European Lipoprotein Club

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Conference Report

European Lipoprotein Club: Report of the 26th ELC Annual Conference, Tutzing, 8–11 September 2003

1. Introduction

The 26th meeting of the European Lipoprotein club was held from 8 to 11 September 2003 at the evangelische akademie in Tutzing and attended by 110 participants from 15 different countries. Dr. J.D. Horton (Dallas, USA) presented the keynote lecture. He discussed the role of sterol regulatory element-binding proteins (SREBPs) in the regulation of cholesterol and fatty acid synthesis and their role in the metabolic syndrome. The first session was focused upon HDL function and regulation, and opened with an overview on regulation of reverse cholesterol transport by Arnold von Eckardstein (Zürich, Switzerland). The next session was focused on lipid metabolism in the brain and was introduced by a lecture from Ingemar Björkhem (Stockholm, Sweden) on "Brain cholesterol—long and intensive secret life behind a barrier". Session III highlighted the state of the art on gene environment action and was opened by Steve Humphries (London) presenting new aspects of smoking interaction with apo E on cardiovascular risk. This was followed by a session on cellular lipid transport. The leading speaker was Bert Groen (Amsterdam, The Netherlands) who updated the audience on the close relationship between reverse cholesterol transport and bile acid metabolism. Inflammation and immune response in atherosclerosis was the topic of session V. Ron Law (Los Angeles, USA) opened this session with a presentation on new targets for PPAR-gamma in the vessel wall. The meeting was closed with the well-known "Varia" session. The young investigator award was this year presented to Arja Kreeft (Leiden, The Netherlands) for her presentation on "identification of dietary response genes in hyperlipidemic mouse models by gene expression profiling.

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

During recent years, the *SREBP*-field has shown considerable progress, in particular due to the numerous contributions of Jay Horton (Dallas, USA). SREBPs function as cholesterol sensors by monitoring the cholesterol content of the membranes of the endoplasmic reticulum. When the intracellular cholesterol level lowers, three proteins are involved in activating SREBP. SREBP cleavage activated protein (SCAP) is the actual cholesterol sensor that will transport SREBP to the golgi apparatus, where it will be cleaved by Site-1 protease (S1P) and Site-2 protease (S2P). Subsequently, activated SREBP migrates to the nucleus where it activates genes through binding to the sterol responsive elements of target genes. Interestingly, three different forms of SREBP exist, i.e. SREBP-1a, SREBP-1c and SREBP-2. A wide range of transgenic models was generated to delineate the precise function of the SREBPs in lipid metabolism. Interestingly, the functions between the different SREBPs differ. It has been observed that in mice, high level expression of SREBP-1a and SREBP-1c leads to activation of the fatty acid metabolism while SREBP-2 preferentially acts on the genes involved in cholesterol synthesis. Transgenic mice expressing activated SREBP-1a develop a massive fatty liver, while SREBP-2 expression leads to a 28-fold increase in cholesterol synthesis. In contrast, by deleting SCAP, the activity of all SREBP activated genes will be down regulated. Recently, direct SREBP target genes have been identified by genome wide expression analysis of the mRNA levels of the livers of these mice (Horton et al., PNAS 2003;100:12027–32). Interestingly, 33 genes were found to be upregulated in the SREBP transgenics and downregulated in the SCAP knock-out mice. Thirteen of these genes were known, and 20 genes were not previously recognized as SREBP target genes. Importantly, 16 of these genes fulfil a role in lipid metabolism, and four of the genes are completely novel. Since these novel genes already meet rigorous selection criteria, it is important to decipher their function and investigate their role in lipid homeostasis.

3. Session I. HDL function and regulation: chaired by Laura Calabresi (Milan, Italy) and Matti Jauhiainen (Helsinki, Finland)

In the beginning of this session Arnold von Eckardstein (Zurich, Switzerland) presented an overview regarding the regulation of *reverse cholesterol transport* process. As is well recognized, low HDL cholesterol is an important risk factor for coronary heart disease (CHD). The function of HDL has been connected with several potentially anti-atherogenic activities: HDL inhibits oxidation of LDL particles, monocyte adhesion to the endothelium, apoptosis of vascular endothelial and smooth muscle cells and platelet activation. On the other hand it stimulates the endothelial

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secretion of vasoactive substances, endothelial nitric oxide synthase (eNOS), and finally mediates the reverse cholesterol transport (RCT) from peripheral cells to the liver. Hence, increasing HDL levels has become an interesting target for anti-atherosclerotic drug therapy. Importantly, the mechanism of HDL modification rather than simply elevating HDL cholesterol determines the anti-atherosclerotic potency of the drug. Levels of HDL and subclass distribution in plasma are regulated on the level of apolipoproteins, lipolytic enzymes, lipid transfer proteins (CETP, PLTP) receptors and cellular transporters. The balanced interplay of these factors contributes to RCT, HDL composition and the anti-atherogenicity of HDL. Regarding the function of apoA-I in RCT, the domains of lipid-free apoA-I that promote cAMP-dependent and cAMP-independent cholesterol and phospholipid efflux were mapped. The cAMP-dependent lipid efflux in J774 macrophages and in HEK293 cells transfected with ABCA1 expression plasmid was decreased by about 80–92% by apoA-I deletion mutant Δ(185–243), 15% by apoA-I Δ(1–41) or apoA-I Δ(1–59). Efflux was restored to 75–80% of the wild-type apoA-I control level by double deletion mutants A-I Δ(1–41)Δ(185–243) and A-I Δ(1–59)Δ(185–243). These results suggest that the central helices of apoA-I can promote ABCA1-facilitated lipid efflux. However, residues 220–231 are necessary to allow the physical/functional interactions between the full-length apoA-I and ABCA1 and the following lipid efflux and HDL maturation. In addition to apoA-I itself other important factors participate in the regulation of RCT. HDL-receptor function (i.e. SR-BI, ABCA1, β-chain of ATP-synthase, etc.) as well as acute/chronic infections affect the efficacy of RCT. Modification of HDL metabolism as a goal to regulate and improve RCT remains an important strategy for the development of novel anti-atherogenic drugs.

