Meeting Summary

The 19th Annual Meeting of the European Lipoprotein Club

Anton F.H. Stalenhoef, Katriina Aalto-Setälä, Victor W. Armstrong, Pascale Benlian, Hans Dieplinger, Steve Humphries, Armin Steinmetz

The European Lipoprotein Club met September 9 to 12, 1996, in Tutzing, Germany. There were 99 participants from 13 European countries and the United States.

Dr Jonathan C. Fox from the University of Pennsylvania, Philadelphia, opened the meeting with a state-of-the-art lecture entitled "Cardiovascular Gene Therapy: Current Concepts." Gene therapy constitutes a novel therapeutic approach to selected cardiovascular diseases and is designed to augment traditional techniques of diagnosis and treatment by targeting the molecular mechanisms of disease.

Dr Fox first presented the general strategies driving the development of gene therapy. In the case of deleterious gene mutations causing disease, the defective gene can be replaced with a normal copy. In a pathophysiologic process that can be alleviated through augmented gene function, a beneficial gene can be overexpressed or a mutated "designer" gene, which is either more active or in some way more beneficial than its natural precursor, can be expressed. Normal genes may also confer therapeutic benefits by virtue of their expression in a novel environment. The expression of harmful genes can also be inhibited by antisense oligonucleotides, antisense RNA expression vectors, ribozymes, or triplex DNA. Dominant negative genes can be expressed, eg, encoding a protein that binds to regulatory partners but is not active. So-called decoy DNA, made up of short segments of double-stranded DNA encoding the recognition sequence for regulatory proteins such as transcription factors, can be delivered to cells, binding the transcription factors and, thereby, competing for their biologic targets and inhibiting their function.

The requirements for successful gene therapy are fourfold. The first of these is the identification of an appropriate molecular target, one that is critical to the development or progression of the disease. The second is the use of an efficient gene transfer vector system, which increasingly takes advantage of unique properties of the target cell or tissue. The third requirement is use of an effective local delivery approach. Finally, and perhaps most important in the final analysis, gene therapy for any disease should confer significant benefit that is both safe and long-lasting. These requirements are often woven together, in that the choice of molecular target implies the cell type or tissue to be treated, and these, in turn, dictate the gene transfer vector system and local delivery technique to be used. With regard to overall efficacy and safety, good animal models are critical to designing and testing potential therapies.

Dr Fox described many vector systems used today. Unfortunately, none of the present systems are flawless, and each has some unique advantages and disadvantages. Plasmid DNA (either naked or in liposomes) have been used in some of the earliest in vivo gene transfer experiments. Plasmid DNA uptake is through inefficient, nonspecific mechanisms, and chromosomal integration, which can only occur in dividing cells and is required for persistent activity, occurs at random sites in the target genome, implying the risk of insertional mutagenesis. The advantages of plasmid DNA include low toxicity, ease of preparation, and absent inflammatory reaction. Because of low efficiency and the need for a dividing target cell, this vector system has been largely abandoned. An exception is the use of plasmid DNA gene transfer of vascular endothelial cell growth factor for arterial insufficiency in peripheral vascular disease, the objective of a phase I clinical trial in the United States. Retroviruses are more efficient gene transfer vectors than plasmid DNA, but the integration, which is required for transgene expression and also depends on target cell division, also occurs at random in the target cell genome, risking insertional mutagenesis as well. Retroviruses infect a wide range of cells, and the expression is stable for months and declines slowly. Their disadvantages include technically difficult preparation, that their integration requires cell division, and that the potential to recombine with viral elements in the environment poses the theoretic risk of malignant transfor-

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The European Lipoprotein Club Organizing Committee: Katriina Aalto-Setälä, University of Helsinki, Finland; Victor Armstrong, Georg-August Universität, Göttingen, FRG; Gerd Assmann, Westfalische Wilhelms Universität, Münster, FRG; Pascale Benlian, INSERM, Paris, France; David Bowyer, University of Cambridge, England; Hans Dieplinger, University of Graz, Austria; Guido Franceschini (Secretary), Institute of Pharmacological Sciences, Milan, Italy; Steve Humphries (Treasurer), Rayne Institute, London, England; Richard James, Cantonal Hospital, Geneva, Switzerland; Luis Masana, Facultat de Medicina, Reus, Spain; Gunilla Olivecrona, Umea University, Umea, Sweden; Rudolf Poledne, Institute Clinical Experimental Medicine, Prague, Czech Republic; Anton Stalenhoef, University Hospital Nijmegen, the Netherlands; Armin Steinmetz, Medizinische Klinik, Philipps-Universität, Marburg, Germany.

Correspondence to Dr A.F.H. Stalenhoef, Department of Medicine, Division of General Internal Medicine 541, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, the Netherlands. E-mail A.Stalenhoef@aig.azn.nl

Selected Abbreviations and Acronyms

ER = endoplasmic reticulum
FCH = familial combined hyperlipidemia
HSPG = heparan sulfate proteoglycan
LRP = LDL receptor–related protein
NEFA = nonesterified fatty acid
NIDDM = noninsulin dependent diabetes mellitus
OVR = oocyte receptor model
PDGF = platelet-derived growth factor
RAP = receptor-associated protein
TG = triglycerides

mation. Retrovirus has been used in a clinical trial for familial hypercholesterolemia. The protocol was quite complex, and thus far, only marginal effects on LDL cholesterol level have been achieved. Adenoviruses have a broad host range and gene transfer is highly efficient. They remain episomal, posing a low risk of insertional mutagenesis. One of the biggest problems with adenoviruses is the immune response. This limits the persistence of expression to about 2 weeks, and there is a potential risk for immuno-mediated host injury. Other viruses being developed for use in gene therapy experiments include adeno-associated virus, herpes simplex, SV-40, vaccinia, sindbis, and lenti viruses such as human immunodeficiency virus (HIV). Semisynthetic vectors are also under development that attempt to combine the cell entry advantages of viral coat proteins with the simplicity of plasmid DNA vectors.

Ex vivo gene therapy means that autologous cells are removed and transduced in cell culture. This technique is useful only in selected situations, but in most cases, this method has just been proof of the principle that gene transfer can be achieved. In vivo gene transfer is the goal on which gene therapeutic techniques will be based in the future. Genes of interest might best be delivered locally, either through direct tissue injection or percutaneous vascular delivery. The gene of interest might also be delivered systemically and the vector targeted using tissue-specific cellular uptake mechanisms or gene expression elements.

In cardiovascular diseases, gene therapy techniques are being tested in lipid disorders, vascular diseases, myocardial diseases, and systemic metabolic diseases. Familial hypercholesterolemia was the first cardiovascular disorder for which gene therapy has been attempted. The advantages of gene therapy for this condition is that it is caused by a single gene mutation and there are good animal models for the disease. Both retro- and adenovirus vectors have been used in experiments with these animal models. Restenosis is another major clinical problem that is being targeted for gene therapy in cardiovascular diseases. Smooth muscle cell proliferation has been identified as a major contributing event in the development of restenosis, and growth factors, transcription factors, and cell cycle mediators are being targeted in gene therapy approaches to this problem.

