The 16th Annual Meeting of the European Lipoprotein Club

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The European Lipoprotein Club met September 13 to 16, 1993, in Tutzing, Germany. There were 97 participants from 14 European countries, Israel, South Africa, and the United States. This report summarizes the presentations of the main speakers and a selection of the remaining contributions in the various sections.

Dr Hans A. Dresel (Boehringer Mannheim GmbH) opened the meeting with a state-of-the-art lecture entitled “New Strategies for the Discovery and Development of Antioxidant Compounds for Treatment of Atherosclerosis.” He briefly introduced the audience to the so-called low-density lipoprotein (LDL)-oxidation hypothesis by summarizing experiments from various groups and results from epidemiologic studies as well as events and mechanisms to explain the promotion of LDL modification in the proatherogenic state. Dr Dresel discussed three strategies for the discovery and development of new antioxidant pharmaceuticals with antiatherosclerotic activity. In particular, he mentioned compounds with dual antioxidant and acyl-coenzyme A cholesterol acyltransferase (ACAT) inhibitory activity, an antioxidant inhibitor of the 15-lipoxygenase enzyme, and carvedilol, a new cardiovascular drug with multiple actions and highly potent hydroxycarbazole antioxidant metabolites.

ACAT inhibitors can be developed as lipid-lowering drugs more rapidly than exclusive antioxidants. BM 150639 is an analogue with close similarity to probucol but without lipid-lowering activity in rats, hamsters, rabbits, Watanabe heritable hyperlipidemic (WHHL) rabbits, and dogs. Its antioxidant activity is comparable to probucol in the LDL oxidation assay with Cu²⁺ ions, but it displays a greater than 10-fold higher potency than probucol in inhibiting LDL oxidation by purified 15-lipoxygenase and by cultured endothelial cells. BM 150639 was compared with probucol in single- and multiple-dose studies in rats. It is much better absorbed than probucol and exhibits protective activity in a rat postischemic kidney model and in a rat acute respiratory distress syndrome model. Treatment of cholesterol-fed atherosclerotic rabbits for 10 days with 0.2% BM 150639 did not alter the serum cholesterol levels but decreased metabolism of radiiodinated LDL in the atherosclerotic lesion by more than 50%. Pilot studies during 8 months with 0.05% BM 150639 and 1% probucol confirmed the known antiatherosclerotic activity of probucol but could not demonstrate inhibition of atherosclerotic progression in the BM 150639–treated animals. The serum levels of both drugs were nearly identical during the whole study period. The reasons for the failure of BM 150639 to inhibit atherosclerosis need to be investigated in further experiments.

Dr Dresel reported further that 15-lipoxygenase–transfected U937 monocytes oxidized LDL during a 48-hour incubation period but did not result in accelerated LDL oxidation when studied either in the fresh state or after induction with interleukin (IL)-4. These studies did not provide evidence for an essential role of 15-lipoxygenase in the LDL oxidation process in monocyte cultures.

Carvedilol was launched as a new vasodilatating β-blocker. Several metabolites are 10- to 100-fold more potent inhibitors of LDL oxidation in P388D.1 macrophage cultures. Treatment of 8-month-old WHHL rabbits with 5 mg carvedilol twice daily for 9 days resulted in a drastic decrease of LDL metabolism in the atherosclerotic thoracic aorta of the rabbits. Carvedilol treatment reportedly antagonizes the growth stimulatory effect of thrombin, platelet-derived growth factor, and epidermal growth factor on cultured smooth muscle cells in vitro and neointima formation in vivo (Ohlstein et al, Proc Natl Acad Sci USA. 1993;90:6189-6193). The antiatherosclerotic activity of carvedilol will now be investigated in clinical studies.

The first session of the meeting on “Modified Lipoproteins and Immunological Reactions in Atherogenesis” began with an excellent presentation from Dr Joseph Witztum (San Diego, Calif). He pointed out that in vitro “oxidized” LDL is not a single, homogeneous entity but rather a complex series of particles at various stages of oxidation, culminating in a lipoprotein particle that is no longer recognized by the LDL receptor but that can be internalized through macrophage scavenger receptors. Although this process can ultimately lead to foam cell formation, the accelerated uptake of these modified lipoprotein particles may in fact represent a defense mechanism of the organism. The damage that finally leads to formation of atherosclerotic lesions may in fact be initiated by products formed much earlier in the oxidation cascade. One caveat to these findings is the fact that the mechanisms leading to in vivo oxidation of LDL may bear little resemblance to the in vitro models.

