PREFACE

A lipoprotein is a biochemical assembly that contains both proteins and lipids. Many enzymes, transporters, structural proteins, antigens, adhesins and toxins are lipoproteins. Examples include the high density (HDL) and low density (LDL) lipoproteins, the transmembrane proteins of the mitochondrion and the chloroplast, and bacterial lipoproteins. This book presents and reviews important data on lipoproteins such as their metabolism and relation to coronary artery disease (CAD), the study of the presence of LP-X in a wide number of liver and biliary tract diseases with cholestatic features, the role of lipoproteins in different stages of women's lives, the role of lipoprotein abnormalities as risk factors for macroangiopathy in type 2 diabetes, and others.

Chapter 1 - Atherosclerosis, along with the resultant coronary artery disease (CAD), is a leading cause of mortality. Significant attention has focused on the role of lipoproteins in the pathogenesis of CAD. Lipoproteins are macromolecular complexes composed of core of lipid molecules (triglycerides and cholesteryl esters) that are covered by a surface monolayer of phospholipids, a small quantity of free cholesterol, and one or more apolipoproteins. Various enzymes and transfer proteins play a key role in lipoprotein metabolism, promoting the exchange of triglycerides (TGs) and cholesteryl esters (CEs) between lipoprotein particles. Genetic polymorphisms that affect blood lipids may have either a greater or lesser than expected effect on vascular disease risk, if those genetic factors also affect other disease mechanisms. Analysis of genetic polymorphisms is therefore particularly useful in families where there is clustering of premature CAD. Whereas these polymorphisms may have a smaller effect in the individual, the higher frequency with which they occur indicates that their impact at the population level may be substantial. This review outlines the various gene polymorphisms which have an effect on circulating lipoproteins and their metabolism and hence may play an important role in pathophysiology of CAD.

Chapter 2 - The proteic component of lipoproteins is more important than the lipidic component in the development of atherosclerosis. Case-control studies showed that apolipoproteins (apo) A-I and apo B are better discriminative factors than lipids for assessing the risk of coronary events. Prospective studies confirmed that the risk of cardiovascular events was 4-5 times higher in patients with elevated apo B levels, when compared to patients with high levels of cholesterol. Those results are theoretically possible since each atherogenic lipoprotein contains one apoB molecule. Measuring the levels of apoB can offer an accurate estimation of total atherogenic lipoprotein level. Statin interventional studies revealed that plasma apoB has a greater prognostic value than HDL cholesterol in assessing the risk for

cardiovascular events. However, a decrease in apoB/apoA-I and total cholesterol/HDL cholesterol ratios can be used as an artificial target in conducting the treatment. Those ratio-derived parameters can only be balanced by increasing the HDL cholesterol plasma levels. Still, apoA-I, apoB, and apoB/apoA-I levels, controlled by genetic, environmental and endocrine factors, reflect the pro and antiatherogenic pathways of lipoprotein metabolism. Consequently, high levels of apoB and elevated apoB/apoA-I ratio, as well as low levels of apoA-I, indicate an increased risk for coronary heart disease.

Chapter 3 - Lipoprotein(a) is an LDL-like lipoparticle having the distinctive multi-kringle apolipoprotein(a). Although the physiological roles of lipoprotein(a) have been somewhat elusive, its pathological effects, closely related to plasma concentrations, have been widely studied.

Several variants of the LPA gene contribute to its differential expression, and to lipoprotein(a) levels and pathogenicity. Although most of the variations in lipoprotein(a) concentrations are under genetic control, a relationship between plasma levels, apolipoprotein(a) phenotypes, anthropometric and biochemical factors, and environmental-associated events has been reported in many studies.

Study of transgenic animals, which bypasses the absence of lipoprotein(a) in common laboratory animals, is an excellent model to examine the function of increased plasma lipoprotein(a) in differing pathological conditions or in cases of dietary intervention.

This chapter offers an overview of some of the non-genetic factors which have modest, albeit significant, effects on lipoprotein(a) levels, also assessing their possible interactions with specific apolipoprotein(a) genotypes. The effects of estrogen-replacement therapy and dietary interventions in the modulation of lipoprotein(a) levels, and the influence of age are evaluated, taking into account their implications in the atherogenic risk.

Lastly, the controversial role of lipoprotein(a) as an acute phase reactant and, in particular, its possible beneficial role in surgical trauma are discussed.