Dr Fox concluded his talk with a view of the future of gene therapy. Vector development and gene delivery are major areas of research at the moment, and much effort is directed toward improved gene transfer technologies. Better knowledge of molecular pathology is also necessary, and rapid advances in our knowledge of molecular targets is being incorporated into gene therapy approaches. The design of clinical trials for gene therapy should appropriately focus on the safety of these methods, even before efficacy can be demonstrated. Finally, for gene therapy to become a clinical reality, the safety, efficacy, and costs of these new techniques will need to be compared with those of both traditional and other novel therapies for gene therapy to provide meaningful benefits for society as a whole.

The major topic of this year's meeting was "Genetics of Lipoprotein Disorders." An overview lecture was given by Dr Steve Humphries (London) who outlined genetic strategies to identify candidate genes and potential functional mutations. It is widely accepted that differences in plasma lipid levels in individuals in the general population are determined by variation at a number of different genes, interacting with environmental factors. Any study attempting to identify candidate genes must, therefore, take into account the possibility of genetic heterogeneity and of gene-gene and gene-environment interactions. The usual strategy to identify genes involved in determining a rare inborn error of metabolism has been to focus on a few "affected" families that are as large as possible. However, for a multifactorial disorder, such a strategy increases the chance of individuals carrying other common mutations that affect the trait "marrying in," which, therefore, results in more confusion than clarification. As such, this approach for common polygenic hyperlipidemia is unlikely to be useful, although it should be useful for rare disorders of lipoprotein metabolism such as Tangier disease. However, genetic heterogeneity will also confound a linkage approach in families. If, for example, such an approach had been used to study families with dominantly inherited levels of elevated plasma cholesterol, in some families the mutation would be caused by a defect in the LDL-receptor gene, whereas in others the defect would be in the apoB gene, and a linkage approach using only one of these markers would be confounded. The strategy of using sibling pairs is a much stronger approach for a multifactorial disorder of late onset; however, parents may be unavailable because of early death, and children may not be useful because they have not yet developed the phenotype. The approach is to collect pairs of siblings who are both affected (eg, hyperlipidemia) and using either random markers or a candidate gene to see if the affected siblings share alleles at the locus more often than expected by chance alone. The statistics for this technique are well worked out, and the approach is robust to genetic heterogeneity. A more powerful way of using siblings is to use a quantitative trait such as plasma levels of triglycerides or of an apoprotein and to look at the between-siblings difference in siblings who share 0, 1, or 2 alleles identical by state. If the mean difference for a group of siblings is smaller in those sharing two compared with those sharing 0 alleles, this is strong evidence for involvement of variation at the gene locus in determining the trait.

Although studies using siblings are extremely useful, a great deal of progress in dissecting the genetic basis of hyperlipidemia has already been made using samples of unrelated individuals. There are two basic strategies, either a "top-down" or a "bottom-up" approach. The top-down approach is when individuals are selected by phenotype (eg, patients versus healthy controls), and the allele frequency at a particular candidate gene locus is

compared between the two groups. Although this approach is relatively easy and has been used in a number of studies, it is not very powerful and can be easily confounded by ethnic mix and stratification. A great deal of the apparent inconsistencies in the literature can be explained by this problem, with some studies showing an association and others not. A more powerful approach is the bottom-up strategy, when individuals are genotyped at a particular locus, and then the phenotype (eg, plasma lipid levels) of those with different genotypes is compared. Although this strategy is more robust to ethnic differences, it will be confounded by gene-environment interaction. If the effect on plasma lipid traits associated with a particular mutation is much larger, for example, in men compared with women, in nonsmokers compared with smokers, in patients compared with healthy individuals, or in obese individuals compared with lean individuals, then the power of a particular sample to detect a statistically significant association will depend crucially on the relative proportions of these different subgroups of individuals. Because a priori these gene-environment interactions are unknown, the apparent inconsistencies between published association studies are most likely the result of this problem, and study designs using homogeneous and clearly defined individuals are ideal. Most polymorphisms are themselves unlikely to be of functional significance, but they may be acting as a marker for a functional mutation elsewhere at the gene locus. The first polymorphism in an apoprotein gene to be published is a very good example of this. The *Sst*I polymorphism originally detected by Southern blotting using an apoAI gene probe, but which is actually in the 3′ untranslated region of the apoCIII gene, was first reported more than 13 years ago (Rees et al, *Lancet* 1983;1:444-446). This polymorphism is associated with hypertriglyceridemia both in top-down and bottom-up studies, and this association has been consistently reported in many different studies. There are more than 50 publications looking at the relationship between polymorphisms in the apoAI-CIII-AIV gene cluster and different plasma lipid traits, and it is curious and disappointing that so far the mechanism of this association has yet to be determined. Although it has been reported that the *Sst*I site might be a marker for functional mutations in the insulin responsive element between the apoCIII and apoAIV genes (Li et al, *J Clin Invest.* 1995;96:2601-2605), a recent paper concluded that, of all the polymorphisms in the gene cluster including those in the insulin responsive element, the *Sst*I was associated with the greatest effect on plasma lipids (Surguchov et al, *Arterioscler Thromb Vasc Biol.* 1996;16:941-947). One of the interesting features of polymorphisms in this gene region is that they appear to be associated with a greater effect on lipid levels in hypertriglyceridemic individuals than in healthy controls, and an example of this using a neutral polymorphism in exon 3 of the apoCIII gene (C1100-T) was presented.

Clearly, understanding the molecular mechanism of this gene-environment interaction would be of great relevance. As further examples of these interactions, the lipoprotein lipase gene is very informative, and several amino acid mutations have been reported in the gene that are relatively common (D9N and N291S). Both of these mutations, which are present in 2% to 4% of the general population in different countries in Europe, cause elevated plasma triglycerides and lower HDL, although the effects are relatively moderate. However, in individuals who are lean, the effect of these mutations is negligible, whereas in those who are obese (eg, body mass index>26 kg/m^2), the triglyceride-raising and HDL-lowering effect of the mutations is much greater. From a statistical point of view, this gene-environment interaction effect is significant and has been shown in several studies. Although the mechanism of this effect is not yet fully understood, it does give a clear example of how knowing an individual's genotype will only be of importance if the environment in which the genotype is found is also known, in this case, degree of obesity.