Dr Witztum described experiments on the apolipoprotein (apo) E–deficient mouse strain, further supporting the hypothesis that oxidized LDL is involved in the pathogenesis of atherosclerosis. These animals rapidly develop atherosclerosis, the extent of which can be readily quantified in the aortas after staining. Both early and late complex lesions can be observed in the same region of the aorta. Using specific antibodies against
malondialdehyde (MDA)-lysine and 4-hydroxynonenal-lysine, adducts that are formed on oxidation of LDL, the presence of this epitope could be demonstrated in lesions. These animals should therefore serve as a valid model to test the effects of antioxidants on the development of atherosclerosis.

Dr Witztum then reviewed the immunologic consequences of modified LDL. With use of a competitive radioimmunoassay, antibodies to MDA-modified LDL (MDA-LDL) could be demonstrated in humans in individuals with and without coronary heart disease. When immunoglobulin (Ig) G fractions against MDA-LDL were isolated from human plasma, they were found to bind to the atherosclerotic lesion. Furthermore, complement colocalized with these immunoglobulins in the lesion. Immunoglobulins bound to MDA-LDL but not to native LDL could also be isolated from the lesions of WHHL rabbits. Fatty streak lesions in the apoE-deficient mouse revealed an intense staining for IgG and IgM antibodies against MDA-LDL, and high titers of circulating antibodies that recognized epitopes on MDA-LDL were also detected in these animals. In a recent study of eastern Finnish men the titer of antibodies to an epitope of oxidized LDL was highly predictive of the subsequent progression of carotid atherosclerosis.

The final part of his talk focused on a novel antioxidant, aminoguanidine. By analogy to its use in preventing the formation of advanced glycosylation end products in diabetes, this compound was conceived as a potential reactant to trap and detoxify aldehydes formed during the oxidation of LDL. Surprisingly, aminoguanidine was also found to be capable of inhibiting copper-induced oxidation of freshly prepared LDL. If, however, the LDL had been stored for some time and therefore contained small amounts of hydroperoxides, then aminoguanidine acted as a prooxidant.

Dr A. Kontush (Hamburg, Germany) described a study aimed at delineating the regions of LDL that are protected by the antioxidants α-tocopherol and ubiquinone 10 (Q-10). The decrease in the fluidity of the LDL particle, measured with the aid of fluorescent probes, was used as an index of oxidative modification. The fluorescent probes used were 1,6-diphenyl-1,3,5-hexatriene (1,6-DPH), which primarily locates in the lipoprotein core, and 1-(4-(3-methylamino)phenyl-6-phenylhexa-1,3,5-triene (TMAO) and 12-(9-anthroyloxy)stearic acid, which are directed to the surface monolayer. Fresh LDL was enriched if necessary with the antioxidants, supplemented with one of the fluorescent probes, and oxidized with copper ions. One mole of α-tocopherol per LDL particle was found to increase the lag phase of oxidation by 4 minutes, while 1 mole of Q-10 per LDL particle increased the lag phase by 96 minutes. Enrichment of LDL with Q-10 significantly protected the LDL core and monolayer against oxidative modification, whereas supplementation with tocopherol primarily protected the LDL-surface monolayer.

Dr A. Tailleux (Lille, France) studied the conformation of LDL oxidized in vitro with copper ions or enzymatically modified with phospholipase A2 and lipoygenase. Conformational changes were investigated by examining altered immunoreactivity to seven monoclonal antibodies against epitopes of apoB-100 and by studying changes in the binding of modified LDL to the B/E receptor on HeLa cells and fibroblasts and to macrophage scavenger receptors. Chemically oxidized LDL displayed an enhanced accessibility of the epitope located in the B/E receptor binding domain that was accompanied by a marked reduction in the binding of the modified lipoprotein to the B/E receptor on fibroblasts and HeLa cells. Enzymatically modified LDL displayed qualitatively similar changes, although to a lesser extent. Both oxidative processes also led to alterations in the accessibility of epitopes far removed from the B/E receptor binding domain, in both the N- and C-terminal regions of apoB-100, which may also influence the interactions between the receptor and its ligand. These conformational changes are also accompanied by an enhancement in the degradation of chemically oxidized LDL by mouse peritoneal macrophages.

