Cholesterol metabolism in mouse models of atherosclerosis and adrenal steroidogenesis

Sluis, R.J. van der

1

GENERAL INTRODUCTION AND THESIS OUTLINE

CHOLESTEROL

Cholesterol, the most prominent sterol in mammals, was originally discovered as a major component of human gallstones by F. Pouletier de la Salle in 1769. In 1815 M.E. Chevreul named the organic molecule "cholesterine" (chole for bile, stereos for solid), which was later adjusted with the chemical suffix of -ol for the alcohol component [1]. Over the past 100 years, cholesterol has been extensively studied and linked to a variety of pathologies and tightly regulated metabolic pathways. The structure of cholesterol consists, in its free form (free cholesterol; FC), of four linked hydrocarbon rings with on one side a hydrocarbon tail, opposing a hydroxyl group [2](**Figure 1A**). The two ends create an amphipathic molecule with a hydrophobic and hydrophilic side. This structure of the cholesterol molecule influences the properties of cellular membranes in animals. The hydrophilic hydroxyl group binds to the phospholipid heads in the cell membrane, turning the hydrophobic hydrocarbon tail towards the core of the membrane bilayer. This improves the membrane fluidity and permeability and allows the cell to change shape [3](**Figure 1C**). An optimal membrane FC/phospholipid ratio is thus essential for membrane rigidity. Any misbalance could influence cellular mobility and eventually induce cell death [4]. Accumulation of membrane-bound FC's are associated with cytotoxic events including intracellular cholesterol crystallization, oxysterol formation [5], and apoptotic signaling pathway activation [6,7]. It is therefore that the majority of cholesterol found in the body exists in its more stable, less cytotoxic, esterified form (cholesteryl esters (CE)). In the plasma compartment, 2/3 of the cholesterol pool exists in the form of CE. Lecithin-cholesterol acyltransferase (LCAT) drives the esterification of FC molecules in plasma, adding a single fatty acid derived from phosphatidylcholine to the hydroxyl group [8](**Figure 1B**). The conversion of FC into CE enables cells to store and/or transport cholesterol, without the risk of FC-induced cytotoxicity. Upon hydrolyzation by cholesteryl ester hydrolase, cholesterol and free fatty acids can be regained for further biosynthesis and metabolism [9].

Besides the eminent role in cell membrane functionality, cholesterol influences a range of pathways i.e. as the precursor for the synthesis of steroid hormones [11] and bile acids [12], and plays a significant role in transmembrane signaling [13] and cellular proliferation [14]. Despite the functional diversity between cholesterol-using pathways, acquisition of cholesterol follows, for most mammalian cells, a comparable pattern. Cholesterol is either synthesized de novo or derived from the blood circulation by cellular uptake.

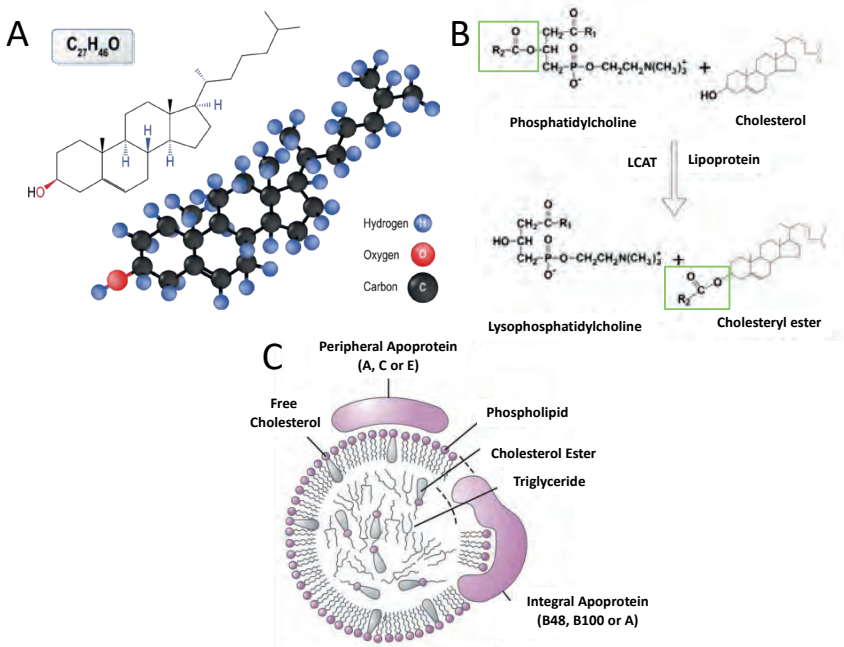

Figure 1: The structural chemical formula and molecular illustration of cholesterol (A). Conversion of free cholesterol (FC) and phosphatidylcholine towards cholesteryl ester (CE) by the enzyme Lecithin-cholesterol-acyltransferase (LCAT) (B). Lipoprotein structure depicting the primary location of FC in the shell of the particle and CE in the core (Adapted from [10] (C)).