Dr H. Wittrup (Copenhagen) reported on the study of the Ser291Asn mutation of lipoprotein lipase in a general population cohort of 9214 individuals (The Copenhagen City Heart Study) and in a group of 948 patients with ischemic heart disease. The mutation was found in 452 heterozygotes and in 7 homozygotes. The mutation was associated with increased plasma triglycerides (+.22 mmol/L; 95% confidence interval, .08-.38) and decreased apoAI levels and HDL (−.17 mmol/L; 95% confidence interval, .12-.23). In contrast with the Gly188Glu mutation which is associated with familial chylomicronemia in homozygotes, a phenotype of only mild dyslipidemia was observed in homozygotes with the Ser291 mutation. On multiple logistic regression analysis, the Ser291Asn mutation was an independent predictor for ischemic heart disease (odds ratio, 1.4; 95% confidence interval, 1.05-1.87) in females, whereas this was not the case in men. Therefore, common mutations that partially disrupt LPL activity were confirmed as a cause of an altered lipoprotein profile in the general population and may predispose to atherosclerosis.

Dr D. Freeman (Durham) reported data of a sibling-pair analysis in normolipidemic families from the United States, examining the genetic predisposition to interindividual variations in plasma LDL cholesterol. Although 50% of the interindividual variation in LDL cholesterol was attributed to genetic factors, major candidate genes (LDL receptor, apoB, LPL, hepatic lipase) were found to have a negligible contribution (<1%). However, the predicted effect of the apoE polymorphism on apoB and LDL cholesterol levels was found. It was concluded that in normolipidemic individuals, apart from the known effects of apoE, the genetic contribution to the variation in plasma LDL cholesterol does not depend on these candidate genes.

Dr M.-C. Blatter Garin and colleagues (Geneva) reported on the effect of sequence polymorphisms in the paraoxonase gene and on the risk of developing coronary heart disease in diabetics. They had previously demonstrated that the Gln192Arg polymorphism, which alters activity, is associated with an increased risk for heart disease in diabetics, and a second polymorphism (Leu55Met) was now studied. No difference in lipoprotein profile was observed, but the polymorphism was associated with highly significant differences in paraoxonase serum concentrations and with increased risk of heart disease. However, a strong linkage disequilibrium was noted between Gln192Arg- and Leu55Met polymorphisms, suggesting that the relationship between the Leu55Met polymorphism and heart disease may be a consequence of this association.

Dr P. Talmud (London) reported a study of the -93T/G transition in the promoter of the LPL gene. The carrier frequency of the G allele was .03 in 1521 healthy Caucasians and .08 in 230 patients with FCH and myocardial infarction. In healthy Afro-Caribbeans, the frequency of the G allele was strikingly increased to .63. In addition, the G allele was found in strong allelic association with the N allele of the Asp9Asn (D9N) variant in Caucasians, all carriers of the N9 allele also being carriers of the -93G allele. Despite the raising effect of the N9 allele on triglycerides, their concentration was lower in carriers of the -93G allele alone. To assess the functional effect of the -93G allele, in vitro studies were conducted in a smooth muscle cell line, A10. The -93G allele resulted in a 24% increase in promoter activity and was found to bind a specific nuclear protein, resulting in a band shift that was not observed with the T allele. These results suggest that the change in promoter activity associated with the -93G allele may be caused by specific interactions with some nuclear factors. This, in turn, may account for the lower triglyceride levels observed in carriers of this promoter mutation.

Dr S. Haubenwallner (Wien) studied the regulation of the transcription of apoCIII in HepG2 cells at two closely related promoter sites recognized by peroxisome proliferation activated receptors and by HNF4, respectively. When cells were incubated with fibrates or ETYA or cotransfected with peroxisome proliferation activated receptor, this resulted in reduction of the level of apoCIII expression. This effect was abolished when the site recognized by HNF4 was mutated, whereas it was unchanged when the cells were transfected with a dominant negative HNF4 mutant. These data suggest that the down-regulation of apoCIII induced by peroxisome proliferation activated receptors in HepG2 is mediated through interactions of peroxisome proliferation activated receptor with the HNF4 recognition site.

Dr S. Ries (Regensburg) presented the characterization of the promoter of the lysomal acid lipase in macrophages and CHO- and HepG2 cells. Within 500 base pairs upstream of the transaction start site, elements recognized by Sp1, AP2, as well as macrophage specific transcription factors were identified. Neither a TATA box nor a CAT box was identified upstream of the LAL gene.

A number of talks described functional tests that detected protein variation. Dr S. Vilaro (Spain) looked at a number of missense mutations in the LPL gene from type I hyperlipidemic patients that all had a major effect in causing intracellular retention of the mutated protein. The mutant N43A prevents *N*-glycosylation at the asparagine 43 of the LPL molecule, and the mutant protein was totally retained in the rough endoplasmic reticulum (ER) of transfected cells. The retention of this nonglycosolated LPL gene induced morphologic changes in the ER that could also affect the intracellular transport of other nonrelated proteins. The mutations G142E and S172C produced nonactive proteins that were also retained inside the cells, and in immunofluorescence and electromicroscopic studies, mutated proteins were retained and degraded in the lysosomes of transfected COS cells. It is of interest that, based on the three-dimensional structure of LPL that has been deduced by comparison with the pancreatic lipase, these two mutations both occur within a very tightly packed region inside the catalytic domain of the protein. The findings suggest that intracellular mis-sorting might be a mechanism of cell quality control of secreted LPL.

Dr F. Benhizia (London) reported on work expressing the three known apoB signal peptide variants in McArdle RH-7777 cells. In the general population, the most common allele of the signal peptide contains 27 amino acids (SP27), whereas about 30% of chromosomes lack three hydrophobic amino acids from the core of the protein (SP24). A further variant that has been found only in Mexican/Americans has an insertion of two hydrophobic amino acids (SP29). In association studies, the SP24 allele has been found to be associated with lower plasma triglycerides, especially when individuals are on a high-fat diet. In studies originally published in 1995, the signal peptide variants had been used to drive secretion of the enzyme invertase in a yeast system, and this work had shown that compared with SP27, SP24 and SP29 were retained inside the cell and did not reach the medium. To confirm this in a homologous system, the three signal peptides had been joined to apoB17, and their transient expression followed in the rat hepatoma cells. The results demonstrated that equal amounts of mRNA were produced from the three variants, and although apoB17 could be seen inside the cells from all three variants, SP24 and SP29 proteins remained within the cell and were apparently degraded, whereas the SP27-directed protein appeared in the medium. These results confirm the functional difference of the three SP variants and support the hypothesis that apoB signal peptide variation is directly involved in determining differences in plasma triglyceride levels.