Dr H. Kleinveld (Nijmegen, the Netherlands) described experiments to determine the factors that protect LDL against oxidation in the basal unsupplemented (no exogenous vitamin supplementation) state. LDL was isolated from α-tocopherol-deficient patients and healthy control subjects. Although LDL of the patients had a significantly lower content of this vitamin than control LDL, the former did not display enhanced susceptibility to copper-mediated oxidation. In contrast, mean lag time was longer, mean oxidation rate slower, and mean maximum diene content lower in the α-tocopherol–deficient LDL. In contrast to control LDL, α-tocopherol–deficient LDL had a lower linoleic acid content and a higher oleic acid content. The ratio of oleic acid to linoleic acid was found to be positively correlated with the lag time and negatively correlated with both the rate of oxidation and the maximum diene production. A high oleic acid content therefore reduced the susceptibility to oxidation, whereas a high linoleic acid content increased the susceptibility.

Dr P. Holvoet (Louvain, Belgium) presented results on the effects of monoclonal antibodies specific for chemically modified LDL (acylated [Ac]-LDL, MDA-LDL) on the uptake of these LDLs by THP-1–derived macrophages. The murine monoclonal antibody mAb4E4 that is specific for both modified LDLs was found to bind to modified LDL in human carotid atherosclerotic lesions. This monoclonal antibody is apparently directed against an epitope that is poorly exposed in delipidated and solubilized apoB-100 from modified LDL. mAb4E4 as well as its F(ab')2 and Fab fragments enhanced the uptake of both Ac-LDL and MDA-LDL by THP-1–derived macrophages, causing a concentration-dependent increase in cholesteryl ester content. Experiments with cytochalasin D revealed that the uptake of the immune complexes was not due to phagocytosis, nor did it occur via the Fc receptor. Their uptake was, however, inhibited when the scavenger receptors were blocked with fucoidin or were downregulated with endotoxins or interferon gamma. He concluded that generation of autoimmune antibodies against modified LDL and subsequent endocytosis of soluble modified LDL immune complexes via scavenger receptors may enhance foam cell formation.

Dr C. Decossin (Lille, France) described her results on the effect of apoAI-containing lipoproteins LpAI and LpAI:AI on the copper-catalyzed oxidation of human LDL. The oxidation kinetics for LDL was followed by measuring the absorption at 234 nm, and the extent of the oxidative changes was estimated by electrophoretic mobility, thiobarbituric acid–reactive substance levels, and fragmentation of apoB-100 assessed by sodium dodecyl sulfate–polyacrylamide gel electro-
Phospholipidosis. The lipoprotein LpAI:AII did not protect LDL against copper-catalyzed oxidation. LpAI that was free of albumin was also unable to protect LDL against oxidation. However, the subtraction of albumin containing LpAI particles inhibited the oxidation of LDL in an albumin dose-dependent manner.

Dr T. Koschinsky (Düsseldorf, Germany) reported the presence of high concentrations of advanced glycosylation end products (AGEs) in LDL of diabetic patients with and without end-stage renal disease (ESRD). In the diabetic subjects mean concentrations of AGEs in both the apoB and lipid components of LDL increased with increasing number of complications, the highest levels being observed in ESRD diabetics. AGE modification may therefore be involved in the development of diabetic microangiopathy and macroangiopathy. Antibodies against AGE-LDL could be detected in approximately 30% of both normal subjects and type 1 and type 2 diabetics. The prevalence of positive titers increased with age in diabetics but not in normal subjects. There was also a significant increase in the IgA titer in diabetics versus normal subjects but not in the IgG or IgM titers. Interestingly, in diabetics with positive autoantibody titers there was a significantly lower proportion of HLA-DR4-positive individuals, suggesting that in both type 1 and type 2 diabetics the immune response to AGEs is related to genetic differences.

Dr Gerd Schmitz (Regensburg, Germany) began the second part of this session with a stimulating lecture entitled "Genetic Diseases of Lipid and Lipoprotein Metabolism Affect the Immunological Reactivity of Mononuclear Phagocytes." In the first part of his talk, he described the differentiation of multipotent stem cells to macrophages and the differentiation-dependent gene expression of macrophages. The recruitment of monocytes by transendothelial migration into the intima in the early lesion and through migration from the vasa vasorum in the advanced lesion plays a central role in the development of atherosclerosis. In the lesion, mononuclear macrophages are found as highly differentiated cells involved in both lipid metabolism and the inflammatory reaction through the secretion of cytokines. In general, the process of monocyte and macrophage differentiation is an irreversible process of cellular development along an increasingly restricted lineage. This process is associated with characteristic changes in gene expression and changes in the expression density of receptors correlated with both the inflammatory reactivity and the lipid metabolism of mononuclear phagocytes. Such receptors are the complement receptors and complement-regulatory proteins, the lipopolysaccharide (LPS) receptors, the Fcy-receptors, the scavenger receptor (type I and II), and the LDL receptor. Concomitant macrophages also acquire tissue-specific functional characteristics; eg, the metabolic phenotype of spleen and peritoneal macrophages is related to their sterile and anaerobic environment or the defense-oriented phenotype of the alveolar macrophage, which resides in an infectious and aerobic environment. Genetic diseases of lipid and lipoprotein metabolism are associated with different risks for the development of the expansion of activated macrophages in the atherosclerotic lesion of the vessel wall. In addition, a selective expansion of macrophages is also observed in other tissues, eg, in xanthomas of the skin in the asymptomatic high-density lipoprotein (HDL) deficiency with plane xanthomas or in the reticuloendothelial system and spleen in Tangier disease with or without the simultaneous development of atherosclerosis.

