Introduction

Arteriosclerosis-related vascular diseases are the principal cause of death in most developed and developing societies, accounting for one third of all deaths in adults. Another cholesterol-related disease, cholelithiasis, is highly prevalent in most Western Countries. Approximately 10-15% of the adult population has cholesterol gallstones in Western Europe and among the Caucasian populations of the United States, representing one of the most costly digestive diseases. A more dramatic impact of gallstone diseases occurs in some developing countries like Chile, where almost 50% of women older than 40 years-old, harbor cholesterol gallstones in their gallbladders. Gallstones are a major risk factor for gallbladder cancer, a neoplasm that is currently one of the leading causes of cancer death among Mestizo women of Amerindian origin, particularly from Chile, Bolivia and Mexico. A similar situation is observed among some aboriginal populations of the Americas, including North American Indians and Mapuche Indians.

Atherosclerosis and cholesterol cholelithiasis are characterized by abnormal regulation of cholesterol trafficking and solubilization, and subsequent development of the arteriosclerotic plaque in the artery walls and gallstone formation in the gallbladder, respectively. Cholesterol metabolism is controlled by many complex polygenetic – environmental interactions that contribute to the regulation of serum lipoprotein cholesterol levels and biliary cholesterol and bile acids secretion, which constitute the only pathway for sterol elimination from the organism. Much of our understanding of cholesterol metabolism has arisen from studies of the pathways controlling cholesterol synthesis and the uptake and degradation of LDL and HDL lipoproteins. Recently, two new members of the ABC transporter family (ABCG5 and ABCG8 heterodimers) have been discovered in the apical pole of the enterocyte and in the canalicular membrane of hepatocytes. Experiments in genetically engineered mice have demonstrated that ABCG5/G8 represent the physiological canalicular transporter of biliary cholesterol and the intestinal secretory mechanism of absorbed dietary plant sterols. Interestingly, mutation of ABCG5 or ABCG8 genes in man causes sitosterolemia, a rare genetic disease characterized by massive absorption of plant sterols and premature arteriosclerosis. The potential pharmacological manipulation of biliary cholesterol secretion represents another important therapeutic target to treat hypercholesterolemia, if this manipulation is simultaneously accompanied by measures aimed to avoid gallbladder cholesterol crystallization. The best theoretical drug should decrease serum lipoprotein cholesterol levels, increase biliary cholesterol secretion and fecal elimination and favoring at the same time gallbladder emptying to prevent gallstone formation.

Key Words:
Arteriosclerosis, Cholesterol cholelithiasis, ABC transporter family, ABCG5 gene, ABCG8 gene.
of arteriosclerosis and gallstone disease, respectively. Interestingly, several polygenic and environmentally determined chronic metabolic conditions including obesity, diabetes, hyperinsulinemia, dyslipidemia of the type low serum HDL cholesterol - high serum triglyceride concentrations are shared associated risk factors of both atherosclerosis and cholesterol gallstone disease. The constellation of these conditions has been included in the so called Metabolic Syndrome5-7. These complex interrelationships strongly suggest the existence of numerous still unknown regulatory genes of lipid metabolism that are functionally linked in the regulation of sterol trafficking and solubilization within the organism and can favour the development of arteriosclerosis and cholesterol gallstone diseases.

Overview of Plasma Cholesterol Transport and Secretion into Bile

The pioneering work by Brown and Goldstein8 from Dallas in 1973 discovered the LDL receptor, as a major determinant of plasma LDL cholesterol concentration. This breakthrough in the understanding of regulation of cholesterol metabolism paved the way for the introduction of statins in the treatment of hypercholesterolemia. Cholesterol, once synthesised or absorbed is distributed through the body compartments and can only be disposed of by the body via the biliary system, either by direct excretion or by breakdown into bile acids to maintain equilibrium in body compartments. Excess serum low density lipoprotein (LDL) cholesterol concentration favours free cholesterol precipitation in the arterial wall initiating the formation of the arteriosclerotic plaque. LDL is the principal cholesterol-carrying lipoprotein (LDL) cholesterol concentration favours free cholesterol precipitation in the arterial wall initiating the formation of the arteriosclerotic plaque. LDL is the principal cholesterol-carrying lipoprotein (LDL) followed by high density lipoprotein (HDL) in human blood. LDL is considered a major offending agent in arteriosclerotic vascular disease. LDL particles are removed from plasma when apoB-100 (the apoprotein component of LDL) binds to LDL receptors (LDL-R) in the sinusoidal compartment of hepatocyte membranes (Figure 1). LDL particles are degraded through the lysosomal pathway after binding to the plasma membrane. The number of