Chapter 4 - Low-molecular-weight aldehydes (glyoxal, methylglyoxal) generated on autooxidation of glucose under conditions of carbonyl stress react much less actively with amino groups of L-lysine and ε-amino groups of lysine residues of apoprotein B-100 in human blood plasma low density lipoproteins (LDL) than their structural analogs — malonic dialdehyde (MDA) resulting on free radical oxidation of lipids under conditions of oxidative stress. Using the methods of EPR spectroscopy and lucigenin-dependent chemiluminescence, it has been shown that non-enzymatic generation of free radicals including superoxide anion radical takes place during the interaction of L-lysine with methylglyoxal at physiological pH values. In the course of analogous reaction of L-lysine with MDA, the formation of organic free radicals or superoxide radical was not observed. Glyoxal-modified LDL aggregate in the incubation medium with a significantly higher rate than LDL modified by MDA, and MDA-modified LDL are markedly more poorly absorbed by cultured human macrophages and significantly more slowly eliminated from the rat bloodstream upon intravenous injection. Studies on kinetics of free radical oxidation of rat liver membrane phospholipids have shown that ubiquinol Q_{10} is the most active lipid-soluble natural antioxidant, and suppression of ubiquinol Q_{10} biosynthesis by β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors (statins) is accompanied by intensification of lipid peroxidation in rat liver biomembranes as well as in LDL of human blood plasma. Injection of ubiquinone Q_{10} protects the human blood plasma LDL against oxidation and prevents oxidative stress-induced damages to rat

myocardium. A unified molecular mechanism of atherogenic action of carbonyl-modified LDL in disorders of lipid and carbohydrate metabolism is discussed.

Chapter 5 - Lipoprotein X (LP-X) is an abnormal subclass of low density lipoprotein (LDL) rich in phospholipid and nonesterified cholesterol but poor in cholesteryl esters, triglyceride, and proteins. The protein component of LP-X is dominated by albumin and apolipoprotein C. LP-X was firstly found in the blood of patients with liver disease with obstructive jaundice. Further studies demonstrated the presence of LP-X in a wide number of liver and biliary tract diseases with cholestatic features, and concentration of LP-X in the blood has been considered as an important and informative marker of cholestasis. In addition, high levels of LP-X were detected in familial lecithin: cholesterol acyltransferase deficiency. Moreover, our recent studies demonstrated the increased levels of LP-X in other diseased conditions including ischemic stroke and familial Mediterranean fever. *In vitro* and *in vivo* studies shown that in addition to antiatherogenic activities detected in primary biliary cirrhosis, at least in three pathologic conditions, e.g. familial LCAT, ischemic stroke and familial Mediterranean fever, LP-X acts as trigger of inflammation. However, the cause of the appearance of lipoprotein X and its involvement in disease-associated pathomechanisms is still unclear. The present chapter summarizes the existing knowledge on LP-X and its implication in disease-associated molecular pathomechanisms.

Chapter 6 - Cardiovascular disease, especially atherosclerosis is a consequence of multiple metabolic disorders. Polycystic ovary syndrome (PCOS) is the most common hormonal disorder of reproductive aged women, affecting 5–10% of this population. PCOS traditionally a reproductive disorder showing hyperandrogenism, infertility and chronic anovulation is now considered as a multifaceted disease with metabolic and cardiovascular long term consequences. Dyslipidemia, hypertension, endothelial dysfunction, low grade chronic inflammation are risk factors for cardivascular problems. Dyslipidaemia is very common in patients with polycystic ovary syndrome. It has been suggested that atherogenic lipoprotein abnormalities may be found in one third of women with PCOS who have normal lipid profile. Determination of high risk population may be important for preventive therapy and may provide reduction of cardiovascular risks. Lifestyle interventions play an important role in the prevention of metabolic complications. Moderate–intensity exercise without significant weight loss was found to have beneficial effects on lipoprotein profiles of women with PCOS.

Metabolic syndrome is associated with increased risk for cardiovascular disease and type 2 diabetes mellitus. The role of lipoproteins is important in the management of the syndrome. Many combinations of drugs have been studied to treat dyslipidemia and reduce cardiovascular risk. Aging and menopause are the main causes that lead to an adverse lipid profile in women. The incidence of coronary heart disease increases after menopause and obesity may exaggerate the unfavorable lipid profile. Diet and lifestyle changes should be recommended as first line therapy. Treatment should be individualised for each patient and if the lipid profile can not be improved drug therapy should be added. The importance of the lipoproteins should be kept in mind to lower coronary heart disease in stages of women life.