In the second part of his talk, Dr Schmitz discussed the characterization of specific cellular phenotypes of mononuclear phagocytes, which may reflect the different atherogenic risk and tissue targeting of monocytes in these genetic disorders of lipid and lipoprotein metabolism. For this purpose the expression of activation and differentiation antigens, cellular adhesion antigens, and functional cell parameters of patient monocytes was analyzed by flow cytometry. It is important for the interpretation of the cellular phenotypes that monogenic disorders, such as the generalized cellular LDL receptor defect in familial hypercholesterolemia (FH), not only affect the cellular lipid metabolism but also are associated with disturbances of eicosanoid synthesis and gene regulation. This leads to a syndrome of polygenic cellular dysregulation during differentiation. Thus, circulating peripheral blood monocytes of patients with FH (in contrast to cells from hypercholesterolemic patients with a normal LDL-receptor expression) were characterized by an abnormal surface antigen density of cellular differentiation antigens with a high density of CD4 and low density of HLA-DR, corresponding to a less mature cellular phenotype. Simultaneously, in FH patients the size of the more mature CD14+/CD16− "premacrophage" subpopulation of blood monocytes was reduced, suggesting a selective depletion of mature cells from the circulation. In a patient with cholesteryl ester storage disease (CESD), who had a 72-bp deletion of the acid lipase gene leading to hypercholesterolemia, early onset of atherosclerosis, and hepatosplenomegaly, the decrease of the CD14+/CD16+ subpopulation was accompanied by the appearance of an abnormal CD14+++/CD16+ double-positive subpopulation, indicating an aberrant maturation of mononuclear phagocytes. Simultaneously, CD1a antigen expression was increased on CESD monocytes, suggesting a dendritic cell-type differentiation of monocytes as a result of the lysosomal metabolic defect. Tangier disease is a cellular defect in processing HDL precursors to mature HDL, which is related to an abnormal cellular signal transduction. The accumulation of mononuclear phagocytes in the reticuloendothelial system and spleen in Tangier disease with or without the simultaneous development of atherosclerosis, are the prominent clinical features of this disease. Tangier monocytes exhibited a selective increase of the alternatively spliced extracellular matrix receptor III (CD44) but a normal expression of the integrin family of adhesion antigens. CD44 expression was further increased after dietary lipid exposure in Tangier patients but not in control subjects, together with the occurrence of a CD1a-positive subpopulation. These results suggest an abnormal regulation of CD44 expression on Tangier monocytes, which may correlate with the reticuloendothelial system accumulation of the abnormal macrophages in Tangier disease. Thus, in several genetic disorders of lipid and lipoprotein metabolism phenotypic defects of circulating blood monocytes have been identified, which may correlate with the immunologic reactivity and tissue accumulation of macrophages. These parameters represent interesting candidates for the analysis of the mechanism of atherogenesis and the characterization of mechanisms for a possible therapeutic intervention.

Dr O. Wiklund (Göteborg, Sweden) reported the effect of modified lipoproteins (oxidized LDL [Ox-LDL] and
Ac-LDL) on the expression of a series of surface epitopes on human monocyte-derived macrophages by fluorescence flow cytometry. During differentiation of monocytes in vitro, the expression of HLA-DR did not change; there was an increasing expression of CD11a, CD11c, and CD14, while CD15 was expressed only in monocytes and early macrophages. Parallel with differentiation, there was also an increase in the binding of Ac-LDL, reflecting an increase in expression of the scavenger receptor. After different times in culture, cells were exposed to Ac-LDL and Ox-LDL for 24 hours. Ac-LDL had no effect on the studied epitopes. On the other hand, Ox-LDL induced a dose-dependent decrease in the surface expression of CD14 (endotoxin receptor), whereas that of CD71 (transferrin receptor) increased. CD14 mediates the release of transcription factor for cytokines. Therefore, the effect of Ox-LDL on LPS-induced tumor necrosis factor secretion was investigated. From cell incubations at day 2 and day 8 with different mixtures of Ox-LDL and LPS, it was clear that the responsiveness of the monocytes to LPS decreased when these cells were preincubated with Ox-LDL. Thus, Ox-LDL has specific effects on macrophage differentiation, which may be important for the function of macrophages in the atherosclerotic tissue.