LIPID METABOLISM

DE NOVO SYNTHESIS OF CHOLESTEROL

De novo synthesis of cholesterol is mainly found in vertebrates and in lower amounts in plants (not in prokaryotes) [15] and acquired via the mevalonate (MVA) pathway. The MVA pathway is a highly controlled enzymatic process, resulting in the stepwise formation of FC [16]. Furthermore, the MVA pathway is a fundamental metabolic network providing several essential elements for normal cellular metabolism and is executed in the endoplasmic reticulum (ER) and cytoplasm of a cell. Despite the presence of the MVA pathway in almost all animal cells, the amount of de novo synthesized cholesterol differs per species and organ. The total cholesterol pool of mice and man are equal, ~2.150 and ~2.200 mg/kg, respectively. However, the human brain holds approximately 23 % (490 mg/kg) of the total cholesterol pool, whereas a mouse brain comprises only 15 % (330 mg/kg) of the total pool of cholesterol. Interestingly, the brain's cholesterol pool consists mainly of CE's that are derived via novo synthesis and are primarily located in myelin sheaths that insulate axons [17-19].

The hepatic contribution to the cholesterol pool derived from de novo synthesis also varies per species: hepatic cells in mice contribute approximately 40 % to whole body cholesterol synthesis, while human liver cells add approximately 10 % to the total pool [20,21].

The newly formed cellular cholesterol is used as a precursor for the formation of metabolites (bile acids, steroids, water-soluble vitamins) included in cellular membranes, or converted to CE by acyl-Co A acyl transferase (ACAT) and stored in lipid droplets [22]. The CE stored within lipid droplets can be reconverted into FC by hormone-sensitive lipase (HSL)[23] and used as a precursor upon demand or fluxed towards the plasma compartment.

Since appropriate cellular cholesterol levels are critical for normal cell metabolism, the regulation of intracellular cholesterol levels are tightly controlled by feedback mechanisms that operate at both transcriptional as well as post-transcriptional levels [24,25]. Low cellular cholesterol triggers the MVA-pathway to upregulate the rate-limiting enzymes, i.e. 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) [25] and stimulates receptor-mediated exogenous uptake. In contrast, high cellular cholesterol levels activate nuclear hormone receptors that in turn trigger transcription of cholesterol efflux-related genes, i.e. ATP-binding (ABC) transporters, and reduce HMGCR expression [26].

The MVA-pathway is an established target for Statins, an extensively prescribed lipid-lowering drug. Statins inhibit activation of HMGCR, resulting in a reduction of hepatic cholesterol synthesis. Furthermore, statins increase the low-density lipoprotein receptor (LDLr) activity and subsequently reduce plasma cholesterol levels in patients that suffer from hypercholesterolemia.

LIPOPROTEINS AND CHOLESTEROL TRANSPORT

Due to the hydrophobic character of CE and triglycerides (TG), their transport throughout the body is facilitated by lipoproteins. Lipoproteins are spherical macromolecular particles consisting of a hydrophobic core and a hydrophilic shell. The lipoprotein shell contains a monolayer of phospholipids, amphipathic molecules, FC and apolipoproteins, enfolding the hydrophobic core of CE and TG. The major apolipoprotein classes are synthesized by the intestine and liver and located in the membrane of lipoproteins. The amphipathic apolipoproteins serve as enzymatic cofactors and receptor ligands, regulating lipoprotein metabolism [27,28]. The function

and presence of apolipoproteins differ per lipoprotein class. Five lipoprotein classes can be distinguished based on their buoyant density: chylomicrons (CM), very low-density lipoproteins (VLDL), intermediate low-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The characteristics of the five lipoprotein classes are described in **Table 1** and the pathways for metabolism of the individual lipoprotein classes are depicted in **Figure 2**.

EXOGENOUS CHOLESTEROL METABOLISM

Approximately 70 % of the total cholesterol pool is derived from endogenous production in the liver, while 30 % is of exogenous cholesterol origin and obtained via dietary intake [30]. Exogenous dietary lipid absorption is achieved via several complex functional steps along the whole digestive track. In short, solubilisation of dietary lipids starts in the duodenum and proximal jejunum parts of the intestine where bile acid micelles hydrolyse CE into FC and fatty acids (FA) and triglycerides into monoacylglycerol and FA. Micelles absorb the FC and FA and facilitate transport into the enterocytes of the small intestine, were the dietary FAs are reconverted into triglycerides (TG) by coupling to the monoacylglycerol backbone. Exogenous FC is converted into CE in the ER by ACAT [31]. The majority of the lipids absorbed in the intestine are triglycerides (95 %), but also nearly 50 % of the total dietary cholesterol is absorbed, while the remainder is excreted via the faeces [32-34].

CHYLOMICRONS

Chylomicrons are essential in the transport of exogenous cholesterol from the intestine towards the liver. Within the ER of enterocytes nascent chylomicron particles are formed as a result of lipidation of one apolipoprotein B48 (ApoB48) molecule with CE, TG and phospholipids, alongside apolipoproteins (**Table 1**). Once the chylomicrons enter the blood circulation via the lymphatic system, circulating Apolipoprotein C (ApoC) is acquired. ApoCs in the membrane of chylomicrons serve as a cofactor for lipoprotein lipase (LPL) that is present on the endothelial cells of adipose tissue and skeletal muscle and hydrolyses the TG content to FA for energy storage [35]. Upon TG hydrolysis, superfluous membrane phospholipids are transferred by the phospholipid transfer protein (PLTP) towards HDL. The chylomicrons exchange Apolipoprotein E (ApoE) and ApoCs at the expense of Apolipoprotein A1 (ApoA1) and Apolipoprotein A4 (ApoA4) with HDL, resulting in formation of smaller TG-poor and Apo-enriched remnant particles [36]. Furthermore, chylomicrons can exchange TG for HDL-derived CE via an interaction with the cholesteryl ester transfer protein (CETP), that is present in humans but not in mice [37,38].