Dr A. Gruber (Innsbruck) reported on the relationship between apo(a) and calnexin in the ER, following the hypothesis that the inverse correlation between the plasma concentration of Lp(a) and the length of the apo(a) protein might be the result of posttranslational mechanisms during apo(a) secretion. In stably transfected HepG2 cells, apo(a) is synthesized as a precursor with low molecular weight in the ER. This precursor is retained for an unusually long time while processing to the mature form occurs, which is found exclusively in the Golgi. Comparing apo(a) isoforms of different sizes, larger allelic variants have longer retention times in the ER than smaller ones, and this longer residence time is possibly the result of an increased time needed for correct folding and glycosylation. Calnexin is a predominant integral membrane protein of the ER and associates transiently with incompletely folded glycoproteins. It, therefore, may be important in apo(a) maturation within the cell; this hypothesis was confirmed using a calnexin antibody, the results of which showed that the important molecular chaperone effects of calnexin are crucially involved in causing variable retention times in different apo(a) isoforms.

Dr T. Bruin (Amsterdam) used molecular modeling and site-directed mutagenesis to study LPL structure and function. The amino acid sequence around P157 is a hydrophobic cavity and is highly conserved in both LPL and pancreatic lipase (PL); conformation of this cavity is stabilized by a conserved network of interactions between α and β strands. It was noted that in rat pancreatic lipase that the equivalent of P157 is replaced by alanine, but in human LPL this substitution produced a nonfunc-

tional protein. However, in rat pancreatic lipase, this substitution is accompanied by substitutions in the α 4 helix (A-V) and in the β 8 strand (V-A). To explore this further, the A137V and V181A substitutions were made in wild-type LPL in combination with the P157A. After expression of mutants in COS cells, results showed that, although neither flanking mutation alone could rescue the deleterious effect of AI57, when both were present, the catalytic activity of LPL was partially restored. Interestingly, the substitution of A137V in wild-type LPL gave a 1.6-fold increase in specific activity, indicating that this substitution is beneficial to the LPL protein. The possibility of using such mutations to create "super" LPL enzymes was discussed.

Dr D. Gaffney (Glasgow) reported on the detection and characterization of two mutations in the apoB gene and their effects on LDL levels. The mutations were A3371V and Q3405E, which were found in 1 and 10, respectively, of roughly 900 hyperlipidemic individuals who were screened. The effect of these mutations on LDL structure and binding to the LDL receptor were examined using the U937 growth assay. As a comparison, patients with the R3500Q, R3500W, and R3531C were also included in the assay. LDL from patients with 3500Q or 3500W both showed a significantly reduced ability to support U937 growth, both being about 60% of normal LDL. LDL from patients with the 3531C mutation showed a smaller reduction (about 80% of normal level), whereas individuals with the 3405E showed about 90%. The results for two related 3371V individuals were unclear, possibly because of high triglyceride levels in the proband. Therefore, although the 3405E mutation appears to be relatively common in patients from Glasgow, it has, at best, a modest effect on LDL function and is, therefore, unlikely to have a major impact in determining plasma lipid levels.

Dr A. Tilkorn (Hamburg) reported studies looking at the effect of induced mutations in LAL in determining its substrate specificity, using an in vitro expression system. Full-length cDNA for LAL was cloned in the baculo virus vector using the signal sequence of alkaline phosphatase to mediate enzyme secretion into the culture supernatant and a carboxyl terminal polyhistidine tag to allow an efficient single-step purification. A systematic replacement of six cysteine residues by alanine showed that several C-A replacements were silent in their effect, whereas a C234A charge produced an enzyme with a marked increase in triglyceride hydrolysis. By contrast, cholesterol oleate hydrolysis was severely attenuated in this mutant, indicating an important role of C234 in substrate specificity. Other studies are underway to further characterize the structure and function of LAL to determine its role in lipid accumulation states, such as foam cell formation and advanced atherosclerotic lesions.

Dr P. Nichols (Belfast) reported a study in which 19 LDL receptor mutations have been identified using DGGE in familial hypercholesterolemia patients in Northern Ireland. As expected from a genetically heterogeneous population, no single mutation was common, although mutations in exon 3, 4, 6, and 10 explained familial hypercholesterolemia in 30% of the patients studied. The most frequent mutation was 932dA in exon 67 (n=6) and D461N in exon 10 (n=6). The total serum cholesterol in probands for families with class I receptor defects (null allele) was almost 2 mmol/L higher compared with families with class 2 (transport) or class 5 (recycling) receptor defects, and this difference was highly statistically significant.

The last section of this session focused on exploring novel mechanisms of lipoprotein metabolism using genetic tools. Dr W. Drobnik (Regensburg) presented biochemical evidence that the genetic defect in Tangier disease is associated with changes in the metabolism and signaling function of sphingolipids. In fibroblasts of Tangier patients, HDL3-mediated efflux of newly synthesized cholesterol, sphingomyelin, and phosphatidylcholine is markedly reduced. This is associated with an impaired HDL3-induced activation of the PI-PLC signaling pathway in these cells and, in confirmation of this protein kinase C activation, was able to normalize HDL3-mediated cholesterol and phospholipid efflux. In a second study, ceramide as well as sphingomyelin were found to be increased by 100 and 40%, respectively, in Tangier fibroblasts. In agreement with the known ability of ceramide to modulate cell proliferation, Tangier fibroblasts showed a 50% reduction in their in vitro growth rate, more than twofold greater percentage of cells in the G2/M phase, and a reduced number of cells in the S phase of the cell cycle. Increased ceramide and sphingomyelin content in Tangier fibroblasts was also associated with an impaired transport of sphingomyelin and glycosylceramide from Golgi to the plasma membrane. Thus, a defect in Golgi transport may result in an accumulation of sphingolipids, such as ceramide, which act as intracellular messengers and modulate cell function. Disturbed sphingolipid transport from the Golgi to the plasma membrane may also influence lipid composition of special membrane domains, eg, caveolae, which are known to be involved in HDL3-mediated efflux and represent centers of signal transduction, therefore, tying up the impaired HDL3-induced lipid efflux and phosphoinositol–phospholipase C activation with disturbed sphingolipid metabolism and transport. Taken together, the biochemical data strongly link the genetic defect in Tangier disease with sphingomyelin metabolism.

Dr B. Angelin (Huddinge) reported on studies of patients with familial hypertriglyceridemia, looking at bile acid malabsorption that resulted in increased bile acid production and enhanced clearance of plasma low-density lipoprotein. Only some patients with familial hypertriglyceridemia show increased bile acid production, and this appears to be mainly caused by a failure of bile acid absorption from the distal ileum. However, as a result of their increased production, their bile pool size and secretion rate of bile acids are normal. Such patients have markedly higher fractional catabolic rates of LDL particles compared with patients with normal bile acid production. The study suggests that the increased bile acid production and subsequent VLDL overproduction, concomitant with an increased hepatic LDL receptor expression, lead to stimulated LDL metabolism and, therefore, lower levels of LDL. A candidate gene involved in this process is the recently cloned ileal bile acid transport protein, and studies of the possibility of mutations in this gene will be of interest.