Dr G. Rothe (Regensburg, Germany) found that modified lipoproteins induce a selective increase of the Fcγ-RIII (CD16) and CD4 expression in the presence of Ox-LDL, suggesting a phagocytic differentiation of monocytes. In contrast, a large increase of the LPS receptor (CD14) and the CD44 adhesion antigen for extracellular matrix was observed in the presence of Ac-LDL. Dr Rothe concluded that modified lipoproteins may be major factors for the modulation of mononuclear phagocytes within the vessel wall through their specific interaction with the differentiation and function of mononuclear phagocytes.

Dr J. Frostegård (Stockholm, Sweden) reported that Ox-LDL enhanced adhesiveness in both endothelial cells and monocytes and induced differentiation of monocytic cells to macrophages. Supernatants from monocytic leukemia exposed to Ox-LDL induced an increased expression of vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, and endothelial-leukocyte adhesion molecule (ELAM) in endothelial cells. Ox-LDL was found to activate T cells as assessed by an increased DNA synthesis and an increased expression of HLA-DR and IL-2 receptors. Ox-LDL induced a significantly lower T-cell activation in patients with atherosclerosis due to tolerance or anergy. Dr Frostegård also discussed the effect of Ox-LDL on the expression of heat-shock proteins, which are involved in the immune response to pathogens and autoimmune reactions. The monocytic cell lines U937 and HL60 treated with Ox-LDL gave a strong increase in the expression of heat-shock protein, analyzed with a fluorescence-activated cell sorter. This increased expression may evoke an autoimmune reaction.

Dr T. Kuusi (Helsinki, Finland and Seattle, Washington) showed that unstimulated human cells display uniform binding of fluorescently labeled Ac-LDL up to 4 days. After this time a varying proportion of the cells developed the appearance of large mature macrophages, whereas others remained small, retaining the morphology of the initial monocytes. Surprisingly, Ac-LDL was taken up to a limited extent by the large macrophages, whereas the small cells displayed intense uptake of Ac-LDL, labeled either in the protein or lipid moiety. These small fluorescent cells were loosely adherent and could be transferred easily to a new plate. Approximately 40% of the degradation of $^{125}$I-Ac-LDL was shown to be due to the small, loosely adherent cells. Thus, human monocytes develop in cell culture into at least two different cell populations with different morphology and probably different functions.

Dr P. Kovanen (Helsinki, Finland) discussed the participation of mast cells in foam cell formation and the mechanism by which mast cells may modify LDL and render it susceptible to uptake by macrophages. He showed that stimulation of rat serosal mast cells induced a 50-fold increase in LDL uptake by preincubated macrophages. The mechanism behind this phenomenon is a novel, mast cell granule-mediated carrier mechanism, in which (1) in the extracellular fluid the exoytosed cytoplasmic secretory granules transform into granule remnants, which consist of heparin proteoglycans and neutral proteases; (2) LDL binds to the heparin proteoglycans of granule remnants; (3) apolipoprotein 100 of LDL undergoes proteolysis by granule remnant neutral proteases; (4) the proteolysed LDL particles fuse on granule remnant surface; and finally (5) the macrophages phagocytose the granule remnants laden with fused LDL particles. In addition, the remnant enzymes cause the proteolysis of HDL particles, thus lessening their ability to induce efflux of cholesterol from the foam cells. Mast cells thus play an active role in the intracellular deposition of choleseryl esters that is characteristic of early atherogenesis.