Next speaker was Ilaria Zanotti (Parma, Italy). She presented data on the reverse cholesterol transport from *macrophages* to feces in vivo. This approach was applied specifically to investigate the ability of apoA-I overexpression to promote macrophage-specific reverse cholesterol transport. J774 macrophages were loaded with cholesterol using acetyl-LDL, labelled with tritiated cholesterol, and then injected intraperitoneally into C57BL/6 mice. Plasma and feces were collected at 24 and 48 h, mice were decapitated and tissues harvested to measure tracer counts. ^{3}H cholesterol was observed in plasma, liver, and feces. In case of mice overexpressing apoA-I, labelled macrophages were injected 3 days after the intravenous injection of apoA-I adenovirus. ApoA-I overexpression caused a significant increase of ^{3}H cholesterol in plasma, liver, and feces. Tracer level in the liver was 35% higher and that excreted into feces over 48 h 63% higher in apoA-I-expressing mice as compared to control group. A substantial amount of the ^{3}H cholesterol in feces was detected in bile acids, indicating conversion of cholesterol into ^{3}H-bile acid in the liver. In conclusion, injection of the ^{3}H cholesterol-labelled macrophage foam cells into mice is a promising method to evaluate reverse cholesterol transport specifically up to feces in vivo, and the data for the first time demonstrates that apoA-I overexpression significantly promotes this process.

Theo van Berkel (Leiden, The Netherlands) introduced novel data on the effects of *scavenger receptor BI* (*SR-BI*) deficiency on lipid metabolism in arterial wall cells and in the liver. SRBI is a key regulator of HDL metabolism. This receptor facilitates the efflux of cholesterol from peripheral cells to HDL but also mediates the selective uptake of cholesterol esters from large HDL in the liver to be directed to the biosynthetic pathway of bile acids. SR-BI deficient mice and wild type controls were used with a Western-type diet containing 15% fat and 0.25% cholesterol. Massive accumulation of cholesterol-enriched large HDL was observed in SR-BI deficient mice suggesting an impaired removal via the liver. SR-BI deficiency did not affect the hepatic cholesterol (ester) amount or the key factors of hepatic cholesterol homeostasis like HMG-CoA reductase, LDL-receptor and cholesterol 7α-hydroxylase. Biliary cholesterol amount reduced about 40%. Expression of ATP half-transporters, ABCG8 and ABCG5 was attenuated by 70 and 35%, respectively. The situation in the arterial wall was totally different. Massive lipid deposition was obvious in the aorta and atherosclerosis. SR-BI deficiency caused a 72-fold increase in mean atherosclerotic lesion area despite the high HDL levels. SR-BI deficiency led to a significant induction of genes involved in adhesion and transendothelial migration of monocytes including P-selectin, E-selectin and vascular cell adhesion molecule-1. These changes coincided with a two-fold increase in CD68 expression indicating an increase of macrophages within the arterial wall. The results clearly demonstrate that SR-BI deficiency differentially affects lipid metabolism in cells of the liver and those of the arterial wall.

Joerg Heeren (Hamburg, Germany) discussed *apolipoprotein E recycling* and its association with cholesterol efflux and high density lipoprotein internalization. During lipolysis triglyceride-rich remnants become enriched with HDL-derived apoE and are rapidly cleared from plasma compartment in a process known to depend on apoE. After receptor-mediated endocytosis core lipids and apoB are delivered into lysosomes whereas endocytosed apoE is recycled back to the plasma membrane. ApoE is then re-secreted and associated with high density lipoproteins. Radioactive pulse-chase experiments indicated that apoE recycling is accompanied by cholesterol efflux, and confocal imaging revealed that there exists co-localization of apoE and cholesterol in early endosomes characterized by the EE antigen 1 (EEA1). During apoE re-secretion, HDL_3-derived apoA-I is found in these early endosome antigen 1, cholesterol-containing endosomes. Using time-lapse fluorescence microscopic technique the trafficking of apoA-I to pre-existing triglyceride-rich lipoprotein (TRL)-derived apoE and cholesterol-containing early ensosomes was evident in the periphery. After re-secretion process, TRL-derived apoE and cholesterol are detected

in extracellular apoE-HDL (HDL_E) particles in the cell culture medium. These results provide evidence for a new intracellular link between TRL-derived apoE, cholesterol transport in the cell, and HDL metabolism especially HDL_E formation.

Carlos Vrins (Leiden, The Netherlands) presented data on the repression of lipoprotein gene transcription by adenovirus mediated gene transfer of zinc finger protein, *ZNF202*. This protein is a transcriptional repressor that binds to promoter elements predominantly located in genes involved in lipid metabolism. In humans, genetic studies indicate that ZNF202 is functionally linked to hypoalphalipoproteinemia. In this study the mouse ZNF202 gene was cloned from liver cDNA of C57BL/6j mice and Ad-ZNF202 were produced. Hepatoma cell line from murine AT3F2 was infected with adeno-ZNF202 and gene expression of lipid related genes was analyzed by real-time-PCR. Expression of the apoE/CI/CII and apoA-I/CIII/A-IV/AV gene clusters was reduced approximately 50%. Expression of hepatic transcription factors HNF-4 and PPARδ as well as microsomal triglyceride transfer protein (MTP) and 27α-hydroxylase were also reduced. In the case of apoE, a more detailed approach was taken and deletion analysis of the mouse apoE promoter was performed. ZNF202 caused a two-fold reduction in apoE promoter activity that was localized to a region spanning nucleotides −705 and −362. Gel mobility shift analysis confirmed association of ZNF202 within the −705/−362 region. The results clearly confirm the repressive effect of ZNF202 on a variety of essential genes associated with lipid metabolism.

Minna Karkkainen (Helsinki, Finland) discussed the apolipoprotein-mediated *activation of phospholipid transfer protein* (*PLTP*). PLTP in human plasma exists in two forms, one catalytically active (high activity) and one inactive (low activity form). While the low active form is associated with macromolecular complexes containing apoA-I, the high activity form is complexed with apoE, which suggest a possible involvement of the apolipoprotein component in modulating the PLTP activity. It was shown that incubation of low activity PLTP with reconstituted discoidal HDL particles containing apoE converts the inactive form into the catalytically active protein. The three apoE isoforms are all able to convert PLTP to the active form, while reconstituted HDL disc containing apoA-I are ineffective. In addition, using surface plasmon resonance technology (Biacore, Pharmacia), it was demonstrated that recombinant PLTP binds to apoE. These results suggest that the movement of PLTP between different plasma lipoproteins could activate/inactivate the protein. In particular, PLTP could enter the circulation in an active form not bound to apoA-I, being then transferred to apoA-I containing particles, becoming therefore inactive.