Dr W. Hofman (Gratz) presented work on transgenic mice in which LPL expression has been knocked-out in adipose tissue and skeletal muscle, but in which, using a cardiac-specific promoter, expression occurs exclusively

in heart tissue. Of major interest is that in these animals, plasma triglyceride levels were almost normal, whereas mice in which the LPL gene is completely deficient die within a few hours of birth as a result of massive hypertriglyceridemia. This suggests a potent role of cardiac LPL in the clearance of plasma triglyceride-rich lipoproteins. The mice expressing LPL exclusively in the heart develop normally with regard to body weight and composition. However, total essential fatty acids in smooth muscle cells and adipose tissue were decreased more than 80%. The lack of LPL in these tissues apparently causes the inability of the cells to take up dietary fatty acids from triglyceride-rich lipoproteins. The small amounts of essential fatty acids found in these noncardiac tissues could originate from two potential sources; one possibility that was raised was that LPL from cardiac tissue might be transported in the blood to muscle or adipose tissue, although this seems unlikely, because LPL activity in whole muscle or in adipose tissue from these mice was extremely low. The alternative possibility was that free fatty acids released from LPL action in the heart could pass through the circulation and be absorbed by the other tissues.

Dr P. Benlian (Paris) continued the discussion about the effect of LPL deficiency by looking at patients with type I hyperlipidemia. Current dogma is that such individuals are at reduced risk of developing coronary atherosclerosis because, although plasma levels of chylomicrons and VLDL are high, these lipoproteins are too large to enter the artery wall, and because LPL metabolism of these lipoproteins is absent, levels of LDL are extremely low. However, to challenge this dogma, four type I patients were presented who had developed atherosclerosis in their fourth or fifth decade. The patients showed evidence of angina and stenosis of the coronary arteries on angiography, and one individual died of a myocardial infarction. Two of the patients were smokers and two were not, although other known risk factors were not obvious. Interestingly, levels of Lp(a) were low in all of these patients. The mechanism of the potential atherogenic processes in type I patients was discussed, and possibilities include lipoprotein retention in the arterial wall, oxidation of the triglyceride-rich lipoproteins, or activation of the thrombotic system. Other patients with type I hyperlipidemia (either apoCII- or LPL deficiency) were discussed, some of whom had survived to old age without developing atherosclerosis or who had no evidence of premature disease on postmortem.

Dr F. de Man (Leiden) described a novel assay to look at the effect of apoE mutations on the binding and lipolysis of VLDL by heparan sulfate proteoglycan (HSPG)-bound lipoprotein lipase. During lipolysis by LPL, lipoproteins interact with vessel wall HSPG, and apoE is considered to enhance the stability of this lipoprotein-HSPG complex. It is, therefore, possible that VLDL from subjects with apoE mutations may show diminished interaction with HSPG and, as a consequence, impaired lipolysis. Lipolysis was studied by quantification of free fatty acid release from VLDL in HSPG-LPL coated plates. Compared with control VLDL-E3, lipolysis of VLDL-E2 was reduced to 80% and lipolysis of VLDL from individuals carrying the E2 Lys146-Gln and apoE3-Leiden was reduced to approximately 60%. This impaired lipolysis was most probably caused by diminished binding of the particles to HSPG-bound LPL, as confirmed by competitive binding studies with ^{125}I-labeled control VLDL. It is, therefore, possible that the increased risk for coronary artery disease in patients with apoE mutations is partly because of a defect of their VLDL and VLDL remnants to bind HSPG-bound LPL.

Dr M. Hofker (Leiden) presented work to explore the potential role of apoC1 in lipoprotein metabolism using transgenic mice. ApoC1 has been reported to activate lecithin-cholesterol acyltransferase in vitro and to inhibit apoE-mediated receptor binding of lipoproteins. Knockout of the ApoC1 gene leads to a mild hypercholesterolemic phenotype during high-fat feeding, and ApoC1 overexpression leads to a moderate increase in plasma lipid levels. The ApoC1 transgene contained a contiguous genomic DNA fragment encompassing ApoC1 and the downstream located hepatic control region. However, on a diet containing sucrose, plasma cholesterol levels, particularly plasma triglyceride levels, became markedly elevated. Turnover studies show that production of VLDL in these animals is within the normal range and the lipolysis of VLDL is also normal, suggesting that the process that is affected must be uptake of the VLDL remnants. Interestingly, overexpression of ApoC1 causes a reduction of VLDL clearance, which is most pronounced in male mice. After the liver has been excluded from the circulation, the VLDL removal is similar in transgenic and nontransgenic mice, indicating that ApoC1 overexpression does not affect the peripheral lipoprotein metabolism and primarily modifies the liver-mediated VLDL clearance. VLDL can be removed by the liver either by LDL receptor- or LRP-mediated processes. In mice in which the LDL receptor gene has been knocked-out, plasma lipid levels are moderately elevated over wild-type litter mains, but when crossed with the ApoC1-overexpressing mice, both plasma cholesterol and plasma triglycerides are extremely elevated (Jong, et al. *J Clin Invest.* 1996;98:2259–2267). These results suggest that in the LDL receptor knock-out mice, in which normally VLDL is cleared by the LRP, the ApoC1 overexpression blocks LRP-mediated uptake. To confirm this, an adenovirus vector containing a construct leading to transient overexpression of the receptor-associated protein (RAP) was used. Overexpression of RAP in the LDL receptor knock-out mouse produced a moderate increase in plasma lipids only, thus, confirming that VLDL clearance is carried out through ApoC1-mediated processes.

Dr K. Willems van Dijk (Leiden) examined the mechanism of apoE3-Leiden and apoE2 in hypercholesterolemia using transgenic mice and adenovirus-mediated gene transfer. Animals in which the apoE2 gene has been knocked-out develop severe hypercholesterolemia, and it was now shown that apoE3-Leiden can rescue this phenotype, whereas crossing with a mouse transgenic for apoE2 does not result in major reduction in plasma cholesterol levels. VLDL apoE3-Leiden shows lower binding to the LDL receptor on HepG2 cells compared with VLDL from wild-type mice, whereas VLDL apoE2 shows binding that is almost as low as that from the apoE knock-out mouse. The possibility that LRP can function as a back-up for removal of lipoproteins that cannot bind to the LDL receptor was investigated using adenovirus-mediated gene transfer of the RAP gene. As

would be expected, in apoE knock-out mice, plasma cholesterol levels were not affected at all when mice were treated with the adeno-RAP construct. By contrast, in the apoE3-Leiden and apoE2 mouse (on an apoE-knock-out background), plasma levels went up threefold when the mice were infected with the adeno-RAP construct. This suggests that for both VLDL apoE2 and VLDL apoE3-Leiden, LRP is involved in their removal. The role of other receptors in VLDL clearance was examined using mice that were infected with an adenovirus containing a construct for the VLDL receptor. On day 5 after infection, plasma cholesterol levels were reduced more than 50% in both apoE2 and apoE3-Leiden mice, mainly accounted for by reduction in the VLDL and LDL fraction. These data indicate that both apoE2 and apoE3-Leiden can function as ligands for the VLDL receptor in vivo and suggest a possible role for the VLDL receptor gene and gene therapy for the treatment of familial dysbetalipoproteinemia.