The topic of the second session of the meeting was “Lipoproteins and Risk Factors in Postmenopausal Women.” It is well recognized that cardiovascular disease is the leading cause of death among postmenopausal women. Consequently, the changes of lipoproteins and risk factors after menopause and the impact of hormone replacement therapy is of major interest and a critical issue with respect to prevention of coronary heart disease (CHD). Dr Marja-Riitta Taskinen (Helsinki, Finland) gave an overview on this topic. In menopause the lipoprotein pattern becomes more atherogenic, with increases in very-low-density lipoprotein (VLDL) triglyceride and LDL cholesterol and lowering of HDL cholesterol compared with the premenopausal state. Estrogen replacement therapy can reverse these adverse changes. In general, changes of lipoproteins induced by progestin administration are opposite to those of estrogens. However, recent data by Nabuls et al. (N Engl J Med. 1993;328:1069-1075) demonstrated that the combination of estrogen with progestin was associated with a better profile than estrogens alone. A controversial issue has been the selection of the optimal progestin. It should be noted that the response is dependent not only on the structure of the compound but also on the dose, duration, and mode of administration. Dr Taskinen highlighted the data from various epidemiologic studies regarding the effects of postmenopausal estrogen on CHD risk. The benefit is confirmed in the majority of these studies. Recent meta-analyses by Stampfer and Colditz demonstrated that overall the relative risk was 0.50 (95% confidence interval, 0.43 to 0.56) in the internally controlled prospective and angiographic studies. The open question is whether added progestin alters the effects of estrogen on CHD risk. Recent data from the Uppsala Health Care Region
Study also suggest that combination therapy reduces CHD risk (Falkeborn et al, Br J Obstet Gynaecol. 1992;99:821-828). The available data point out that changes of lipoproteins explain only part of the benefits. Emerging evidence indicates that estrogens have direct effects on endothelial cells and that these positive actions are not negated by the combination with progestins (Haarbo et al, J Clin Invest. 1991;87:1274-1279). In particular, the action of estrogens on coronary artery dynamics is a topic of intense research.

Dr J. Heinrich (Münster, Germany) demonstrated that postmenopausal women in the PROCAM Study had higher blood pressure and body mass index than premenopausal women, consistent with previous studies. Postmenopausal women showed increased levels of fibrinogen, factor VIIc, antithrombin III, and protein C. The data also demonstrated that plasminogen activator inhibitor-1 activity was age dependent in women. Overall, these changes may predispose the postmenopausal women to the risk of thrombosis and may partly explain the excess CHD risk.

Dr T. Kuusi (Helsinki, Finland) reported that subcutaneous estradiol treatment rapidly normalized serum estrone and estradiol but had no effects on either serum sex-hormone binding globulin (SHBG) or serum testosterone concentrations. This was followed by a decrease of serum LDL cholesterol, whereas the HDL cholesterol was unaffected. Surprisingly, hepatic lipase was unaffected by subcutaneous estradiol treatment lacking the first-pass hepatic metabolism of poreral treatments, thus explaining the lack of effect by this treatment on serum HDL. The decrease of LDL cholesterol in this study suggests that the hepatic LDL receptors are highly susceptible to regulation by estrogens.

Dr U. Arca (Rome, Italy) presented studies on LDL metabolism in postmenopausal women with LDL cholesterol above 160 mg/dL but no signs of FH or remnant hyperlipoproteinemia. Their LDL cholesterol/apoB ratios were similar to those in normocholesterolemic postmenopausal control women, indicating an increase of LDL particle number as a cause of LDL elevation. The kinetics of autologous 125I-LDL revealed a significant reduction of the fractional catabolic rate of LDL in postmenopausal hypercholesterolemia, whereas the production rate of LDL was comparable to that of control women. Dr Arca also summarized previous knowledge about LDL kinetics in different female age groups (<30, 30 to 50, and >50 years). With increasing age, the 125I-LDL fractional catabolic rate is decreased 0.435, 0.361, and 0.281, respectively. Downregulation of the metabolic LDL receptor takes place along with increasing age in women.

The third topic of the meeting was “Genetic Disorders Affecting LDL Metabolism.” The aim of this part of the meeting was to explore what has been learned about the extent to which the degree of hyperlipidemia in a patient and the severity of the associated cardiovascular disease is determined by genetic and environmental factors.

In the first session Dr Anne Soutar (London, England) summarized what is known about the structure and function of the LDL receptor protein and its gene and the way in which different mutations can produce a wide variety of defects in the protein, ranging from those that would result in a total absence of LDL-receptor function (receptor-negative) to those that would result in a recep-
with increasing age in the same way as in unaffected individuals. Dr V. Gudnason (London, England) reported the observation that mutations in exon 4 of the gene were particularly common, with no fewer than 15 different base substitutions or minor deletions lying in a stretch of only 50 bases. Since this region codes for a crucial region of the binding domain of the protein, this frequency could represent selection bias, and in support of this was the observation that patients with a mutation in this region of the gene had significantly higher lipid levels than those with a mutation elsewhere. Dr Gudnason showed that patients with a null allele had a slightly lower total cholesterol, higher HDL cholesterol, and lower triglyceride levels than those with a defective allele and might, therefore, be at lower risk of developing coronary disease.