Jessica Lie (Rotterdam, The Netherlands) discussed the *role of the two plasma lipid transfer proteins*, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), *in atherogenesis*. Diet-induced atherosclerosis was studied in LDLR ± mice expressing both human CETP and PLTP. Following a high fat, high cholesterol diet, VLDL + LDL cholesterol levels were raised in animals expressing only CETP; the expression of PLTP decreased both HDL and VLDL + LDL cholesterol induced by the diet. Susceptibility to atherosclerosis was increased in animals expressing both the transfer proteins, probably due to the lowering of HDL cholesterol and in spite of the reduced VLDL + LDL cholesterol. The measurement of two antioxidant enzymes carried by HDL, paraoxonase and platelet activating factor acetyl hydrolase, showed a significant reduction in animals expressing CETP and PLTP.

The last two presentations of the session dealt with the non-lipid related antiatherogenic properties of HDL. Jerzy-Roch Nofer (Munster, Germany) discussed the mechanism responsible of the *HDL mediated enhancement of NO availability*. Endothelial NO is generated by a constitutive NO synthase (eNOS), primarily localized to caveolae, which becomes activated in response to multiple stimuli. The incubation of cultured endothelial cells with HDL activates eNOS in a process that involves the binding of apoA-I to the scavenger receptor-BI (SR-BI). The HDL interaction with SR-BI leads to an activation of the Akt, an ubiquitous kinase responsible for the phosphorilation and activation of eNOS. The active HDL components in the activation of eNOS are lysosphingolipids, and in particular sphingosylphosphorylcholine, sphingosine 1-phosphate, and lysosulfatide. Lysosphingolipids are known to interact with "endothelial differential genes" (EDG), a family of heptahelical receptors coupling to several trimeric G proteins, and have been involved in different pleiotropic properties of HDL.

The *cardioprotective effects of synthetic high density lipoproteins* (sHDL) were discussed by Monica Gomaraschi (Milano, Italy). Isolated rat hearts, which underwent a 20 min low-flow ischemia followed by a 30 min reperfusion, were perfused with sHDL, made of phosphatidylcholine and apolipoprotein A-I, during the 10 min immediately before ischemia or in the first 10 min post-ischemia. The administration of sHDL (at the doses of 0.5–2.0 mg of protein/ml) caused a rapid, dose-dependent improvement of post-ischemic cardiac function. The preservation of post-ischemic cardiac function by sHDL was mediated through a reduction of cardiac tumor necrosis factor-α content, and an enhanced cardiac production of prostaglandin E_2 and I_2. This new observation indicates that sHDL may provide a novel therapeutic approach to clinical conditions in which myocardial ischemia/reperfusion occurs.

4. Session II. Lipid metabolism in the brain: chaired by J. Nimpf (Vienna, Austria) and M. Rudling (Stockholm, Sweden)

Ingemar Björkhem (Stockholm, Sweden) opened the session lipid metabolism in the brain. Björkhem, who is a devoted steroid biochemist, initially commenced his research

on regulatory events on bile acid metabolism, an area he is still very active within. During the last 2 decades he has made many contributions on the role of oxysterols as regulators in cholesterol metabolism. Interestingly, in addition to these forms of steroids direct regulatory actions they also function as transporters of cholesterol. The oxysterol, *24-hydroxycholesterol* is generated by the action of CYP 46. Normal human plasma contains about 70 ng/ml and the half-life is 12 h.

It was shown, from studies on humans, that there is an arteriovenous difference of 24-hydroxycholesterol over the brain with about 10% higher venous levels so that there is a net outflow of about 6 mg of 24-hydroxycholesterol daily that is transported from the brain into the blood. The human brain contains 120 g of cholesterol and the turnover of cholesterol is very slow. In rat brain the half-life for 24-hydroxycholesterol is 0.5 days as compared to 3–4 months for brain cholesterol. The significance of the transport of 24-hydroxycholesterol out from the brain was illustrated by the fact that mice deficient of CYP 46 have a 40% reduced synthesis of cholesterol in their brains. In infant plasma the levels are five times higher than adult levels. During the first years there is a gradual decline so that an adult level is established at 4 years age. This probably reflects the great reorganisation of CNS during this period. The levels in brain dead are about 50% of normal and they tend to be slightly lower in patients with Alzheimer disease or CNS infections. The relatively broad interindividual variations do yet not allow for any major diagnostic use of plasma assays of 24-hydroxycholesterol. It was also shown, in studies on humans, that there is a significant difference in the arterio-venous levels over the liver indicating the importance of this organ for the extraction and subsequent removal of 24 hydroxycholesterol from the body. In human liver every day approx. 7 mg of 24-hydroxycholesterol can be further hydroxylated in the 27-position or converted into bile acids in order to leave the body in the stool, again highlighting the key role of the liver for the cholesterol balance in the entire body.

Christian Göritz (Strasbourg, France), who previously has found that cholesterol from *glial cells* promotes synapse formation, showed that purified neurons synthesize cholesterol and that this is strongly suppressed by the presence of glial cells. Endogenous cholesterol can also be released from neurons. Further data were shown indicating that neurons in primary culture can produce A-I. The results support the concept that cholesterol is shuttled between astrocytes and glial cells. However, the specific transport form is yet to be found.

Neil Schachter (New York, USA) presented interesting in vitro data regarding the possible role of *cellular fat stores* (*TG and cholesterol*) *in Alzheimer disease* (AD) development. In AD beta-amyloid accumulates which is determined by beta and gamma secretase cleavage of the amyloid precursor protein. In contrast, a third alpha secretase reduces beta amyloid by direct cleavage. In early AD a mutation in presenilin 1 (PS-1) is common. PS-1 is the major gamma secretase. It was shown that cellular TG and cholesterol loading resulted in increased PS-1 that was due to a reduced degradation of PS-1. An elevated PS-1 should increase gamma-secretase and reduce alpha secretase resulting in increased beta amyloid formation. It was further shown that astrocytes could secrete HDL- and VLDL-like particles that may associate with apoE4 that should more efficiently increase cellular TG levels than apoE3. It remains to be seen to what extent this interesting concept is valid in vivo.

Monique Mulder (Maastricht, The Netherlands) gave a talk on *apolipoprotein E* and its role in maintaining the integrity of the blood–brain barrier. A high-fat diet enhances the permeability of the blood–brain barrier in apoE-knockout mice but not in control mice. Besides developing severe atherosclerosis, these mice are less efficient in acquiring the spatial Morris water maze task. Since apoE3-Leiden-transgenic mice, which also develop severe atherosclerosis, do not show disturbed blood–brain barrier function, atherosclerosis could be ruled out as direct cause for these disturbances.