LDL-R in hepatocytes depends on the hepatic cholesterol concentration and is under tight genetic control. Statin drugs (lovastatin, simvastatin, atorvastatin, etc.) are potent inhibitors of cholesterol synthesis, decrease hepatic cholesterol content and simultaneously increase LDL-R, an effect that markedly enhance hepatic LDL uptake and decrease plasma cholesterol LDL concentration. This mechanism explains most of the pharmacological effects of statins in the efficacious treatment of hypercholesterolemia and prevention of cardiovascular diseases9.

More recently, Krieger and collaborators10 from Boston discovered the SR-BI receptor that removes cellular cholesterol from peripheral tissues by a mechanism that depends at least in part on the gene for Tangier disease (ABCT). The delivery of cholesterol by plasma HDL occurs from the so called peripheral tissues to adrenal glands for steroid hormone synthesis and to liver for excretion. The delivery of peripheral tissue cholesterol in HDL to the liver is called reverse cholesterol transport (Figure 1). The HDL cholesterol in the liver can be secreted into the bile as cholesterol or in the form of bile acids. The role of HDL in arteriosclerosis has been hallmarked by a series of experiments in gene-manipulated mice in which the expression of the SR-BI receptor was manipulated in mouse models of arteriosclerosis. The absence of SR-BI (in knockout mice) dramatically accelerates the onset of arteriosclerosis11, whereas overexpression of SR-BI by the liver either in transgenic mice or in mice subjected to a virus-based gene-therapy protocol suppresses arteriosclerosis12,13. A major hint in the therapy and prevention of arteriosclerosis through the reverse cholesterol pathway is the decreased risk of the disease by increasing serum HDL cholesterol concentration14.

Hepatic cholesterol is originated from the uptake of serum lipoproteins and chylomicron and VLDL remnants and new synthesis from acetate. Cholesterol is stored as cholesterol esters in cytosolic lipid droplets. The hepatic secretory pathways include VLDL and HDL newly synthesized particles, bile acids and biliary cholesterol. Regulation of these processes is based on a tightly tuned interplay of a number of protein factors that maintain whole body cholesterol homeostasis.
In the majority of patients with cholesterol gallstone disease, the primary pathophysiological event is biliary cholesterol hypersecretion from the liver. This abnormality has been found in normal weight Caucasians and Chileans, obese patients, and in subjects using estrogens and progestins15. The higher content of biliary cholesterol, which cannot be completely solubilized by bile salt/phospholipid complexes, precipitates in the gallbladder triggering the gallstone formation sequence16-18. The interaction of genetic and environmental pathogenic factors is thought to modulate biliary cholesterol secretion by interfering with the regulation of hepatic cholesterol uptake, trafficking, and storage. Target interference could theoretically occur in a number of steps of hepatocellular cholesterol transport. Among a number of possibilities, potential malfunctions could be related
to sinusoidal lipoprotein receptors, VLDL and HDL production and secretion, intracellular sterol transport proteins (i.e., SCP-2, Niemann-Pick type C1 (NPC1) protein), cholesterol and bile acids canalicular transporters and metabolic nuclear receptors of the type of LXRs and FXR.

Intracellular sterol transport proteins could mediate cholesterol movement to the canalicular membrane. A number of genetically engineered mouse models have established the physiological relevance sterol carrier protein-2 (SCP-2) to preferentially deliver free cholesterol to the canalicular pole of the hepatocyte19,20. A iso in mice, it has been demonstrated that NPC1, an intracellular protein involved in trafficking of endocytosed lipoprotein cholesterol, mediates cholesterol delivery to bile21.