Chapter 7 - Plasma lipoprotein cholesterol homeostasis is regulated by genetic predispositions and environmental factors. Pathological plasma lipoprotein cholesterol levels play a key role in the development and pathogenesis of human atherosclerotic cardiovascular diseases. Animal models showing aberrant plasma cholesterol levels are used for the identification and analysis of novel causative genes/alleles. Plasma cholesterol was

successfully used as a screening parameter in several phenotype-driven N-ethyl-N-nitrosourea (ENU) mouse mutagenesis projects for the systematic, genome-wide, large-scale production and analysis of novel mouse mutants as animal models for inherited human diseases. This was done by random mutagenesis of a large number of mice using ENU and subsequent high-throughput screening of their offspring for increased or decreased plasma cholesterol levels. Transmission of the altered phenotype to subsequent generations led to the establishment of lines showing hypercholesterolemia or hypocholesterolemia. Thus, novel panels of ENU-derived mutant mouse lines were established to be used for the identification of genes/alleles selectively influencing plasma cholesterol homeostasis which may have counterparts relevant for human diseases.

Chapter 8 - Hypertriglyceridemia, diminished HDL content and the presence of small dense LDL are common features of atherogenic lipoprotein phenotype. Besides, the accumulation of nascent HDL with pre-beta electrophorertic mobility is suggested to reflect the deficiency of reverse cholesterol transport. The original approach was used to follow 11–12 individual lipoproteins by capillary isotachophoresis (CITP) of parallel plasma aliquots pre-stained with lipid-specific fluorescent probe or fluorescein-labeled apoE. The slow HDL with pre-beta mobility (sHDL) and fast LDL with the increased negative charge (fLDL) were precisely localized in lipid and apolipoprotein CITP profiles. For 10 patients with ε3/ε3 genotype and plasma triglyceride content varying in a wide range, the fLDL content was positively associated with VLDL-cholesterol while negatively with HDL-cholesterol, thus evidencing the fLDL accumulation in hypertriglyceridemia. Besides, apoE content in sHDL increased at the decrease of HDL cholesterol level. The affinity parameters of apoE binding to individual lipoproteins were measured at plasma titration by apoC-III, followed by residual apoE detection. These parameters for sHDL and fLDL were associated positively, assuming the common metabolic pathway(s). This metabolomic-like approach is assumed to permit the overall, fast and quantitative determination of atherogenic lipoprotein phenotype. Also, both the contribution of pro- and anti-atherogenic cytokines into dyslipidemia manifestation and the specific cytokine-lipoprotein associations at metabolic syndrome and type 2 diabetes are considered.

Chapter 9 - Oxidative modification of LDL alters its structure allowing LDL to be taken by scavenger receptors. Scavenger receptors, are unregulated receptors that recognizes the highly modified forms of LDL and is responsible for the rapid lipoprotein uptake by intimal macrophages. Smooth muscle cells (SMCs) can also express scavenger receptors, providing an additional link between oxidized lipoproteins and the formation of SMC-derived foam cells during atherosclerosis. In our lab the author obtained the transdifferentiation of chick aortic SMC to a macrophage-like state after cholesterol feeding. A diet rich in cholesterol leads to high levels of cholesterol in SMC, repressing the synthesis of LDL receptor, not affecting to the scavengers. Cultured of SMC isolated from arterial hypercholesterolemic chicks (SMC-Ch) and from control-fed chicks (SMC-C) were used here to develop a comparative study of LDL receptors and cholesterol homeostasis. The author first described the effects of LDL and modified LDL (ox-LDL and acetyl-LDL) on the homeostasis of cholesterol of SMC-Ch and a comparative study in SMC-C versus SMC-Ch and SMC-Ch versus macrophage. Scavenger receptors, particularly important in the formation of atherosclerotics foam cells, are up expressed by cholesterol feeding. Moreover, these results show the study of the LDL receptor in chicks SMC and its regulation by cloroquine and 25-hydroxycholesterol, as well as a specific Dil-LDL fixation 33 % higher in SMC-Ch than in

SMC-C, suggesting that these receptors play an important roll in cholesterol feeding-induced atherosclerosis and foam cell formation, in vascular smooth muscle cells.