In the next talk, Anita van den Hoek (Leiden, The Netherlands) presented a report on the effect of *neuropetide Y* (*NPY*) on liver-specific insulin resistance in mice. NPY stimulates food intake and decreases energy expenditure and it has been suggested to play a role in the pathogenesis of obesity and diabetes. To clarify NPYs role in diabetes, mice were treated with NPY and glucose, glycerol, and free fatty acid turnover and VLDL production were measured. The results of these experiments showed that NPY causes liver-specific insulin resistance with respect to VLDL and glucose metabolism, independent of food intake and weight gain.

The session was closed with a contribution from Johannes Nimpf (Vienna, Austria). He presented data on the molecular mechanism of the *Reelin-mediated signal transduction pathway* in neurons. Reelin, which is secreted by Cajal-Retzius cells acts as guidance cue for migrating neurons in the developing central nervous system. The Reelin signal is relayed into target neurons via ApoER2 and VLDL receptor, two members of the LDL receptor family. Binding of Reelin to the receptors leads to phosphorylation of Dab1 an intracellular adaptor molecule that binds to the cytoplasmic domains of ApoER2 and VLDLR. Data presented here demonstrate that the primary effect of the Reelin/receptor interaction is the dimerization of Dab1 that leads to its phosphorylation. In contrary to the proposed theory, this event seems to be independent of any co-receptor.

5. Session III. Gene environment interaction: chaired by Philippa Talmud (London, UK) and Vincent Mooser (Philadelphia, USA)

Steve Humphries (London, UK) opened the session on gene:environment interactions by outlining the *basis of gene:environment interactions*, namely that predisposing

gene variants are risk-associated only in the presence of environmental stress, and illustrated this with an example of smoking:genotype interaction. While smoking roughly doubles life-time risk of CHD, the question explored was whether the increased CHD risk of *ApoE* ε4 allele was modulated by smoking. This was convincingly shown in a large prospective study of over 2700 healthy UK men, where, compared to non-smokers of all genotypes, (hazard ratio set at 1), ε3 homozyotes who smoked had a hazard ratio of 1.68, reflecting the effect of smoking on risk, but ε4 carriers who smoked had a 3.17 risk of CHD. This ε4:smoking interaction may reflect the reduced anti-oxidant activity of apoE4 as measured by total antioxidant activity (TAOS). When genotype and environmental interactions were added to a ROC curve, derived from the PROCAM risk algorithm, they showed an increase in the overall risk prediction, while genotypes alone did not. A second aspect of gene:environment interaction studies, the so-called 'stress the genotype' approach, was illustrated with the effect of angiotensin converting enzyme (*ACE*) genotype on the change in left ventricular mass (LVM), assessed by magnetic resonance imaging, in army recruits after a 10 week training period. Men homozygous for the *ACE* deletion allele could not maintain LVM homeostasis during training and showed a significant increase in LVM compared to those homozygous for the *ACE* insertion allele. Furthermore, the interaction of the bradikinin 2 receptor promoter variant with *ACE* genotype on LVM confirmed that the likely role of the *ACE*:LVM association was via the kinin pathway. These 'observational' and 'stress the genotype' gene:environment studies provide novel pathophysiological insights into CHD.

Martin Hersberger (Zurich, Switzerland) addressed the question whether maleness was a risk factor for CHD by examining *genetic variation in the androgen receptor*. The numbers of the CAG repeats in exon 1 of the androgen receptor (AR) correlated significantly with HDL-cholesterol levels in men. This effect was independent of known confounding factors in a multiple regression model. Despite the negative effect of the AR alleles with lower repeat numbers on HDL-cholesterol levels, there was no association of AR repeat numbers with the presence of CHD in a small case-control study ($n = 346$). These data argue against an important role of AR mediated regulation for the male gender disadvantage in cardiovascular risk.

Evelyn Orso (Regensburg, Germany) presented novel data regarding *cellular functions of apolipoprotein A-IV* (apoA-IV) in small intestinal mucosa. There is a direct correlation between the number of differentiated epithelial cells involved in apical/lumenal lipid absorption and basolateral lipoprotein secretion and the formation of junctional complexes (e.g. tight- and adherens junctions). By using a yeast-two-hybrid screening approach apoA-IV was identified as an interacting protein with α-catenin (component of adherens junctions) as well as with cyclin C (involved in the control of RNA polymerase II holoenzyme and in the expression of mitotic cyclins), linking apoA-IV to adherens junctions and cell cycle modulation. Overexpression of apoA-IV in Caco-2 and HT-29 intestinal cell lines was paralleled with (1) a more differentiated phenotype of adherens junctions, (2) a cell cycle arrest in the S-phase and (3) an up-regulation of a set of ATP-binding cassette transporters involved in basolateral transport functions, thus suggesting a modulatory role for apoA-IV in junctional control, cell cycle regulation of mucosal cells and in the modulation of intestinal (lipoprotein) secretion.

In order to identify novel candidate genes and regulatory pathways involved in the *cholesterol raising effect of cafestol*, Arja Kreeft (Leiden, The Netherlands), who won the young investigator award, used gene expression profiling to identify novel candidate genes stimulated by cafestol. Cafestol has been identified as the cholesterol-raising factor in coffee. The cholesterol-raising effect of cafestol is pronounced in transgenic APOE3Leiden (E3L) mice, a model for hyperlipidemia. RNA from livers of E3L mice fed a moderate high fat diet (control group) and those fed a moderate high fat diet plus 0.04% cafestol (treatment group) for 4 weeks were hybridised onto cDNA microarrays (GEMs 2.03, Incyte Genomics). The relative expression of ∼9900 genes/ESTs was studied in response to cafestol. A total of 648 transcripts were identified whose expression changed in at least one mouse as compared to the control group using a *z*-test ($P < 1 \times 10^{-6}$). Annotation of these genes using the Gene Ontology database showed that major processes were strongly affected by cafestol, namely lipid metabolism, detoxification processes, the immune response and amino acid metabolism. Of the 648 genes, 23 genes were highly regulated in all four mice. An interesting observation was that one of these genes was the FXR target gene cholesterol 7α-hydroxylase (*Cyp7a1*), a key enzyme involved in the conversion of cholesterol to bile acids in the liver. Currently, ongoing studies are being performed in E3L mice to reveal more insight in the molecular mechanism underlying the cholesterol raising effect of cafestol.