Dr D. van der Westhuizen (Cape Town, Republic of South Africa) widened the discussion to beyond Europe and, perhaps of more importance, to a population in which a founder gene effect has increased the frequency of FH to 1 in 50, compared with 1 in 500 in most populations, and has resulted in only a few different mutations being responsible for the disease in the majority of patients. In this population it is possible to draw significant conclusions about the effect of a particular mutation on the phenotype of the disease, bearing in mind that the individuals concerned are also likely to have a genetic background in common that could influence coronary risk. From studies in heterozygous FH Afrikansers with one of three different mutations, Dr van der Westhuizen and collaborators from the University of Stellenbosch were able to show that the nature of the mutation did indeed influence the plasma LDL cholesterol concentration but found that this in itself was not a particularly informative predictor of premature CHD, although it was for tendon xanthomas.

The next part of the session focused on variation in the apoB gene in determining levels of LDL cholesterol. Dr A. Attie (Madison, Wis) summarized studies on pig allo-antisera to eight distinguishable alleles of the apoB protein, one of which was associated with elevated plasma cholesterol levels (Rapacz et al, Science. 1986;234:1537-1577). In this hypercholesterolemic animal, skin fibroblasts showed normal LDL-receptor activity, while LDL from the mutant pig showed defective clearance and significantly reduced binding affinity for the LDL receptor, suggesting that the mutation resulted in production of LDL particles that were dysfunctional, and implying that there may be a mutation in the apoB gene itself. The apoB gene from the pig carrying this mutant allele, called Lpb5, was examined for sequence changes in exons 26 to 29, with a total sequence analysis of 10.5 kb. Thirteen different mutations altering amino acid sequences were identified, but none of these single amino acid changes were unique to the gene from the mutant Lpb5 pig. However, comparison with the sequence of other alleles revealed that a haplotype defined by a change to Asp3164 and Ala3417 was unique to the gene from the mutant Lpb5 pig, raising the possibility that these two amino acids acting together may affect the function of the LDL particle (Purcell et al, J Lipid Res. 1993;34:1323-1346). There are similarities between this observation and the situation in human apoB, where two amino acid changes (Pro122 to Leu and Asn3491 to Ser) together define one of the functional variants of apoB that is detected by antibodies, the Ag(x) epitope of the antigen series Ag(x,y) (Dunning et al, Am J Hum Genet. 1992;51:208-221). The nature of the dysfunctional LDL was explored further by examining the pH of the two chemically distinct pools of lysine residues in pig apoB, one of which in human apoB has been shown to be involved in binding to the LDL receptor. In pigs with the Lpb5 mutation, these two pools of lysine residues can also be detected, but the pool with the pH of 8.9 is reduced significantly in the dense LDL subfractions. The buoyant LDL in the mutant pig binds well to the receptor, and it appears that the mutation is in some way causing the production of increased amounts of dense LDL. To examine this hypothesis further, turnover studies were carried out, which gave the surprising result that in the mutant pig there is an expansion of the pool size of buoyant LDL caused by an increase in direct production of this buoyant LDL, but that this does not appear to be the cause of the large increase in the pool size of the dense LDL (Checovich et al, Arterioscler Thromb. 1991;11:351-361). Further analysis demonstrated that there were two classes of animals that carried the mutant Lpb5, some of which had low cholesterol and some of which had high cholesterol levels. In the mutant pig, all LDL was cleared through nonreceptor pathways, suggesting that the receptor is highly downregulated in this animal. Since earlier studies in skin fibroblasts demonstrated apparently normal receptor activity, it appears that this mutation is effective only in vivo, or possibly acting only in the liver. Breeding experiments and complex segregation analysis with the data from the large pedigrees available have produced evidence for a separate locus determining hypercholesterolemia in this strain of pigs, with this mutant gene acting in a recessive fashion. The exact nature of this mutation is unknown. To examine further the effects of the apoB mutations identified, an in vitro expression system for apoB is required, using constructs with the appropriate sequence changes. Dr Attie presented data from the baculovirus/insect cell system, which he believes will be extremely useful in the future for carrying out such expression studies for apoB. The virus contains a natural strong promoter for the capsid protein, is tolerant of large insertions, and gives a high degree of expression in cultured insect cells. The system has the added advantage that the virus can be used to infect the Manduca sexta caterpillar larvae, which then produce large amounts of the protein in hemolymph. The hemolymph contains a lipoprotein particle called lipophorin, which has a density in the "HDL" range and is used in triglyceride metabolism in the insect, and later in the moth, for transport of triglycerides to provide energy for flight. The particle contains two catterpillar apoproteins, and it is of interest that their x-ray crystallography structure shows similarities with that of human apoE. The system has been used to express human apoE (Gretch et al, Proc Natl Acad Sci U S A. 1991;88:8530-8533) with up to 200 g/mL of apoE present in the hemolymph. Experiments are in progress to optimize the expression of two truncated apoB constructs: apoB-17, which is poorly associated with lipids, and apoB-48, which produces stable lipoprotein particles in the hemolymph. The system is also being developed for expression of full-length apoB-100.