In the first of two smaller sessions, focused on "Lipoproteins and the Kidney," Dr H.-J. Gröne (Marburg) gave a lecture on the role of lipoproteins in nephrosis and glomerulosclerosis. He defined nephrosclerosis as a degeneration of proglomerular arteries and arterioles with consecutive glomerulosclerosis. Foam cells are not normally observed in nephrosclerosis, and arterial hypertension has been thought to be a major causative factor of this disease. However, examination of human renal biopsies has revealed the presence of foam cells in the capillaries and the mesangium of the glomerulus. The following factors are associated with the development of glomerulosclerosis: mechanical (arterial and glomerular hypertension), hormonal (endothelin, angiotensin II, vasoactive peptides), immunologic (immune complexes), and metabolic (hyperglycemia, hyperlipidemia). In a rat model, a synergism between arterial pressure and hyperlipidemia was observed. The extent of glomerulosclerosis in hyperlipidemic animals fed a high-fat, cholesterol-rich diet was enhanced in the clipped hypertensive kidney, whereas no glomerulosclerosis was seen in the unclipped hypotensive kidney. In cellular studies, human mesangial cells were found to have a much higher affinity for β-VLDL than for LDL, and the maximal capacity for the uptake of β-VLDL could be increased by the vasoactive peptides endothelin-1, angiotensin II, and platelet-derived growth factor (PDGF). Further experiments demonstrated that LDL could provoke a significant but not pronounced increase in cellular DNA synthesis as measured by ^{3}H-thymidine uptake. In the presence of PDGF, there was a pronounced synergistic induction of cell proliferation. He went on to describe experiments in the guinea pig in which LDL at a concentration of 50 μg/mL was found to induce the early expression genes c-*fos* and c-*jun* but not c-*myc*. Both LDL and β-VLDL were also able to stimulate the expression of PDGF. Guineas pigs fed a fat-rich diet were found to have lipid deposition in the glomerulus. Animals treated with a low dose of lovastatin, which did not alter VLDL and LDL levels, showed reduced mesangial cell proliferation and sclerosis, presumably because of the inhibition of the prenylation of proteins involved in cell proliferation.

The monocyte is an essential cell for the development of glomerulosclerosis: myeloperoxidase is found in large amounts in the human kidney and, therefore, provides the potential for the formation of hydroxychlorite and subsequent modification of LDL. With use of a Mab specific for hydroxychlorite-modified LDL, this modified lipoprotein could be demonstrated in biopsies from human kidneys. Furthermore, HOCL-modified LDL was shown to induce expression of cytokines such as IL-8 in the human monocyte.

Dr J. Joles (Utrecht) discussed plasma lipoproteins and the development of glomerulosclerosis and renal disease in animal models. He posed the question as to whether glomerulosclerosis and atherosclerosis have a common pathogenesis and noted that individuals with homozygous familial hypercholesterolemia and severe premature atherosclerosis do not appear to be at increased risk for renal disease. He suggested that rather than LDL, the triglyceride-rich lipoproteins chylomicrons, VLDL and IDL, are of primary importance in the development of glomerulosclerosis. Evidence for such a hypothesis comes from studies in an animal model, the analbuminemic rat. Male rats are generally more prone to developing renal disease than females. However, in the case of the analbuminemic rat, females are profoundly hyperlipidemic and develop proteinuria and glomerulosclerosis after uninephrectomy. Male anulbuminemic rats on the other hand are less hyperlipidemic and are more resistant to developing renal damage after uninephrectomy. The importance of triglyceride-rich lipoproteins in the pathogenesis of renal disease in this animal model was further substantiated through studies in the ovariectomized analbuminemic female rat. Ovariectomy of female analbuminemic rats markedly decreased plasma triglyceride levels by lowering hepatic triglyceride secretion, leading to a profound and persistent reduction in their hyperlipidemia. Animals that had been ovariectomized during the first 12 weeks did not develop proteinuria.

Dr J. Moorhead (London) presented investigations on the influence of cytokines and calcium channel blockers in the expression of LDL and scavenger receptors on human mesangial cells in culture. The mesangial cell is a multipotent cell that can both oxidize LDL and release cytokines. Tumor necrosis factor-α, transforming growth factor-β, PDGF, and interleukin-1 (IL-1) were all found to stimulate LDL receptor expression in a dose-dependent manner. Tumor necrosis factor-α, PDGF, and IL-1 also effectively induced scavenger receptor expression, but in contrast tumor growth factor-β inhibited the expression of this receptor. The induction of both the LDL receptor and the scavenger receptor by tumor necrosis factor-α, and PDGF as well as the induction of the LDL receptor by tumor growth factor-β apparently require protein synthesis, as suggested by experiments in which cells were pretreated with cycloheximide. In contrast, the induction of the two receptor genes by IL-1 was not affected by cycloheximide. These results suggest that tumor growth factor-β may protect human mesangial cells from lipid accumulation by stimulating LDL receptor expression while at the same time inhibiting expression of the scavenger receptor. The second part of his talk was devoted to the influence of calcium channel blockers on the expression of the LDL receptor in mesangial cells. Calcium channel blockers are known to retard the course of glomerulosclerosis, but the mechanism is still unclear. Human mesangial cells were, therefore, exposed to three kinds of calcium channel blockers,

nifedipine, diltiazem, and verapamil. Northern blot analysis showed that all three calcium channel blockers could stimulate the expression of LDL receptor mRNA in a dose-dependent manner. LDL receptor promoter activity was also increased, whereas the stability of LDL receptor mRNA was unaffected. These results suggest that calcium channel blockers may reduce lipid deposition and oxidation by upregulating LDL receptor or regulating the downstream events in cellular degradation and efflux of LDL.