The *ATP binding cassette* (*ABC*) *transporters ABCG5 and ABCG8* play an important role in regulating intestinal plant sterol absorption. Mutations in these transporters cause the rare inheritable disease sitosterolemia. Jogchum Plat (Maastricht, The Netherlands) reported on the significant association of a common ABCG8 variant T400K with cholesterol-standardised serum campesterol and sitosterol concentrations in 112 normo-cholesterolemic healthy subjects. Interactions between the ABCG8 T400K genotype with changes in serum plant sterol concentrations were evaluated after a daily consumption of 3.8–4.0 g plant stanols, which lower plant sterol levels. The reduction in TT subjects was significantly greater as compared to that of carriers of the K, with no significant associations with serum total or LDL cholesterol levels. Thus genetic variation in ABCG8 not only explains cross-sectional differences in serum plant sterol concentrations, but also determines a subject's responsiveness to changes in serum plant sterols during interven-

tions known to affect plant sterol metabolism. This finding may be relevant for drug treatment regimes known to affect serum plant sterol concentrations such as for example statins, ezetimibe, etc.

6. Session IV. Cellular lipid transporters: chaired by Geesje Dallinga-Thie (Utrecht, The Netherlands) and Arnold von Eckardstein (Zürich, Switzerland)

This session was opened by Bert Groen (Amsterdam, The Netherlands) with a lecture entitled 'The ins and outs of reverse cholesterol transport'. Reverse cholesterol transport has been classically defined as the process whereby HDL acts to take up excess cholesterol from the peripheral tissues into the liver for removal from the body via secretion into the bile. The liver is the major principle organ for cholesterol synthesis in the body, and the amount of cholesterol synthesized varies widely among different species. HDL is the major carrier for cholesterol in rodents. However, with the use of knock-out mouse models for genes involved in reverse cholesterol transport like apoA-I and ABCAI, new models can be generated that lack HDL as a major carrier for cholesterol. The ABCAI knock out mice resembles the phenotype seen in patients with Tangier disease, making it possible to investigate the role of HDL in reverse cholesterol. It turned out that in the absence of HDL reverse cholesterol was unchanged. Apparently, other as yet unidentified cholesterol carriers can take over and ABCA1 activity does not play a role in overall reverse cholesterol transport. In vitro studies with fibroblasts from patients with low HDL levels revealed evidence for a role of a-specific non-ABCAI-mediated cholesterol efflux to HDL particles, thereby enabling reverse cholesterol transport in the absence of a functional ABCA-I transporter. Although ABCA-I may not be important for bulk reverse cholesterol transport it is important in macrophages. Its absence induces foam cell formation, thereby contributing to the initiation of atherosclerotic disease.

Cholesterol excretion from the body occurs via secretion into the bile. This pathway is under control of several ABC half-transporters. The two halftransporters ABCG5 and ABCG8 form a heterodimer and mediate cholesterol transport into the bile, whereas ABCB4 (MDR2) is involved in transport of phosphatidylcholine, and ABCB11 is responsible for bile salt export. The molecular mechanism by means of which cholesterol secretion is regulated is not yet clear. From genetically engineered mouse models, the indications are that ABCG5/G8 may primarily promote an activated state of cholesterol so that acceptors like mixed bile salt-phospholipids micelles can easily pick it up. However, floppase activity by ABCG5/G8 cannot be excluded. Both halftransporters are also expressed in the small intestine where they transport both plantsterols and cholesterol from the enterocytes into the lumen and thus play an important role in regulation of reverse cholesterol transport. Indeed, activation of the transporters by agonists of the Liver X receptor strongly stimulates cholesterol excretion in the feces.

Next, Torsten Plösch (Groningen, The Netherlands) presented the results of his study on the role of the ABC-half-transporter ABCG5 in sitosterolemia in mice. The question he asked was whether deficiency of ABCG5 alone is sufficient to cause sitosterolemia in mice. To test this hypothesis ABCG5 knock-out mice were generated. Lack of ABCG5 resulted in elevated plasma levels of campesterol and sitosterol, and reduced plasma cholesterol concentrations. Furthermore the plant sterols amounted up to 40% of the liver sterol content. Treatment of the knock-out mice with a synthetic agonist of the LXR receptor resulted in even more elevated plasma sterol levels, whereas biliary sterol secretion was not affected, thereby illustrating the important role of ABCG5 in the intestine. In contrast, in the ABCA1 knock-out mice no plant sterols were detected in plasma, suggesting a role for ABCA1 in facilitating the uptake of plant sterols.

Tina Rubic (Munchen, Germany) presented data on the effect of niacin on the transcription of genes involved in reversed cholesterol transport. Niacin has been reported to lower plasma LDL and increase HDL levels by inhibition of hepatic VLDL secretion. However, it is also known that niacin promotes prostaglandin D_2 formation in monocytes, thereby mediating an anti-inflammatory response by transcriptional regulation of PPARγ-mediated genes. Niacin significantly stimulated translocation and transcription of PPARγ, CD36, and ABCA1 in monocytes, hepatic and endothelial cells, whereas the transcription of the LDL receptor was not affected. Furthermore niacin significantly reduced cellular cholesterol content and enhanced HDL-mediated cholesterol efflux from the cells. The effect of niacin on CD36 expression could be blocked by acetylsalicylic acid, revealing a role for PGD_2/15d-PGJ_2 mediated-pathway, whereas the effect on ABCAI was blocked by a protein kinase A inhibitor, suggesting a cAMP-dependent dependent route. These data are in favor of a role of niacin in promoting reverse cholesterol transport at the cellular level thereby stimulating regression of atherosclerotic disease.

The liver consists of three major different cell types, namely parenchymal, endothelial, and Kupffer cells. It is not known whether they differ in their regulation of uptake, processing and excretion of cholesterol. Hoekstra (Leiden, The Netherlands) used realtime PCR to quantify the mRNA expression of ABC transporters and genes involved in the regulation of cholesterol metabolism in rat liver. They found that liver parenchymal cells, as compared to endothelial and Kupffer cells express higher levels of SR-BI (~three-fold), PPARalpha and PPARgamma (8–20 fold), CYP7A1 (>100 fold) and ABCG5/G8 (~5 fold). Liver endothelial cells show a high expression of CYP27, LXRbeta, PPARdelta and ABCG1. In Kupffer cells, the expression levels of LXRalpha, ABCA1 and in particular ABCG1 were high. A 70-fold higher expression of ABCG1 in Kupffer cells than

in parenchymal cells suggests a major role for ABCG1 in these cells. Upon a high cholesterol diet, both ABCA1 and SR-BI levels are downregulated in parenchymal cells but up-regulated in Kupffer and endothelial cells. In contrast, ABCG1 expression is upregulated in parenchymal cells only. The authors conclude that the cellular localization should be taken into account for a proper interpretation of metabolic responses to changes in hepatic gene expression.