Table 1: Plasma lipoprotein characteristics, all lipid values are expressed as a percentage of the total weight; abbreviations: CM: Chylomicrons, VLDL: Very low-density lipoproteins, IDL: Intermediate-density lipoproteins, LDL: Low-density lipoproteins, HDL: High-density lipoproteins, FC: Free cholesterol, CE: cholesteryl ester, TG: Triglyceride, PL: phospholipids, P: Protein, Apo: Apolipoprotein (Adapted from [27,29]).

Lipoprotein class	Size (Å)	Density (g/ml)	Diameter (nm)	FC	CE	TG	PL	P	ApoA	ApoB	ApoC	ApoE
CM	800-5000	<0.95	75-1200	1-3	2-4	80-95	3-9	2	A1,A3,A4	B48	C1-C4	E
VLDL	300-800	0.95-1.006	30-80	4-8	6-22	45-65	10-20	8	A5	B100	C1-C4	E
IDL	250-350	1.006-1.019	25-35	8	42	20-50	15-25	19	-	B100	C1-C4	E
LDL	180-280	1.019-1.063	18-25	6-8	45-50	5-15	20-25	22	-	B100	-	-
HDL	50-120	1.063-1.210	9-12	3-5	5-20	5-10	20-30	40	A1,A2,A4,A5	-	C1-C4	E

PLTP, a plasma glycoprotein and, like CETP, a family member of the lipopolysaccharide (LPS)-binding proteins [39], is involved in the metabolism of both the ApoB-containing lipoproteins chylomicrons/VLDL/LDL and HDL. PLTP deletion results in a marked decrease in plasma levels of ApoB-containing lipoproteins [40] as well as HDL particles [39].

Hepatic clearance of the remaining chylomicron remnants commences with sequestration in the hepatic space of Disse via an ApoE-dependent route. ApoE, associated with chylomicrons, VLDL and its remnants and subclasses of HDL, binds with a high affinity to the LDL receptor (LDLr), LDLr-related protein (LRP) and heparan sulphate proteoglycan (HSPG) thereby facilitating the uptake and clearance of the ApoE-containing lipoproteins. The liver subsequently converts the remnant cholesterol content either into bile acids or re-uses it for VLDL synthesis.

ENDOGENOUS CHOLESTEROL METABOLISM

A third source for cellular cholesterol is derived from the endogenous cholesterol metabolism pathway. Endogenous cholesterol metabolism starts with the synthesis of VLDL within the ER membrane of hepatocytes. Here a single copy of ApoB100 is lipidated with TG and de novo produced or exogenous derived cholesterol [41-43]. The TGs that are packaged into VLDL particles are free fatty acids that are synthesized in the ER. The free fatty acid influx are either derived from the 1) intestine 2) chylomicron remnants or 3) produced by the adipose tissue and taken up from the circulation by hepatic receptors [41-44]. Since hepatic VLDL metabolism is dependent on the availability of TGs, the de novo synthesized ApoB100 molecules undergo degradation when they are not lipidated. The transport of TGs and CEs between membranes is executed by the soluble protein microsomal triglyceride transfer protein (MTTP). MTTP is vital for the assembly of VLDL and chylomicrons and is found in both the liver as well as intestines. Any disruption in the production of MTTP leads to abetalipoproteinemia, characterized by the absence of lipoproteins containing ApoB, low blood TG and cholesterol levels [45].

Once the VLDL particles enter the circulation newly synthesized ApoE and ApoCs are subsequently incorporated into the particle. Animal models revealed that deletion of ApoE or introduction of dysfunctional ApoE induces hypercholesterolemia and subsequently an increased susceptibility for atherosclerotic lesion development [46-48].

Next, circulating VLDL particles interact with LPL at the surface of the endothelial cells to reduce its TG content in a similar manner as with chylomicrons. The remaining VLDL remnants, also known as intermediate-density lipoproteins (IDL), contain less TGs and are either removed from the circulation through hepatic clearance via the LDLr or LRP1 or converted by LPL and hepatic lipase (HL) into low-density lipoproteins (LDL). The LDL particle still contains the ApoB100 molecule but no other apolipoproteins and is subjected to LDLr-mediated internalization and subsequent degradation of the particle [27].