Dr F. Kronenberg (Innsbruck) discussed "Lp(a) and renal disease" and covered several clinical and metabolic aspects of this atherogenic lipoprotein. He first gave an overview on the metabolism and genetics of Lp(a), including the exclusive genetic control of Lp(a) plasma levels in the general population. These Lp(a) concentrations are inversely correlated with a genetic size polymorphism of apo(a) because of a highly variable number of identical kringle 4 domains. Because elevated Lp(a) levels have been found to be associated with atherosclerotic complications such as coronary heart disease and stroke, Lp(a) can be regarded as a genetically determined risk factor for those diseases. However, nongenetic influences on Lp(a) plasma concentrations such as hormonal regulation and effects secondary to certain diseases such as renal insufficiency also occur. In the second part of his talk, he concentrated on the significance of elevated Lp(a) plasma concentrations in patients with end-stage renal disease. He discussed the statistical problems associated with case-control studies on Lp(a). Because Lp(a) plasma concentrations show a skewed distribution in most populations and span a wide range, analysis of small sample groups ($n<100$) may lead to nonrepresentative results. Case-control studies fulfilling the criteria $n>100$ have consistently shown an Lp(a) elevation of between 30 and 100% in patients treated by hemodialysis or continuous ambulatory dialysis, higher levels generally being observed in the latter group. Prospective studies measuring Lp(a) before and after renal transplantation consistently showed a decrease in Lp(a) independent of the mode of immunosuppressive therapy. This together with the unchanged apo(a) phenotype pattern clearly demonstrated that the elevation of Lp(a) in renal patients has a nongenetic background and is caused by the disease itself. Next, he reported on arteriovenous differences in Lp(a). In a study of 100 patients with normal renal function, Lp(a) concentrations were lower in the renal vein than in the renal artery, consistent with extraction of Lp(a) from the circulation by the healthy human kidney. Three studies have reported the occurrence of apo(a) fragments with molecular weights ranging from 30 to 215 kDa in urine. These observations suggest that mechanisms other than glomerular filtration might be involved in the generation of urinary apo(a) fragments. The lecture ended with an overview on studies into the significance of Lp(a) as an atherosclerotic risk factor in renal disease. Only a few large studies are presently available linking Lp(a) to the high risk of atherosclerosis seen in these patients. There is evidence from some studies, however, that the apo(a) phenotype may be of major importance for risk assessment, at least in hemodialysis patients.

Dr T. Willnow (Berlin) presented recent data on the possible involvement of Gp330/megalin in developing neural tissues. This protein is a 600-kDa endocytic receptor and a member of the still-growing LDL receptor gene family. The receptor is expressed on the apical surface of the epithelial tissues of the lung, intestine, neuroectoderm, and the proximal tubules of the kidney, where it mediates the endocytic uptake of various macromolecules including cholesterol-carrying lipoproteins. Megalin has been identified as the major autoantigen in an induced glomerular nephritis rat model (Heymann nephritis). During embryonic development, megalin is expressed in the yolk sac, the amniotic ectoderm, and the neuroectoderm. To elucidate its physiologic role, megalin-deficient mice were constructed by gene-targeting. Homozygous-deficient animals are born alive but die perinatally. They suffer from severe developmental malformations of the forebrain that include fusion of the forebrain hemispheres, lack of olfactory bulbs, and a common ventricular system. Similar syndromes in humans and animals are caused by insufficient supply of cholesterol during development. Before the formation of the circulatory system, lipids are supplied to the developing embryo by transport across the yolk sac and diffusion-dependent uptake by embryonic tissues. It was, therefore, proposed that megalin might be part of the maternal-fetal lipid transport system, mediating uptake of lipids and lipid soluble vitamins into the developing neuroepithelium.

Dr J. Nimpf (Vienna) described detailed epitope studies on megalin and another target antigen in the Heymann nephritis model, RAP. This 40-kDa protein is related to several members of the LDL receptor family. In the chicken oocyte receptor model (OVR, which binds VLDL and vitellogenin), RAP and members of the LDL receptor family share a common immunologic epitope. Antibodies against recombinant RAP cross-reacted with OVR and other members of the LDL receptor family including LRP (LDL-receptor related protein) and megalin. The interaction of this antibody with OVR was comparable with the binding of physiologic ligands to this receptor: binding of the antibody was Ca^{2+}-dependent, was abolished under reducing conditions, and inhibited the binding of VLDL and vitellogenin. By using recombinant fragments of RAP, the cross-reacting epitope could be localized to the carboxyl-terminal end of this protein. After immunization of rabbits, anti-OVR antibodies arose at the same time as anti-RAP antibodies, indicating that the OVR-specific activity was not caused by anti-idiotypic antibodies. These results suggest the existence of a common epitope on both proteins as well as on other members of the LDL receptor family and favor the model of cross-reactive epitopes for the development of Heymann nephritis.

Dr P. Tarugi (Modena) reported on the synthesis of apolipoproteins B and AI by the chicken kidney. This organ was found to secrete these apolipoproteins as constituents of IDL, LDL, and HDL, the relative amounts of apoB and apoAI being about 50% of that produced by the liver. It could be shown immunohistochemically that both proteins are primarily synthesized in proximal and distal convoluted tubules of the kidney cortex but not in collecting tubules. The high rate of renal apolipoprotein production in the chicken appears to be related to the direct uptake of intestinal lipids by the tubular epithelial cells via the renal portal system. Ten minutes after infusion of a ^{14}C-cholesterol-contain-

ing lipid emulsion into the terminal ileum, radioactivity was found in the kidney, suggesting that intestinal lipoproteins are directly transported to the kidney via the caudal-mesenteric vein branch of the renal portal system. These results suggest that chicken renal lipoproteins significantly contribute to the plasma lipoprotein pool.

In the session on "Causes and Consequences of the Metabolic Syndrome," Dr E. Ferrannini (Pisa) gave a comprehensive overview on the different facets of the metabolic syndrome, limiting the discussion to data derived from human studies. He first showed that plasma insulin concentrations (fasting as well as postglucose) display a skewed distribution in the general population (such as blood pressure or triglyceride levels). By using a statistical definition of hyperinsulinemia, there is a marked enrichment in the prevalence of NIDDM, hypertension, obesity, and dyslipidemia in the hyperinsulinemic segment of the population in comparison with normoinsulinemic subjects. A similar clustering is observed when the analysis is limited to a nonobese cohort. In studies in which insulin sensitivity has been measured directly (by the insulin clamp technique), an inverse relationship is found between insulin sensitivity and insulin levels. Although this relation indicates that insulin levels are a proxy for insulin action, the scatter of the association is such that the two variables cannot be used interchangeably.

The relationship between insulin and glucose levels characteristically takes the form of an inverted U, indicating that increasing degrees of glucose intolerance are associated with increasing insulin resistance up to a breakpoint (around 8 mM), after which β-cell function fails to keep up with insulin resistance and progressive fasting hyperglycemia develops. This biphasic function is observed both cross-sectionally and longitudinally; thus, in long-term follow-up studies, both insulin resistance and β-cell impairment are independent predictors of NIDDM.

Another metabolic change consistently associated with hyperinsulinemia and insulin resistance (in both diabetic and nondiabetic subjects) is an increased concentration of fasting and postprandial nonesterified fatty acid (NEFA). In a population-based survey (the San Antonio Heart Study), NEFA cluster with almost all the features of the metabolic syndrome. An excess of endogenous or exogenous NEFA can lead to impaired peripheral glucose uptake (by substrate competition) as well as enhanced gluconeogenesis and endogenous glucose release (as a result of increased uptake and oxidation of NEFA in the liver). Conversely, insulin resistance of lipolysis (as occurs in lean NIDDM patients) leads to excessive hydrolysis of triglycerides and availability of NEFA for oxidation. Thus, raised NEFA can be at the same time the cause and the consequence of insulin resistance. Increased flow of NEFA to the liver also triggers VLDL-triglyceride synthesis, which is associated with the increased formation of atherogenic, small dense LDL particles. This pattern (pattern B) of LDL particle distribution has been shown to be independently associated with insulin resistance in nondiabetic individuals.