ABCA1 primarily facilitates the release of cholesterol and choline-phospholipis to apoA-I. Previous data suggest that *ABCA1* is not an active transporter but may function as a regulator protein. This is in analogy to the cystic fibrosis transmembrane conductance regulator (CFTR) and the sulfonylurea receptor 1 (SUR1) which, like ABCA1, contain classical PDZ binding domains that associate with PDZ domain proteins. For CFTR it was previously shown that it directly interacts with syntaxin 1A and that overexpression of syntaxin 13 blocks trafficking of CFTR through the early secretory pathway. The similarity of ABCA1 and CFTR interactive proteins prompted Maa Bared (Regensburg, Germany) to investigate whether syntaxins may be important in ABCA1 shuttling in endocytic vesicles. They investigated both the mRNA and protein expression of all 15 members of the syntaxin family in human monocytes, in-vitro differentiated macrophages, in macrophages incubated with atherogenic enzymatically modified LDL (E-LDL) for 24 h and in cholesterol loaded cells subsequently treated with HDL for 24 h to allow cholesterol efflux. Incubation with E-LDL induced syntaxin 3, 6 and 13 and subsequent addition of HDL reversed this upregulation for syntaxin 3 and 13. ABCA1 protein is induced by sterols and E-LDL also induced syntaxin 3, 6 and 13 macrophages. Immunoprecipitation revealed that only syntaxin 13 directly interacts with ABCAI, syntaxin 3 and 6 were not found associated with ABCAI in macrophages or foam cells. ABCA1 and syntaxin 13 were also co-localized in Lubrol WX insoluble lipid rafts which reside in the plasmamembrane and in phagosomal membranes. Finally, evidence was presented which shows the involvement of syntaxin 13 in ABCA1 processing through the early secretory pathway, the raft compartment and maturing phagosomes in the non-clathrin adapter protein-3 (AP-3) and AP-4 compartment. Thus, the interaction of syntaxin 13 and ABCA1 resembles to the one already shown between syntaxin 1A and CFTR.

Chylomicron retention disease (CMRD), Anderson disease and CMRD with the neuromuscular disorder Marinesco-Sjogren syndrome are three inherited disorders of severe fat malabsorption, all associated with failure to thrive in infancy. The conditions are characterized by deficiency in fat-soluble vitamins, low blood cholesterol levels and a selective absence of chylomicrons from blood. Affected individuals accumulate chylomicron-like particles in membrane-bound compartments of enterocytes, which contain large cytosolic lipid droplets. The distinction between CMRD and Anderson disease was derived from differences in the partitioning of lipid between membrane and cytoplasmic compartments. Reasoning that CMRD, Anderson disease and CMRD-MSS have a common genetic basis with phenotypic variation arising from mutational heterogeneity, Carol Shoulders (London, UK) carried out a genome-wide screen in six families. A locus on chromosome 5q31.I segregated with affected status in six affected families. *SARA2*, belonging to the SarI-ADP-ribosylation factor family of small GTPases, which govern the intracellular trafficking of proteins in COP-coated vesicles, was identified in the region of apparent homozygosity. Eight mutations in SARA2 were finally associated with these three severe disorders of fat malabsorption. The Sar I family of proteins initiates the intracellular transport of proteins in COPII (coat protein)-coated vesicles. These data suggest that chylomicrons, which vastly exceed the size of typical COPII vesicles, are selectively recruited by the COPII machinery for transport through the secretory pathways of the cell.

Neutral lipids are stored within cells in the form of cytoplasmic lipid droplets. Triglycerides (TG) represent the storage form for energy in mammals, whereas cholesteryl esters (CE) serve as storage molecules for cholesterol. *Adipocytes* contain, in addition to large quantities of TG, considerable amounts of cholesterol. Steyrer (Graz, Austria) have previously found that in murine 3T3-442-A cells the amount of CE remains constant during the differentiation process, whereas the amount of unesterified cholesterol (FC) increases concommitantly with the TG content of the cells. They now present data on the existence of TG-free CE-droplets (CED) within adipocytes. In contrast to TG-filled droplets (TGD), the CED membrane lipids had a higher ratio of SM:PC. Also the fatty acid composition of the phospholipid moiety of CED and TGD differed. Using biosynthetic tracer studies CE of CED was found to originate preferentially from endogenous FC synthesis, but not from extracellular lipoprotein sources. Western blot experiments revealed only a partial structural similarity in the protein cages of CED and TGD. Both CED as well as TGD harbour BiP, a lumenal ER protein, and Ribophorin, an integral ER protein. Calnexin, another integral ER protein, and Vimentin were not detectable in the CED protein cage, but were present in TGD. Notably, DGAT-2 was identified exclusively in TGD, but not in CED. In summary the results indicate the presence of distinct storage droplets for CE and TG within adipocytes and imply that the two lipid storage entities might originate from distinct sites of the ER.

7. Session V. Inflammation and immune response in atherosclerosis: chaired by Bart Staels (Lille, France) and Marten Hofker (Maastricht, The Netherlands)

Ron Law (Los Angeles, USA) started his presentation by introducing the metabolic syndrome as a syndrome predisposing to type 2 diabetes and cardiovascular disease. Upon coronary artery bypass grafting (CABG), patients suffering from type 2 diabetes are at increased the risk for

restenosis. *PPARgamma* is a transcription factor belonging to the nuclear receptor superfamily that modulates peripheral insulin resistance and influences vascular function. PPARgamma acts by regulating target genes involved in these (patho)physiological processes. Ron Law discussed the effects of PPARgamma agonists on restenosis. In rats, PPARgamma expression is induced in the neointima developing after balloon injury. Treatment with thiazolidinedione (TZD) PPARgamma agonists inhibited this hyperplasia and restenosis after balloon injury. TZDs are effective irrespective of existing insulin resistance, indicating that they may exert direct vascular effects. TZDs inhibit smooth muscle cell (SMC) proliferation by preventing SMC entry into the cell cycle at the G1 → S phase. TZDs inhibit phosphorylation of the retinoblastoma protein, thus resulting in the sequestering of E2F, rendering it inactive. One mechanism is via maintenance of elevated levels of the cyclin-dependent kinase inhibitor (CDKI) p27, via decreased protein expression of minichromosome maintenance proteins (MCM) 6&7, which are involved in the initiation of DNA replication at the beginning of the S phase.