REVERSE CHOLESTEROL TRANSPORT AND HDL

The process of extrahepatic acquisition of cholesterol and subsequent transport towards the liver is called reverse cholesterol transport (RCT). The sole lipoprotein involved in RCT is HDL and its metabolism involves complex interactions of membrane-bound and circulating plasma proteins that can be divided into five major processes (schematically depicted in **Figure 2**) (**1**) Production and secretion of ApoA1 mainly by the liver and intestine [49,50]. (**2**) Via an ABCA1-dependent pathway the ApoA1 particle incorporates cellular phospholipids leading to the formation of lipid-poor discoidal pre-β HDL particles. Consequently, targeted hepatic ApoA1 deficiency in mice results in 83 % lowering of the plasma HDL level [51,52]. (**3**) Once released into the circulation, lipid-poor pre-β HDL particles take up excess amounts of FC from peripheral cells via ABCA1 and to a lesser extend ABCG1-mediated efflux to form FC-enriched discoidal particles. The pivotal role of ABCA1 in the biosynthesis of HDL is demonstrated in ABCA1 deficient (Tangier disease) patients and ABCA1 knockout mice. These patients and mice display an inadequate transport of cholesterol towards the lipoprotein resulting in hypercatabolism of lipid-poor nascent HDL particles and the subsequent development of virtual HDL deficiency [53,54]. (**4**) Esterification of HDL-FC, initiated by LCAT in the plasma, stimulates maturation of the pre-β HDL particles into spherical HDL3 particles [51]. Next, HDL3 particles are converted into larger HDL2 particles via a PLTP-driven acquisition of phospholipids, alongside the attraction of apolipoproteins released during lipolysis of TGs in VLDL and chylomicrons. (**5**) Circulating HDL2 is transported back to the liver where scavenger receptor class B type I (SR-BI) facilitates selective uptake of CE without parallel internalization and degradation of the HDL particle [55]. Based on this important function SR-BI it is considered to be the main lipoprotein receptor for HDL [56,57]. In addition, it facilitates the bidirectional flux of

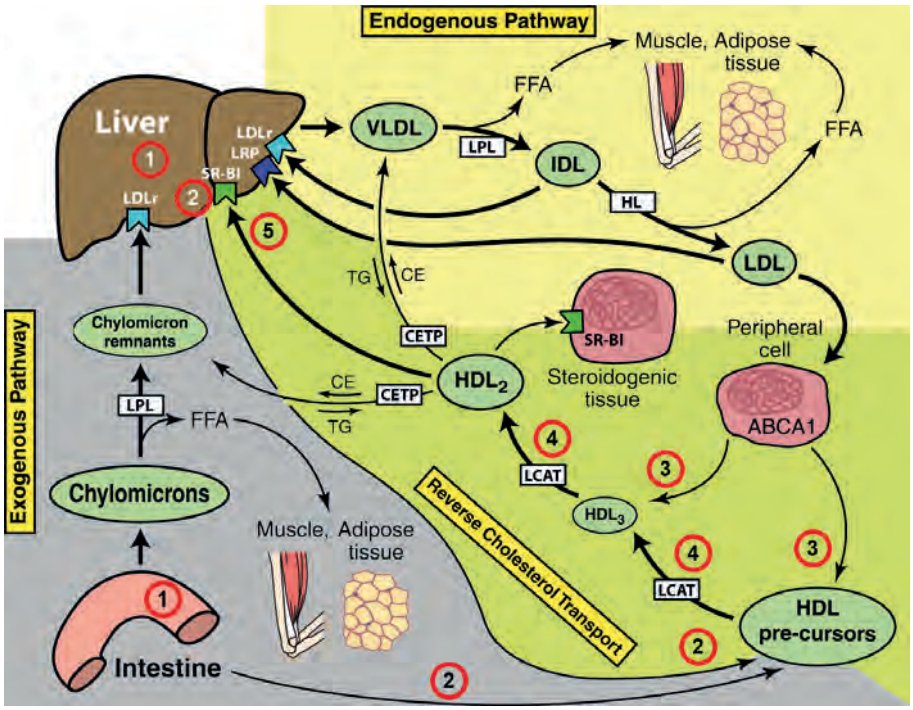

Figure 2: Overview of the basic pathways in plasma lipoprotein metabolism (Adapted from [60]) The numbers 1-5 represent the schematic steps of HDL metabolism during reverse cholesterol transport (see main text for explanation).

FC. In line with its function as main lipoprotein receptor for HDL, in vivo deficiency of SR-BI in mice is associated with impaired serum decay and hepatic uptake of [^{3}HCEt]-HDL, resulting in FC and CE accumulation in HDL particles and the appearance of enlarged HDL particles [58].

In addition to the clearance by SR-BI, HDL-cholesterol (HDL-C) can also be removed from the circulation via alternative routes. Firstly, HDL particles can be enriched with ApoE obtained from extrahepatic tissues or the blood circulation. ApoE on HDL enables removal from the circulation via hepatic LDLr or LRP1-mediated whole particle uptake [59]. A second alternative HDL-cholesterol clearing route is, as previously noted, the transfer of CE from HDL2 towards VLDL and LDL through a CETP-mediated exchange. It should, however, be noted that CETP expression is not present in the majority of rodents (mice and rats).