Translocation of lipids across the hepatic canalicular membrane requires the activity of ATP-binding cassette (ABC) transporters including the phospholipid flippase ABCB422 responsible for biliary phospholipid secretion, and the bile salt export pump sister P-glycoprotein ABCB1123. Studies on ABCB4 have allowed a better understanding of biliary cholesterol transport and solubilization in phospholipid-rich vesicles, the principal carrier of newly secreted biliary cholesterol24.

A series of recent studies are elucidating the role of a series of nuclear receptor factors including LXRs, FXR, PPARs and RXR, in the regulation of lipid and carbohydrate metabolism. Ligands for these metabolic receptors include oxysterols, several compounds from dietary origin, and intermediates of metabolic pathways, drugs and presumably, other unknown environmental factors. Liver X receptors (LXRs) are the body’s key sensing apparatus for maintaining triglyceride and cholesterol homeostasis by regulating cholesterol catabolism, storage, absorption, and transport through the transcriptional control of the key target genes involved in these processes. The identification of the LXR-regulated-genes has been facilitated by the use of genetically engineered mouse models25,26.

The transcriptional control of intrahepatic cholesterol trafficking requires tight coordination between nuclear receptors and other transcription factors and co-regulators. Altered signalling, either through chronic ligand excess or genetic factors, may cause an imbalance in these homeostatic circuits, contributing through these mechanisms to the pathogenesis of metabolic diseases such as obesity, insulin resistance and type 2 diabetes, hyperlipidemia and arteriosclerosis, and gallbladder disease. Further studies should exploit the fact that many of these nuclear receptors are designed to respond to small molecules and turn them into therapeutic targets for the treatment of these disorders.

**Discovery and Role of Canalicular ABCG5 and ABCG8 Heterodimers**

Bile is the only quantitatively significant pathway of sterol elimination from the body as neutral sterols and bile salt synthesis-derived acidic sterols, a fraction of which are either reabsorbed or excreted through the faeces to maintain whole body cholesterol homeostasis. The study of the metabolic sources and molecular regulatory mechanisms of biliary lipid secretion has been a major area of interest in the field of lipoprotein and lipid metabolism to better understand the molecular and metabolic basis of atherosclerosis and cholesterol cholelithiasis. In this context, until recently a major question in the area was the necessity or not of specific canalicular cholesterol transporters in liver. Now a series of studies in mice have finally unravelled the mystery about the existence of specific canalicular cholesterol transporters.

A key discovery in the search for the long sought canalicular cholesterol transporters came from the finding that mutations in the genes encoding human ABCG5 and ABCG8 transporters, sterolin-1 and sterolin-2, respectively, cause sitosterolemia27,28. This rare disease is characterized by increased plasma plant-derived sterols, hypercholesterolemia, premature arteriosclerosis, xanthomatosis and impaired biliary cholesterol secretion29. Sterolin-1 and sterolin-2 function at the apical pole of enterocytes and hepatocytes to exclude primarily plant sterols from the organism, probably by preventing initial entry at the intestinal level, and by rapidly excreting any residual amounts that enter the bloodstream, by rapid uptake by the liver and excretion into bile. Now, in a series of outstanding series of experiments using transgene techniques in mice, Hobbs group30 from...
Dallas has demonstrated that ABCG5 and ABCG8 control hepatointestinal cholesterol transport in mice. In human ABCG5/G8 transgenic mice these transporters were expressed primarily in the liver and small intestine, mirroring the tissue expression pattern of the endogenous murine genes. Human ABCG5 and ABCG8 overexpression in mice markedly altered cholesterol transport through the enterohepatic circulation: fractional absorption of dietary cholesterol was decreased, whereas biliary cholesterol secretion and saturation were increased. In ABCG5/G8-overexpressing mice compared to control animals, hepatic cholesterogenesis was higher indicating a compensatory response to both decreased availability of intestine-derived remnant lipoprotein cholesterol and increased biliary cholesterol output.

