) and hydrophilic (TUDCA) bile salts, a similar relative increase of phospholipid and cholesterol excretion was observed in wild type and transgenic mice respectively, although at a lower absolute level in the latter. Wildtype mice fed a high cholesterol diet showed a strong induction of ABCG5/G8 but this response was absent in LXRα deficient mice. Interestingly, the LXRα deficient mice showed a similar increase in hepatobiliary cholesterol excretion as wild type mice upon high cholesterol feeding indicating another route for hepatobiliary cholesterol excretion independent of ABCG5.

Kees Rodenburg (Utrecht, The Netherlands) presented data on the function of the insect lipoprotein receptor and how it relates to LDL receptor functioning. The lipoprotein of insects, high-density lipophorin (HDLp), is homologous to that of mammalian low-density lipoprotein with respect to

its apolipoprotein structure. In addition, the endocytic receptor for HDLp has been identified (insect lipophorin receptor, LpR) that is the first insect homologue to the human LDL receptor (LDLR). Upon intemalization, mammalian and insect lipoproteins share endocytic vesicles. In contrast to LDL, which is degraded in lysosomes, HDLp follows a transferrin-like recycling pathway. In human hypercholesterolemia class 5, LDLRs remain in complex with LDL after endocytic uptake but the complex is degraded in lysosomes. Receptor mutants and hybrids between LpR and LDLR were used for their endocytic and recycling characteristics. Most receptor constructs were able to internalize their ligands. However, one lipoprotein binding hybrid receptor was unable to recycle and did not colocalize in endocytic recycling compartment (location of transferrin). Another hybrid receptor behaved similar to wild-type LpR. After ligand endocytosis, the LDL-internalizing mutants and hybrid receptors were not able to recycle. It was suggested that the lysosomal degradation of full-length receptor (i.e., complexed with ligand) needs communication between the intracellular domain with the extracellular EGF-precursor homology domain. The data also suggest that hypercholesterolemic class 5 mutations can be divided in two separate subclasses and the cysteine-repeats are involved bifunctionally in ligand binding/dissociation. Thus, studies on ligand recycling by LpR produces information that can be used to evaluate the (mal) function of LDLR.

The next speaker in the Varia session was Ken Lindstedt (Helsinki, Finland). Locally activated mast cells may participate in the weakening of atherosclerotic plaques by secreting heparin proteoglycans, cytokines and chymase. Chymase has been shown to be involved in death (apoptosis) of arterial smooth muscle cells (SMC) thereby predisposing to plaque erosion or rupture. The mechanism behind the chymase-mediated apoptosis of SMC was the object of this study. Integrin-mediated SMC survival is critically dependent on the NF-κB-mediated expression of bcl-2, an antiapoptotic protein. This study demonstrated by means of RT-PCR, Western blot, EMSA, immunohistochemistry and confocal microscopy that chymase disrupted focal adhesion-dependent NF-κB-facilitated survival signalling. The amount of NF-κB/p65 in SMC declined and nuclear translocation of this complex was eliminated. Also endotoxin (LPS)-induced nuclear targeting of NF-κB/p65 was inhibited by chymase. Bcl-2 mRNA expression was downregulated by chymase, which caused opposite translocation of bcl-2 and bax between mitochondria and cytoplasm. Deficiency of bcl-2 launched apoptosis by activation of caspase 9. The data strongly suggest that chymase-secreting mast cells mediate SMC apoptotic processes through disruption of NF-κB-facilitated outside-in survival signalling pathway and this may enhance atherosclerotic plaque erosion and rupture.

The last speaker in the Varia session was Andreas Niemeier (Hamburg, Germany). Dietary lipids and lipophilic compounds are important for bone formation. Bone mineralization depends on the function of gamma-carboxylated osteocalcin in anabolic osteoblasts. Lipophilic Vitamin K is a crucial factor for the gamma-carboxylation. Very little is known on the mechanisms that facilitate the uptake of lipids to bone. The potential function of the low density lipoprotein receptor-related protein (LRP, or chylomicron remnant receptor) and apoE in the uptake of Vitamin K was investigated as a possible route to the osteoblast and for bone formation. In human osteoblast-like cells expression of LRP1 was demonstrated. In vivo also human osteoblasts on normal bone sections showed positive expression for LRP1. LRP does not only bind chylomicron remnants (CR), but also the lipases that are directly involved in the generation of these remnants. This was also shown here since after Vitamin K incorporation into CR, uptake of Vitamin K was mediated by LRP1 in an apoE- and lipoprotein lipase-dependent fashion. Because LRP is an apoE-binding receptor, a decrease in uptake of CR (and Vitamin K) was demonstrated in osteoblasts isolated from apoE deficient mice. Furthermore, reduced levels of serum gamma-carboxylated osteocalcin forms were detected in apoE knockout mice. Besides their roles in facilitating post-prandial CR uptake by the liver, LRP and apoE are crucial in regulating osteoblast function.

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