A positive correlation between hyperinsulinemia/insulin resistance and elevated blood pressure has been found in many population studies, including children and adolescents. A family history of NIDDM is associated with a higher prevalence of hyperinsulinemia *and* hypertension (an example of co-tracking). In nondiabetic subjects, higher glucose levels are a good predictor for the development of hypertension 18 years later. Among lean hypertensives, a higher frequency of impaired glucose tolerance can be observed than among normotensives. Overall, high insulin levels in normal subjects have been shown to predict the three main components of the insulin resistance syndrome, i.e., NIDDM, hypertension, and the hypertriglyceridemia/low HDL-cholesterol couple.

The list of cardiovascular risk factors included in the metabolic syndrome increases steadily. New members include abdominal obesity, increased plasminogen activator inhibitor-1 and fibrinogen levels, micro-albuminuria, hyperuricemia, and raised Na/Li counter transport and cytosolic calcium levels. For some of these associations, the mechanisms have been elucidated. Thus, in healthy as well as in hypertensive subjects, insulin causes an increased tubular reabsorption of uric acid in coupling with its antinatriuretic effect. Although insulin antinatriuresis can contribute to raising or maintaining high blood pressure, the antiuricosuric effects of insulin might relate to the hyperuricemia that is frequently observed in NIDDM or essential hypertension. NIDDM patients with hypertension are more insulin-resistant than normotensive patients, and typically, their blood pressure is salt-sensitive. Insulin also lowers intracellular calcium concentrations in sensitive tissues. In smooth muscle cells, this translates to lower tension development and sensitivity to agonists. In platelets, a blunted calcium spike in response to agonists corresponds to decreased platelet aggregation. Platelets from insulin-resistant subjects fail to reduce intracellular calcium in response to insulin and are more prone to aggregation. The mechanism(s) underlying the association of insulin resistance with microalbuminuria have yet to be described.

The insulin resistance syndrome is not a disease but rather a risk syndrome. Because many, if not all, of its components are significant, independent predictors of atherosclerotic cardiovascular disease, the syndrome can be viewed as a complex of physiologic interrelations, which transduces the impact of environmental influences (stress, diet, smoking, alcohol, etc) into pathogenic mechanisms. Against a background of genetic predisposition, clinical diseases—hypertension, dyslipidemia, and NIDDM—develop, eventually leading to cardiovascular disease.

Dr P. Arner (Huddinge) reported from studies on gender differences in visceral fat lipolysis and their relation to metabolic complications in obesity. Although abdominal fat constitutes only about 10% to 15% of the total fat mass, it is metabolically extremely active and fatty acids released directly reach the liver via portal vein. Omental fat cells recovered during elective surgery from 29 obese males and 34 obese females showed a twofold higher release of fatty acids and glycerol in male tissue because of 12 times higher lipolytic β-3-adrenoceptor sensitivity and 17 times lower antilipolytic α-2 adrenoceptor sensitivity. Also, lipolysis induced by agents acting at the PKA or hormone-sensitive lipase level were twofold increased in males over females. The observation of increased ability of cAMP to activate

hormone-sensitive lipase in males cannot be explained up to now. Studies on totally removed omental fat tissue (≈1-2 kg), which was done in addition to gastric banding procedures, showed that cathecholamine-induced fatty acid release from total omentum was 2 times more rapid in men than in women. These findings explain diminished features of the metabolic syndrome in women compared with men with the same degree of obesity.

Overproduction of VLDL, a feature of FCH, is also associated with insulin resistance. Dr A. Stalenhoef and colleagues (Nijmegen) investigated directly the insulin sensitivity in nonobese patients with FCH and their nonaffected first-degree relatives, using the euglycemic hyperinsulinemic clamp technique. During the clamp, forearm blood flow was measured by venous occlusion plethysmography. The patients and relatives had normal glucose tolerance according to an oral 75-g glucose tolerance test. Mean whole body glucose uptake and insulin sensitivity index were lower in the FCH patients than in the nonaffected subjects. The forearm blood flow increased only in the nonaffected subjects. This study demonstrated that nonobese subjects with FCH are, on average, insulin-resistant, which may partly be explained by a decreased insulin-induced vasodilation in skeletal muscle.

Dr J. Reckless (Bath) studied the concentration and composition of lipoprotein subfractions (fasting and postprandially after a 75-g fat load) in patients with and controls. Glucose, insulin, and NEFA were measured and insulin resistance and β-cell function calculated. The data clearly reflected a relative insulin lack, with increased NEFA flux, decreased catabolism of triglycerides (TG)-rich lipoproteins, TG enrichment of all fractions and an increased proportion of large VLDL, an increase in small LDL (Sf 3-6), and reduction of HDL cholesterol.

Dr B. Eliasson (Göteborg) investigated variables of insulin resistance in healthy middle-aged men, who were chronic smokers or users of nicotine-containing chewing gum. Smokers were insulin-resistant as measured with the clamp technique; they had impaired clearance of TG after a meal, despite normal fasting lipid levels. The proportion of small, dense LDL was increased, as was postheparin hepatic lipase activity. The degree of insulin resistance was correlated to smoking habits. The degree of insulin sensitivity and HDL cholesterol and apoAII concentrations increased significantly 8 weeks after smoking cessation. Chronic nonsmoking users of nicotine-containing chewing gum were also insulin resistant, the degree of which was positively correlated to nicotine consumption as determined by plasma cotinine levels. Thus, nicotine replacement for smoking should be of limited duration.

Dr D. Brümmer (Hamburg) reported on oral fat tolerance tests in patients with mixed hyperlipidemia and controls. He reported abnormal TG clearance in the patients, which was dependent on fasting TG levels and body mass index but independent of their apoE phenotype. Several patients displayed mutations in the LPL gene. Postprandial insulin levels were increased in 8 of 14 patients and correlated with body mass index.

The 20th Annual Meeting of the European Lipoprotein Club was held September 8 to 11, 1997, in Tutzing, Germany. It began with the Anniversary Lectures on Recent Developments in Lipoprotein Research, followed by sessions on: 1) Lipoprotein Receptors (Old and New); 2) Significance of Lipoprotein Heterogeneity (Metabolic and Pathological Aspects); 3) Novel Methodologies for Lipoprotein Research. For information contact Prof. Guido Franceschini, Secretary ELC, Centro E. Grossi Paoletti, Institute of Pharmacological Sciences, Via Balzaretti 9, 20133 Milan, Italy. Fax: 39/2/29007018. E-mail francesc@isfunix.farma.unimi.it