Moreover, PPARgamma activation promotes caspase-mediated apoptosis of SMC via the induction of GADD (Growth Arrest and DNA Damage Gene) 45, Egr1 and p21. GADD45 activates the Jun kinase (JNK) pathways leading to apoptosis induction. PPAR enhances Oct1 protein binding to the GADD45 promoter.

Finally, Ron Law discussed data from preclinical animal models demonstrated that TZDs decrease atherosclerotic lesion size by inhibiting macrophage accumulation in LDLR −/− mice as well as in angiotensin II-induced high fat-fed male LDL-R −/− mice, a model of more advanced lesions. One possible mechanism that was proposed by Ron Law, is the inhibition of Egr1, a pro-inflammatory transcription factor expressed in atherosclerotic lesions.

In conclusion, PPARgamma provides exciting opportunities to develop compounds useful to confer vascular protection in Type 2 diabetes mellitus. Novel pharmacological developments are non-TZD partial agonists that have less metabolic effects, but are more active on vascular function.

Menno de Winter (Maastricht, The Netherlands) started his presentation by discussing the pro-inflammatory *NF-kappaB pathway*, that has been shown to be activated in atherosclerotic lesions. The NFkappaB transcription factor complex regulates >100 genes, several of which are implicated in atherogenesis. Activation of the IKK complex leads to IkappaB phosphorylation and release of the p50 and p65 subunits of NF B that move into the nucleus. p50 homodimers can exert anti-inflammatory actions by blocking p50/p65 heterodimer formation.

Menno de Winter evaluated the phenotype of LDL-R−/− mice that were transplanted with bone marrow from mice deficient in NFkappaB1 that codes for the p50 subunit. p50−/− bone marrow transplanted mice had approximately 50% lower atherosclerotic lesion areas, but the lesions displayed a severe inflammatory phenotype characterized by increased levels of T and B cells with presence of secondary lymphoid structures indicative of severe inflammation. Macrophages isolated from p50 deficient mice produced higher amounts of TNFalpha, displayed normal oxLDL uptake under basal conditions, but much lower LPS-induced oxLDL uptake due to repressed SRA expression.

A general discussion resulting from this work was whether a smaller lesion but with an inflammatory phenotype should be considered good or bad.

Johan Kuiper (Leiden, The Netherlands) discussed the functions of the *Interleukin 9* (IL-9), a T_{H2}-type cytokine member of the IL-2 family. IL-9 acts as a growth factor that activates the JAK-STAT pathway. It has been implicated in the pathogenesis of asthma, a T_{H2}-type disease, and protects against intestinal parasites as well as against septic shock by suppressing TNFalpha, IFNgamma and IL-12 and inducing IL-10.Atherosclerosis is an associated inflammatory disease, characterized by a T_{H1}-type unbalanced immune response. Johan Kuiper studied a possible role for IL-9 in the atherosclerotic process. LDLR−/− mice fed a Western diet and in which atherosclerosis was induced by a collar were treated daily with IL-9. IL-9 did not influence plasma cholesterol levels, but decreased atherosclerotic lesion size without changing plaque composition. To study a role for endogenous IL-9, mice were vaccinated with an IL-9/ovalbumin complex leading to the induction of IL-9 blocking antibodies. In the same collar-induced atherosclerosis model, IL-9 vaccination led to a >2 fold increase in atherosclerosis. Mechanistic studies suggested that IL-9 may act by inhibiting LPS-induced VCAM1, ICAM1, P-selectin, E-selectin and MCP1 expression, suggesting that IL-9 may prevent monocyte recruitment to the arterial wall. These data indicate that IL-9 may be an anti-atherogenic cytokine.

Sepsis is an exaggerated response to infections generally provoked by bacteria. No effective cure is currently available. Lipopolysaccharide (LPS) is a major pathogenic component of Gram negative bacteria, which activates cells via the TLR4 receptor leading to an inflammatory response. LPS binds to and is neutralized by lipoproteins. Patrick Rensen (Leiden, The Netherlands) discussed the role of apo E as a protein participating in the reorientation of LPS from macrophages to hepatocytes. Apo E may thus be a physiological mediator of the response to LPS. Apo E was shown to bind to different LPS serotypes from Salmonella Minnesota. Moreover, apo E prevented the LPS-induced TNFalpha response in vivo. Similar effects were observed on IL-1beta and IL-6. Apo E protected against septic death in D-gal sensitised mice. Moreover, apo E−/− mice exhibited an exacerbated response to LPS. LPS treatment resulted in a rapid increase in plasma apo E concentrations, mainly in the HDL fraction. Interestingly, plasma apo E levels were significantly higher in patients that survived sepsis in which it was associated with HDL.

These observations suggest that apo E plays a role in the defense against septic shock. Since the TLR4 receptor is present in atherosclerotic lesions, the inhibition

of LPS activity by apo E may also be of relevance for atherogenesis.

Karl Lackner (Mainz, Germany), discussed the role of oxidative stress in atherogenesis. Glutathion peroxidases convert H_2O_2 to H_2O and lipid peroxides to corresponding alcohols, and inactivate peroxynitrite. Patients with CAD of the Atherogene population ($n = 636$) were followed for an average of 4.6 years. Individuals that underwent a cardiovascular event displayed lower glutathion peroxidase-1 (Gpx-1) levels in red blood cells (RBC). Patients in the lowest quartile of plasma Gpx-1 were at an approximately three-fold increased risk to develop cardiovascular events compared to the highest quartile. After adjustment for known risk factors, the association of GPx-1 with CAD risk did not change appreciably. Thus RBC GPx1 activity is a strong, independent determinant of future cardiovascular events. The operative mechanisms are presently unknown, but may include alterations in LDL oxidation and endothelial function. Finally, GPx-1 inhibits 5-lipoxygenase activity in monocytes, an enzyme which has been implicated in atherogenesis recently by several research groups.