ADRENAL GLUCOCORTICOID SYNTHESIS

Cholesterol serves as the precursor for the synthesis of steroid hormones, including the in the adrenal cortex produced glucocorticoids (e.g., corticosterone in mice and cortisol in human) and mineralocorticoids (e.g., aldosterone), gonads produced androgens (testosterone), ovarian produced progestin and estrogen (e.g., progesterone and estradiol), and neurosteroids produced in the brain. Besides the common precursor for steroidogenesis, cholesterol, steroid hormones have a common precursor steroid, pregnenolone (**Figure 3B**) [61]. In accordance, high expression of genes linked to cholesterol metabolism are found in the steroid hormone producing organs including the expression of LDLr, SR-BI and ApoE. The adrenal derived glucocorticoids (GC) are synthesized within the zona fasciculata of the adrenal cortex. The action of GCs is transduced upon binding to the GC receptor (GCr) and mineralocorticoid receptor (MCr). Both receptors are members of the nuclear hormone superfamily and structurally and functionally highly related. The GCr is expressed ubiquitously throughout the body and is solely activated by GC. Expression of MCr can be found in specific tissues, particularly in kidney, and is activated with a similar high affinity by both the mineralocorticoid, aldosterone, as well as GC. Albeit the similarities between the two receptors, they differ in biological activity. GCr is linked to immune function and stress response, metabolism and cellular proliferation and differentiation, whereas the role of MCr is more restricted, primarily controlling blood pressure and promoting sodium reabsorption in kidney. Whereas both receptors have different biological activities, both GC and aldosterone bind and activate the MCr. This could theoretically result in an unwanted situation where the MCr is occupated and activated by the more abundantly present GC. To avoid the occupation of the MCr by GCs, tissue-specific expression is found of the GC-inactivating enzyme, 11 beta hydroxysteroid dehydrogenase type 2 (HSD11B2). HSD11B2 converts the GC, cortisol into cortisone (corticosterone to 11-dehydrocorticosterone in rodents), thereby increases the circulating levels and allows aldosterone to activate the MCr. Aldosterone by itself is found in a much lower concentration in the blood and has a very low affinity for the GCr [62, 63]. Once GC bind to GCr or MCr they undergo conformational changes that initiates translocation of the GCr with its chaperone complexes towards the nucleus where it triggers genomic mechanisms on the GC response elements (GREs), as reviewed by Kadmiel et al. [64]. Importantly, an discrepancy in the plasma levels of GC results in pathological disorders known as respectively Addison's disease (relatively low plasma GC levels) or Cushing's syndrome (relatively high plasma GC levels).

A classic role of GCs is the initiation of an anti-inflammatory response upon stress activation. This makes treatment with synthetic as well as natural occurring GCs an interesting therapeutic strategy for a variety of inflammatory conditions. As such, since 1940, GCs have been used to treat symptoms of chronic inflammatory conditions like rheumatoid arthritis, asthma, skin infections, ocular infections, multiple sclerosis and have been given as an immunosuppressant to patients following organ transplantation [65,66].

GC synthesis is regulated via dynamic circadian rhythms and upon stress-induced hypothalamic-pituitary-adrenal (HPA) axis activation [67]. Activation of the HPA axis is followed by the release of pituitary gland-derived adrenocorticotropic hormone (ACTH). ACTH interacts with the melanocortin-2 receptor (MC2R) a family member of the G protein-coupled receptors (GPCR) that is primarily present on the adrenal cortex. Binding of ACTH to the MC2R results via activation of adenylyl cyclase (AC) and instant increase of cytoplasmic adenosine monophosphate (cAMP) [68]. The formation of cAMP activates protein kinase A (PKA) leading to the phosphorylation of hormone-sensitive lipase (HSL) and steroidogenic acute regulatory protein (StAR). The intracellular presence of HSL stimulates the conversion of CEs stored in lipid droplets to FC. The released FC is transport into the mitochondria by StAR, where it is subsequently used as substrate for steroidogenesis. cAMP also stimulates HMGCoA synthesis and increases the mRNA expression of genes involved in the receptor-mediated cholesterol uptake pathways (SR-BI / LDLr), thereby priming the adrenal for an adequate synthesis of GCs [69, 70].

FC is a key component in the synthesis of GCs, therefore multiple sources are available to maintain the subsequent influx that is needed upon HPA axis activation. As schematically depicted in **Figure 3A** cholesterol needed for steroidogenesis in the adrenal could be obtained via **1**) SR-BI derived from HDL **2**) de novo synthesis of cholesterol, and **3**) uptake of cholesterol via the LDLr. However we recently showed that the contribution of the cholesterol taken up via the LDLr to the adrenal lipid pool is marginal [71].