Chapter 10 - Type 1 (T1D) and Type 2 (T2D) diabetes are independent risk factors for cardiovascular disease (CVD), stroke and coronary artery disease. The higher risk of CVD associated with diabetes has been related to hyperglycaemia, hyperinsulinaemia and to alterations of the levels and/or heterogeneity of the major lipoprotein classes. An increase of small and dense low-density lipoproteins (LDL) and of glycated apoproteins has been described in patients with diabetes. These compositional and physicochemical changes reflect alterations in the susceptibility to lipid peroxidation and impaired interactions with cell receptors. In the past decade, homocysteine (Hcy) has been recognized as a risk factor for cardiovascular disease. Several studies have observed a relationship between Hcy levels and chronic complications of diabetes, and it has been reported that hyperhomocysteinaemia is associated with coronary heart disease in diabetes.

The mechanisms by which hyperhomocysteinaemia may be involved in the development of atherogenesis are only partially understood, but induction of oxidative damage and endothelial injury have been suggested. It has been demonstrated that Hcy-induced vascular damage could be related to homocysteine-thiolactone (Hcy-thiolactone), an Hcy reactive product formed in human cells from the enzymatic conversion of homocysteine to the corresponding thioester. The interaction between LDL and Hcy-thiolactone induces the formation of homocystamide-LDL adducts (Hcy-LDL). Homocysteinylation of lipoproteins is accompanied by structural and functional alterations and it has been suggested that homocysteinylation could increase the atherogenicity of lipoproteins and contribute to abnormal interactions with cells. The aim of this study is to further investigate the role of homocysteinylation of LDL in the pathogenesis of diabetic vascular complications. The author compared the effect of homocysteinylation of LDL isolated from healthy subjects or T1D patients in good metabolic control on NO production, intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ and Na^+/K^+-ATPase activity from human aortic endothelial cells (HAEC) in culture.

In fact, in a previous study, the author demonstrated that a short term interaction with LDL from Type 1 diabetic patients causes alterations of the plasma membrane surface and of cellular functions in endothelial cells in a possibly atherogenic way.

In the present work, the author showed that $[Ca^{2+}]_i$ from HAEC incubated with Hcy-LDL from diabetic patients was greater than after incubation with Hcy-LDL from control subjects and untreated LDL from diabetic patients ($P<0.001$) whilst NO production and Na^+/K^+-ATPase activity had an opposite trend.

These results show that the compositional changes in Hcy-LDL from diabetic subjects have cytotoxic effects on human endothelial cells. Therefore, the author suggest that the higher oxidative damage exerted by LDL from diabetic patients on endothelial cells could contribute to the high risk of cardiovascular disease associated with Type 1 diabetes.

Chapter 11 - Female-specific very-high-density lipoprotein, vitellogenin, is naturally synthesized in the liver under estrogen control, but can be also detected in male and immature ovipara including fish under the influence of excess doses of estradiol-17 β (E_2) and/or estrogenic compounds. In this study, the author successfully purified and characterized the intact vitellogenin by a combination of the density gradient ultracentrifugation and the DEAE-Toyopearl column chromatography from a wild female silver eel *Anguilla japonica*, which was captured in the local river. The vitellogenin from a wild female silver eel was very-high-density lipoprotein, however, its behavior in the ion exchange column

chromatography was considerably different from that of E_2-treated eels. The vitellogenin from E_2-treated eels was eluted with 0.1–0.2 M NaCl. In contrast, the vitellogenin from a wild female silver eel was poorly eluted under the same condition, but it was mainly eluted with 0.5 M NaCl. The different charge situation in both vitellogenins affected their behavior in the ion exchange column chromatography. The protein and lipid ratios of the vitellogenin from E_2-treated eels were 86.9 and 13.1%, respectively, and the major lipid component was phospholipid (9.5%). Its density calculated by the chemical composition was 1.28 g/ml. On the other hand, the density of vitellogenin from a wild female silver eel was 1.30 g/ml, reflecting its low lipid (4.6%) and high protein (95.4%) ratios. Compared with the lipid components of the vitellogenin from E_2-treated eels, the major lipid components of the vitellogenin from a wild female silver eel were triacylglycerol (2.3%) and total cholesterol (2.2%) but not phospholipid (0.22%). These results indicated that the charge situation in the vitellogenins from E_2-treated and wild female silver eels depended on their protein and lipid ratios.