The mechanisms of plaque rupture/stability are not yet well defined. Oxidized LDL-specific antibodies locate to atherosclerotic lesions and quantification of radiolabelled *MDA2 antibodies* provides a measure of atherosclerotic lesions as demonstrated by non-invasive imaging in WHHL vs NZW rabbits. Dr. Torzewski (Mainz, Germany) addressed the question whether MDA2 antibody uptake in plaques correlated with markers of atherosclerosis in LDL-R−/− mice and WHHL rabbits. A perfect concordance between plaque progression and antibody uptake was observed, whereas this correlation was not absolute in the regression group of LDL-R−/− mice. Reduced MDA2 uptake was accompanied by enhanced collagen and SMC staining. In rabbits, lesions yielding weaker staining also appeared as more stable plaques. Ox-LDL antibodies thus preferentially detect lipid-rich, rather than collagen/SMC-rich plaques and may provide a way to quantitate unstable plaques.

8. Session VI: chaired by Fredrik Karpe (Oxford, UK) and Folkert Kuipers (Groningen, The Netherlands)

The role of *apolipoprotein-CI* (*apoCI*) is unclear and Berbee (Leiden, The Netherlands) showed data from the combination of transgenic mouse models and other in vivo experiments which demonstrated that apoCI has a direct antilipolytic effect. Mice transgenic for human apoC were overtly hypertriglyceridaemic. It has previously been suggested that another significant role of apoCI is to modulate apoE-mediated remnant particle removal, but this effect was ruled out by studying the phenotype in apoE knockout mice made transgenic for apoCI; these mice were still overtly hypertriglyceridaemic. Whereas the post-heparin lipoprotein lipase (LPL) activity was unaltered in the apoCI transgenic mice, apoCI appeared to inhibit LPL directly. This was verified by a three-fold decrease in the removal rate of [^{3}H]triglyceride emulsions injected intravenously into apoCI transgenic mice and confirmed by other in vitro studies. These experiments clearly demonstrate an interaction between apoCI and LPL, but it is still unclear if alterations of apoCI plasma or lipoprotein concentration within a physiological range are major determinants of lipolysis.

Joost Luiken (Maastricht, The Netherlands) presented data on molecular *transport mechanisms of glucose and fatty acids into cardiomyocytes* by the dedicated glucose transporter GLUT4 and the fatty acid transporter CD36. Insulin translocates GLUT4 and CD36 through similar mechanism whereas the translocation of CD36 upon muscle contraction is mediated through a distinct mechanism. Obese Zucker rats showed sarcolemmal abundance of CD36 which did not readily recycle upon stimulation. These data offer a molecular mechanism for the cardiac substrate preference in diabetic states.

Mitro (Milano, Italy) showed data on the transcriptional regulation of *CYP7AI* gene encoding for the rate-limiting step in bile acid synthesis, i.e. 7alpha-hydroxylase. One might anticipate an overwhelming role of FXR, the nuclear bile acid receptor, but the regulation of CYP7AI appears to be more complex. Bile acids impaired recruitment of PGC-1 and CREB-binding proteins, which are cofactors in the PPARgamma activation. Interestingly, PEPCK, which is a model gene for PPARgamma, was also repressed. PEPCK is key in gluconeogenesis. This may suggest a novel fed-to-fasted transcriptional cycle of transcriptional regulation, but the implication of bile acids in the regulation glucose homeostasis remains to be established.

Groenendijk (Leiden, The Netherlands) have studied the effect of a novel class of *bile acid reabsorption inhibitors* (*BARIs*) in the apoE*3Leiden transgenic mouse model. Reduction in bile acid reabsorption increased the fecal output of bile acids and neutral sterols. The plasma cholesterol concentration showed an expected reduction and apoB production was reduced. When conventional methods are used to interfere with bile acid reabsorption, the plasma triglyceride concentration normally increases, however, this was not observed using these novel BARIs. The mechanism behind this potentially advantageous hypolipidaemic profile is still unknown.

The bile acid-activated farnesoid X-receptor (FXR, NR1H4) controls expression of several genes considered crucial in the maintenance of bile acid homeostasis. Kuipers and co-workers (*Groningen*) have evaluated the consequences of FXR-deficiency on bile formation and on the kinetics of cholic acid, the major primary bile acid in mice, using a novel stable isotope dilution procedure. Kinetic parameters were related to the expression of relevant bile acid transporters in liver and intestine. It was found that cholate synthesis (+85%) and cholate pool size (+67%) were increased in FXR(−/−) mice, coinciding with a 2.5 fold increase in cholesterol 7*alpha* hydroxylase (*Cyp7A1*) expression. Surprisingly, despite a complete absence of

ileal bile acid-binding protein (Ibabp) mRNA and protein, fractional turnover rate and cycling time of the cholate pool were not affected and the total amount of cholate absorbed from the intestine was increased. Thus, absence of FXR is associated with defective feedback inhibition of hepatic bile salts synthesis; the absence of Ibabp does not negatively interfere with the enterohepatic circulation of bile acids.

The recently discovered *apolipoprotein AV* has a role in the control of plasma triglyceride levels, but mechanisms of action are unclear. Two groups idependently addressed this issue using different experimental approaches. Willems van Dijk and co-workers (Leiden, Amsterdam) overexpressed the human apo AV in hyperlipidemic mouse models using adenovirus mediated gene transfer. Injection of adenovirus encoding the human apoAV into hyperlipidemic APOE2 knock-in mice resulted in a decrease by 80% of plasma triglyceride levels and a concommitant reduction of plasma cholesterol levels. Similar results were obtained in APOE3Leiden transgenic mice. No change in hepatic VLDL-triglyceride production was observed in APOAV expressing mice, but apoAV significantly enhanced the rate of LPL-mediated triglyceride hydrolysis. It was concluded that apoAV stimulates the lipolytic conversion of triglyceride-rich lipoproteins to stimulate remnant formation which would explain the finding that apo AV overexpression reduces both triglyceride and cholesterol concentrations in hyperlipidemic mice.

Merkel (Hamburg, Germany) reached a similar conclusion, based on their studies employing APO AV transgenic mice. These mice displayed decreased total and VLDL-triglyceride concentrations in their plasma when fed a chow-diet. Plasma decay of i.v. administered radiolabeled chylomicrons was substantially faster in the APO AV transgenics and their uptake in liver and adipose tissue was increased. Hepatic VLDL-triglyceride production was moderately increased in the transgenic mice. It was concluded that apo AV decreases plasma triglyceride concentrations by accelerating chylomicron catabolism rather than decreasing VLDL production.

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For information about the preliminary program and abstract forms please contact, Prof. Dr. Marten Hofker, European Lipoprotein Club secretary, and please visit the website of the ELC: http://www.elc-tutzing.org.

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