Adrenal steroidogenesis starts within the mitochondria of the zona fasciculata of the adrenal where FC is converted via a stepwise enzyme controlled pathway into GC's (**Figure 3A**). Most enzymes involved in the steroidogenesis pathway are either family members of cytochrome P450 or hydroxysteroid dehydrogenases (HSDs) and function uni-directionally. P450scc, encoded by CYP11A1, initiates the first step

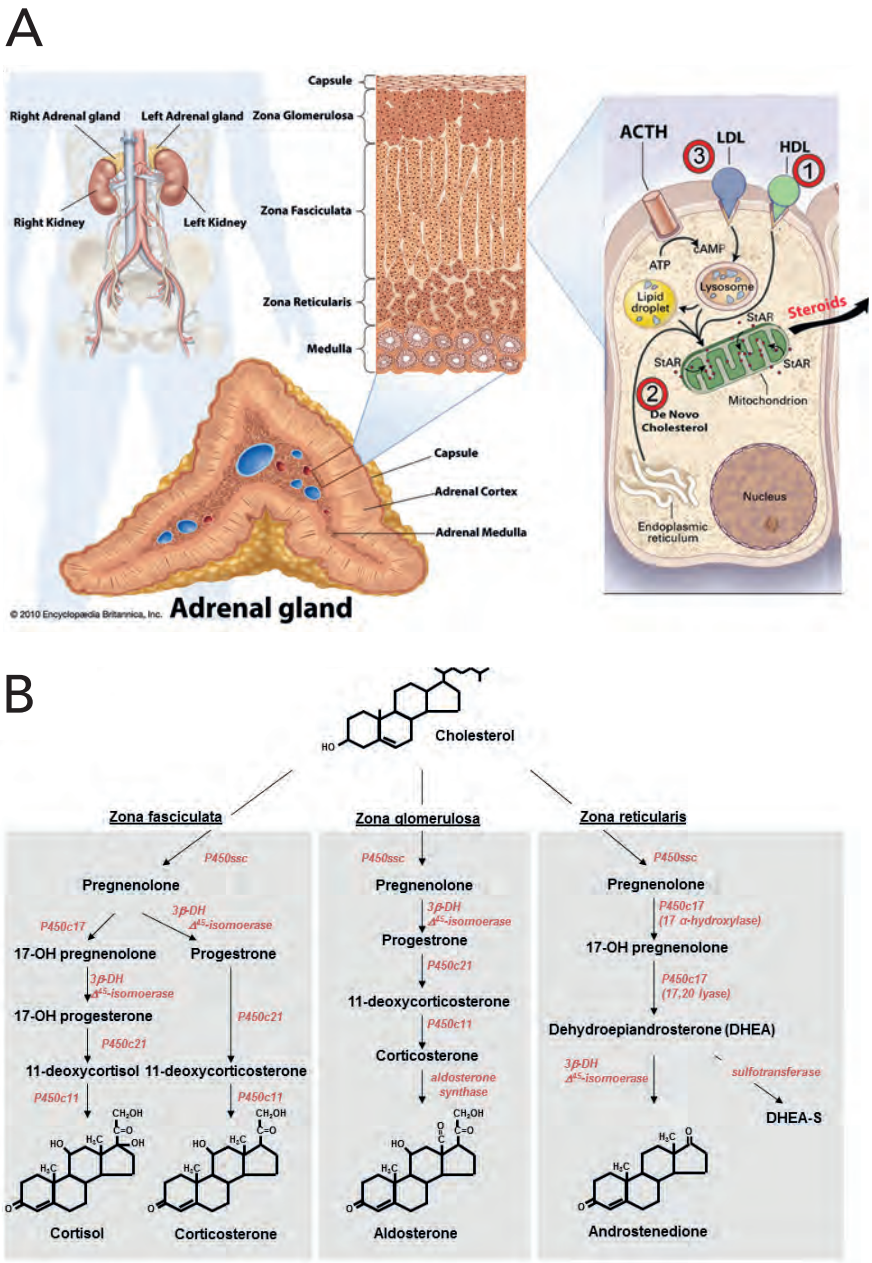

Figure 3: Overview of the pathways involved in adrenal synthesis of steroids, A) Schematic representation of the adrenal and production of the adrenal hormones in the zona fasciculata cells (Reproduced with permission from [78] © Massachusetts Medical Society, adapted and used with permission by the courtesy of Encyclopaedia Britannica, Inc., © 2010 [79]), B) Steroidogenic pathways in the different zones of the human adrenal. Note not all intermediate steroids, pathways and enzymes are shown (Adapted from [80]).

in steroidogenesis, i.e. the conversion of cholesterol into the steroid precursor, pregnenolone. Next, as summarized in **Figure 3A and B**, enzymatic modulation of the intermediate steroids leads to the formation of the cortical hormones: the glucocorticoids cortisol (in humans) and corticosterone (in mice), mineralocorticoid aldosterone as well as androgens (in humans, not in mice) [72]. Limiting factors in the production of GC's are, next to substrate availability, the availability and activity of steroidogenesis-related enzymes [73, 74].