The next two topics focused on aspects of familial defective apoB-100 (FDB). Dr A. Tyjéerg-Hansen (Denmark) presented a summary of published data on FDB
patients in terms of both biochemical and clinical characteristics. In both men and women carrying the mutation, there is a significant increase in the plasma cholesterol and LDL cholesterol levels with age of diagnosis, which parallels very well the rise in lipid levels in the population as a whole. On average, LDL cholesterol levels in FDB patients are 3.0 mmol/L higher than expected for their age and sex from the general population, and this is lower than the average difference reported for patients with a clinical diagnosis of FH, which is approximately 4.7 mmol/L. In comparison with FH, HDL cholesterol levels in FDB patients are normal, as are triglyceride levels, and this is explicable by the normal metabolism of apoE-containing lipoproteins. The frequency of coronary artery disease increased sharply as a function of age in patients with FDB; at age 60 the frequency had risen to about 70% in both sexes, which is similar to that in FH patients. By comparison, the corresponding frequencies of coronary artery disease in the general population were 14% and 9% in men and women, respectively. In FDB the frequencies of tendon xanthomas and of arcus corneae rose sharply as a function of age in both men and women after first appearing at age 35 and 40 years, respectively. At age 60, approximately 40% had tendon xanthomas and 35% had arcus corneae irrespective of sex, showing a pattern very similar to that reported for FH patients. Surprisingly, the frequencies of arcus corneae and xanthelasma were not strikingly higher than those found in the general population sample. The frequency of this disorder varies widely in different countries and even within countries. In Germany, the United Kingdom, and Holland the frequency may be as high as 1 in 500 to 1 in 700 in the general population and may be higher in places such as Switzerland.

Dr. März (Frankfurt, Germany) presented details of a 54-year-old man who is homozygous for FDB. Total and LDL-cholesterol concentrations in the patient were 8.8 mmol/L and 7.1 mmol/L, respectively; thus, hypercholesterolemia was less severe than in homozygous FH patients. Surprisingly, the frequencies of arcus corneae and xanthelasma were not strikingly higher than those found in the general population sample. The frequency of this disorder varies widely in different countries and even within countries. In Germany, the United Kingdom, and Holland the frequency may be as high as 1 in 500 to 1 in 700 in the general population and may be higher in places such as Switzerland.

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Dr. L. Havekes (Leiden, the Netherlands) presented work on transgenic mice carrying the human apoE3-Leiden gene. This apoE variant is caused by duplication of codons 120 to 126 in the protein, and in human subjects it is associated with a dominant form of type III hyperlipidemia. Mice with a 27-kb insert were generated containing human apoE, apoCI, and part of the apoCI pseudogene, with this construct containing the liver-specific transcription control element. Several lines were established with either low or high expression of the transgene. At 6 to 12 weeks of age lipid levels reached maximum values, roughly twofold to threefold higher than control mice. The transgenic mice were extremely sensitive to cholesterol and high-fat diets, once again dependent on the level of expression of the transgene. The responses to diet were more severe in males than females during the first 12 weeks of life. Of major interest was the observation that the high-expresser mice developed atherosclerotic lesions in the aortic arch after 12 weeks on the cholesterol and high-fat diet. These lesions varied from fatty streaks containing foam cells to severe atherosclerotic plaques containing cholesterol crystals and necrotic calcified tissue. Development of atherosclerotic lesions was positively correlated with plasma levels of cholesterol and the expression of the apoE3-Leiden gene. Clearly, this transgenic mouse model is extremely useful for studying the relation between the variation in one particular gene and diet, plasma lipid levels, and the development of atherosclerotic lesions.

The 17th Annual Meeting of the European Lipoprotein Club is scheduled for September 12 to 15, 1994, in Tutzing, Germany. It will begin with a state-of-the-art lecture on "Assembly of ApoB: Role of Microsomal Triglyceride Transfer Protein" by Dr. J. Wetterau (Princeton, NJ), followed by three sessions that will examine (1) determinants of postprandial lipoprotein metabolism, (2) interactions of postprandial lipoproteins with peripheral cells, and (3) lipoproteins/arterial wall interactions.

The European Lipoprotein Club Organizing Committee
The 16th annual meeting of the European Lipoprotein Club.
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