Unexpectedly, cholesterol supersaturated bile samples of ABCG5/G8-overexpressing mice (which increased biliary cholesterol output by 700%) were turbid and exhibited a white floating lipid layer after centrifugation even though no signs of cholesterol crystallization or gallstone formation were observed. Taken together, these series of breakthrough studies showed for the first time that the apical expression of ABCG5 and ABCG8 in the liver and intestinal epithelial cells controls biliary neutral sterol secretion and intestinal cholesterol absorption, respectively. Sterols (cholesterol and plant sterols) are secreted by ABCG5 and ABCG8 transporters from hepatocytes and enterocytes to the canalicular and the intestinal lumen, respectively. The generation of a mouse strain lacking expression of ABCG5 and ABCG8 recently obtained by the same group culminates this series of elegant experiments demonstrating definitively that ABCG5 and ABCG8 transporters are essential for determining biliary cholesterol secretion and fecal neutral sterol elimination from the body.

The detailed molecular mechanism by which ABCG5 and ABCG8 heterodimers facilitate biliary and intestinal cholesterol secretion remains to be established. By homology to a number of ATP-dependent canalicular membrane ABC transporters, it is plausible that the ABCG5/G8 heterodimers is a primary sterol transporter that translocates cholesterol in an ATP hydrolysis-dependent manner from the inner to the outer leaflet of the canalicular membrane making cholesterol available for efflux into biliary acceptors, such as phospholipid vesicles and bile salt micelles. Further studies are also required to determine whether cholesterol transported by ABCG5/G8 is delivered to the heterodimer complex by interaction with intrahepatic cholesterol transport proteins such as SCP-2, or is directly derived from the canalicular cholesterol pool.

A remarkable finding of these ABCG5/G8-overexpressing mice was the absence of cholesterol crystals and gallstones in spite of the presence of cholesterol supersaturated gallbladder bile. Paradoxically these mice did not develop gallstones. The more plausible explanation is that ABCG5 and ABCG8-overexpressing C57BL/6J × SJL mice lack key essential unknown lithogenic gallbladder factors required for gallstone formation, including biliary cholesterol crystallization factors and or abnormal gallbladder emptying, two steps that are essential in the multifactorial pathogenic chain in cholesterol gallstone formation.

**Future Perspectives**

From a human disease perspective, these series of outstanding experiments performed in Dallas by Hobbs and collaborators should have major epidemiological, clinical and therapeutically significant implications. Recent studies have shown that ABCG5/G8 heterodimers expression is modulated by dietary cholesterol through the LXRs nuclear receptors and that LXRs expression could be modulated by insulin, linking in this manner overall regulation of fuel metabolism with cholesterol trafficking. Additional studies of the relationships of ABCG5 and ABCG8 gene polymorphisms and their metabolic surrogates (e.g., plasma and biliary plant sterol levels) with altered hepatic-biliary and intestinal cholesterol transport are required to assess the importance of these ABC transporters in human arteriosclerosis and gallstone disease. A nether relevance of the work by the Dallas group is related to the potential pharmacological manipulation of neutral sterol disposal through the selective regulation of the ABCG5/G8 heterodimers ex-
pression and/or activity. If biliary cholesterol output could be stimulated without increasing the risk of forming gallstones or decreased without altering overall cholesterol homeostasis, drugs aimed to modulating ABCG5/G8 function might open new pharmacological possibilities for prevention and or therapy of arteriosclerosis and cholesterol gallstone diseases and their complications.

References

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Cholesterol Management Guide 7. In adults 40 to 75 years of age without diabetes mellitus and with LDL-C levels ≥70 mg/dL (≥1.8 mmol/L) at a 10-year ASCVD risk of ≥7.5%, start a moderate-intensity statin if a discussion of treatment options favors statin therapy. Risk-enhancing factors favor statin therapy (see No. 8). If risk status is uncertain, consider using coronary artery calcium (CAC) to improve specificity (see No. 9). If statins are indicated, reduce LDL-C levels by ≥30%, and if 10-year risk is ≥20%, reduce LDL-C levels by ≥50%. The liver and (small) intestine are key organs in maintenance of cholesterol homeostasis: both organs show active de novo cholesterogenesis and are able to transport impressive amounts of newly... Sudhop T, von Bergmann K (2002) Cholesterol absorption inhibitors for the treatment of hypercholesterolemia. Drugs 62:2333–2347 PubMed Google Scholar. Sudhop T, Lütjohahn D, Kodal A et al.