HPA axis activation is a potent and ad hoc system that primes the body within minutes in response to a stressor. However, chronic exposure is harmful resulting in pathophysiological changes including, obesities, depression, and increased susceptibility to atherosclerosis [75, 76]. It is therefore that a properly functioning feedback-mechanism is required in response to the release of GC's. GC's execute a direct inhibitory role on the expression of corticotropin-releasing factor (CRF) in the brain and pituitary gland, thereby suppressing the stimulation of ACTH synthesis and adrenal steroidogenesis [77]

ATHEROSCLEROSIS

An imbalance in the circulating cholesterol levels has been implicated in many diseases, such as cancer [81], diabetes mellitus type 2 (T2DM) [82,83], and Alzheimer's disease (AD) [84,85]. Among the cholesterol-associated diseases, cardiovascular diseases (CVD) are the most frequent cause of death in the Western society [86]. The underlying pathology driving CVD is atherosclerosis, an ongoing process of thickening of the vessel wall leading to deprivation of oxygen and nutrients in distally located tissues. Atherosclerosis is characterized as a chronic inflammatory disease, driven by high cholesterol levels. As depicted in **Figure 4**, development of atherosclerotic lesions starts (**1**) with the infiltration of LDL particles into the vascular wall driven by physical forces (hypertension), chemical insults (hyperglycemia) or genetic alterations [87]. Within the vessel wall, LDL particles become oxidized (**2**) through either non-enzymatic or enzymatic pathways. In addition, it has been proposed that the interaction with endothelial cells, smooth muscle cells or (monocyte-derived) macrophages drives the oxidation of the LDL particle (oxLDL). Notably, the general hypothesis is that oxidation of LDL particles is not possible in the circulation due to strong anti-oxidant defense present in plasma and on the lipoproteins [88].

Modified LDL particles stimulate the expression of adhesion molecules such as P- and E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on the endothelial lining of the arterial wall (**3**) [89,90]. Subsequently, monocytes react to the inflammatory trigger and migrate into the sub-endothelial space via diapedesis (**4**) [91]. Upon entering the sub-endothelial space, monocytes differentiate into macrophages and internalize the modified LDL particles via e.g. scavenger receptor A- or CD36-mediated uptake (**5**) [92,93]. To maintain cellular cholesterol homeostasis and avoid cellular cholesterol-induced toxicity, lipid-laden macrophages actively efflux excess cholesterol via ABCA1, ABCG1 and SR-BI towards HDL particles thereby inducing the RCT process, as previously described [94]. As soon as cholesterol influx exceeds cholesterol efflux, macrophages turn into immobile lipid-laden cells with a "foamy" appearance (foam cells) (**6**). The formation and accumulation of foam cells in the sub-endothelial space are the hallmark for atherosclerotic lesion initiation, called fatty streaks [95]. Lesion progression is a dynamic process characterized by cell proliferation and migration as well as cell death [96]. It is therefore that the lesion content changes during the progression of the disease. Early lesions primarily contain macrophage derived foam cells whereas more advanced lesions are characterized by a variety of foamy cell types e.g. dendritic cells and smooth muscle cell- derived foam cells [97] and the development of a necrotic core. In more advanced lesions, smooth muscle cell (SMC) migration is triggered by the inflammatory response from the media into the intima (**7**). SMCs start proliferating and produce a fibrous cap covering the plaque (**8**) [98, 99]. Over time the plaque advances, narrowing the vessel lumen and hampering the blood flow (**9**) [87]. A common CVD event as a consequence of atherosclerotic lesion development is blockade of the blood flow by a thrombus that is either the result of a ruptured or eroded advanced lesion. The composition between the two advanced lesion phenotypes differs. Ruptured plaques display a thin fibrous cap, more inflammatory cells and contain a lipid core that is exposed to the blood stream upon rupture. Whereas eroded plaques are enclosed with a thick fibrous cap and do not contain a necrotic core [100]. The development of these different types of advanced plaque phenotypes depends on age and sex. Analysis of the carotid artery revealed that women are more prone to develop eroding plaques, whereas men carry more vulnerable rupture-prone lesions [101,102].

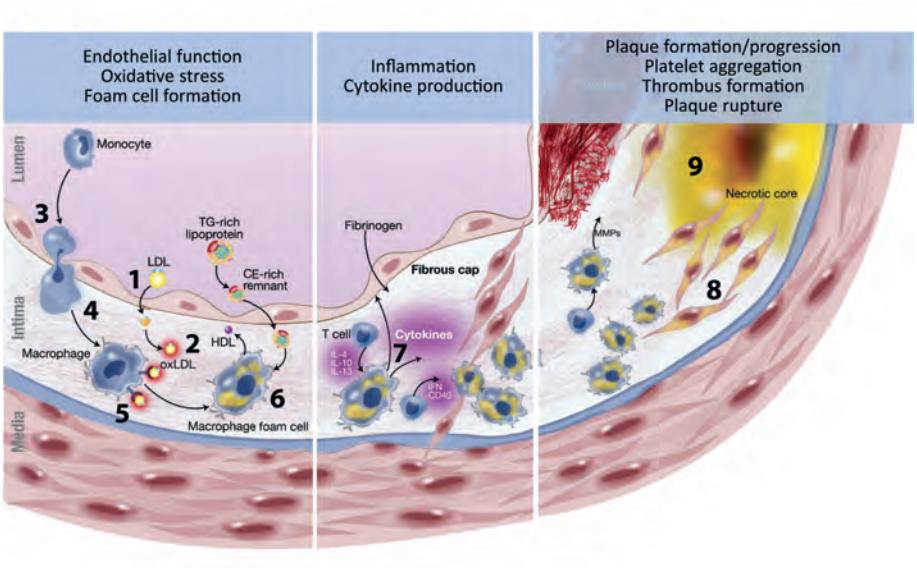

Figure 4: Schematic overview of atherosclerotic lesion development, see text for explanation (Reproduced and adapted with permission from [103], © Massachusetts Medical Society).