Chapter 12 - Apolipoprotein B100 (apoB100)-containing lipoproteins are the principal fat carriers in blood and serve as transport system and source of cholesterol for most tissues in the body. They adopt the shape of globular microemulsion particles consisting of a non-polar ("fatty") core of cholesteryl esters, triacylglycerols and an amphiphilic coating of phospholipid, cholesterol and a single copy of apoB100, which plays a crucial role in the receptor-mediated recognition, uptake and degradation of its carrier. Although the size and composition of the lipoprotein particles change during conversion from very low density lipoprotein (VLDL) to low density lipoprotein (LDL), the amphiphilic apoB100 molecule remains bound to the lipids and does not exchange between lipoprotein particles. This exceptional feature of apoB100 may be attributed to hydrophobic regions in the amino acid sequence of apoB100, which penetrate the lipid core and adhere tightly to surrounding lipids. Apart from this, apoB100 has to display inherent conformational flexibility necessary for the association to differently sized lipoprotein particles of apparently different lipid packing densities. It is now becoming clear that this intrinsic mobility of apoB100 is necessary for protein function in lipid metabolism, but is a stumbling block for high resolution structure determination, which is still a scientific challenge. Although new insights into the molecular organisation and dynamics of apoB100 are forthcoming, many open questions remain unanswered. In this chapter current conceptions on apoB100 structure and dynamics are discussed and most recent advances in this field are presented.

Chapter 13 - The envelope *of E. coli* cells serves as a barrier between cell contents and the environment and also as the means of communication between them, providing the flows of nutrients, energy, and information. Most secreted soluble proteins of this gram-negative bacterium, with the exception of some protein toxins, are localized in the periplasm.

In the cells with enhanced synthesis of alkaline phosphatase, as our studies have shown, there is a disturbance of the outer membrane barrier properties without any genetic interference. This is evidenced both by excretion of periplasmic proteins that the author have revealed and by increase in ethidium bromide fluorescence in the presence of cells secreting PhoA into the medium. The author have shown the negative correlation between protein and LP secretions. The data obtained by new approaches once again confirm that phospholipids are precursors of envelope components, as lipoprotein, on the one hand, and involved in protein secretion across the cytoplasmic membrane, on the other hand. Under the disturbance of phospholipid metabolism, both of these processes are suppressed, apparently as a result of

their enhanced competition for phospholipids. It is notable that the author have observed intensification of secretion into the medium even against significant decline of protein secretion across the cytoplasmic membrane. In this case, the protein overproduction or increased secretion already could not be the reason of changes in cell envelope. The main reason of higher outer membrane permeability was primarily the significant changes in metabolism and composition phospholipids which are metabolic precursors and outer membrane components.

Chapter 14 - Macroangiopathy in diabetes mainly consists of an accelerated form of atherosclerosis. Despite the high prevalence of risk factors, no more than 25% of the additional cardiovascular risk in diabetes can be explained by known risk factors.

Lipoprotein abnormalities play a fundamental part in this context. In particular, high VLDL concentrations, smaller LDL particles, and reduced levels of the HDL_2 subclass are hallmarks of type 2 diabetic patients with extrahepatic insulin resistance, hypertension, obesity and microalbuminuria, and they may help to explain why type 2 diabetic patients are prone to cardiovascular disease. Low Apolipoprotein AII levels in diabetic patients also seem to be relevant, since these patients frequently have reduced concentrations of HDL cholesterol, and these apo anomalies may predict fatal vascular events in such patients.

As for small, dense LDL particles, which are more susceptible to oxidation and glycation, with a fundamental role in atherosclerosis, several studies have confirmed that the concentration of plasma triglyceride is the most important determinant of variability in LDL size. Oxidized LDL play a major part in the formation and development of atherosclerotic plaques. Oxidative modifications make LDL immunogenic and autoantibodies against oxidized LDL reportedly predict the progression of coronary artery disease. Judging from our data, humoral immune response could play a different role in different stages of the development of atherosclerosis.

Patients with coronary heart disease, be they diabetic or not, very often have low levels of HDL cholesterol, but little information are available on the possible qualitative changes in their HDL. In a recent investigation, the author demonstrated that 2D gel electrophoresis, followed by enzymatic digestion and MALDI MS, is an effective tool for characterizing Apolipoprotein AI changes, and modified-Apo AI levels are considerably higher in individuals with type 2 diabetes.

In conclusion, there is evidence of a close connection between lipoprotein abnormalities and macroangiopathy in type 2 diabetes and this could help to explain the additional cardiovascular risk associated with diabetes.