Table 2: Differences between human and mouse plasma lipoprotein content.

Parameter	Human	Mouse	
		Wild type	ApoE KO
HDL-C	50 mg/dl	50 mg/dl	48 mg/dl
LDL-C	150 mg/dl	10 mg/dl	100 mg/dl
CETP	Present	Absent	Absent
Atherosclerosis	Susceptible	Resistant	Spontaneous lesions

ATHEROSCLEROSIS MOUSE MODELS

Development of atherosclerosis is under the influence of various environmental as well as genetic modulating factors. Studying atherosclerosis in unregulated human cohorts is therefore difficult. The development of animal models has provided a controlled setting that enables studying the mechanisms and processes driving atherosclerotic lesion development. The most extensively used animal in CVD research is the mouse, chosen for its low maintenance costs, quick reproduction rate, and high availability of models with an atherosclerotic phenotype [104]. However, it should be acknowledged that the lipoprotein metabolism of mice differs from man in crucial aspects, influencing the overall pathogenesis atherosclerosis [105] (**Table 2**).

A major difference in lipid metabolism between mice vs humans is the lipoprotein profile. In wildtype mice the predominant lipoprotein is HDL, whereas humans display an LDL phenotype. A possible reason for this difference is the absence of CETP in mice [106,107]. Importantly, as a result of the major difference in the plasma lipoprotein profile, mice as opposed to humans do not develop atherosclerosis without major interference like genetic modification or atherogenic diet feeding.

APOE KNOCKOUT MICE

One of the most commonly used models for atherosclerosis is the total body ApoE knockout (ApoE KO) mouse, published in 1992 by two separate groups; by N. Maeda [46,47] and J. Breslow [108]. ApoE is synthesized by many tissues and cell types including liver, brain and macrophages [109-111]. The most profound role of the ApoE ligand is facilitating binding of ApoE-containing lipoproteins to the hepatic receptors; LRP1 and LDLr [112]. An additional key role for ApoE is found in the brain and adrenals, where ApoE facilitates intercellular cholesterol transport [12,114]. A targeted mutation of the ApoE gene in mice results in severe hypercholesterolemia, driven by the accumulation of ApoB-containing lipoproteins [46,47]. In line with the notion that relatively high levels of VLDL/LDL-cholesterol are pro-atherogenic, ApoE knockout mice display spontaneous development of atherosclerotic lesions already upon feeding a standard diet low in cholesterol and fat. Early stage lesion formation is measured in young mice around 8 weeks of age, with a strong progressive development of the lesions between 12 and 38 weeks [115]. Feeding ApoE knockout mice a high-fat, high-cholesterol diet further increases plasma cholesterol levels and accelerates lesion development [116,117].

LDLR KNOCKOUT MICE

A second commonly used model for atherosclerotic lesion formation is the LDLr total body knockout mouse. The LDLr is a cell surface receptor expressed primarily on mammalian hepatic cells that binds and internalizes lipoproteins carrying ApoE and/or ApoB100, thereby regulating the plasma cholesterol levels [118-120].

The association between elevated LDL-C level and an increased CVD occurrence is reflected in patients with familial hypercholesterolemia, an autosomal disorder caused by mutations in either the LDLr, ApoB or PCSK9 genes [121]. Mice with a homozygous deficiency of the LDLr gene (LDLr knockout) display a 2 to 3-fold increase in plasma cholesterol levels. Despite the relatively high plasma cholesterol levels, LDLr knockout mice do not spontaneous develop atherosclerotic lesions. However, feeding LDLr knockout mice either a high cholesterol diet (1 % cholesterol 4.4 % fat) or a Western-type diet (0.2 % cholesterol and 21 % fat) increases the plasma cholesterol concentration to a level that does stimulate atherosclerotic lesion development [122].

SR-BI KNOCKOUT MICE

SR-BI is a key player in the metabolism of the anti-atherogenic HDL particles [123]. High expression of SCARB1, the gene encoding for SR-BI, is primarily found in the liver, steroidogenic tissues and endothelial cells [124-126]. Subjects with specific mutations in the gene encoding for SR-BI display elevated levels of HDL [127-132]. To study the role of SR-BI in cholesterol metabolism and in particularly HDL and the RCT pathway in more detail, SR-BI knockout mice were generated [133]. These total body SR-BI knockout mice develop a range of pathologies, including reticulocytosis [134], reduced platelet counts [135,136], increased serum oxidative stress levels [137], as well as a reduced maximal output of adrenal derived glucocorticoids [138]. The most profound phenotype of SR-BI knockout mice is, however, the appearance of cholesterol-enriched large HDL particles. Paradoxically, the increase in HDL-cholesterol levels in SR-BI knockout mice does not confer atheroprotection, but actually results in an increased susceptibility to atherosclerosis development upon Western-type diet feeding (0.25 % cholesterol and 15 % fat) [139]. It is generally assumed that this controversy is due to the loss of the (atheroprotective) functionality of the HDL particles.

The aforementioned strains are either widely used for studying lesion progression or described in this thesis. Yet there are numerous other strains used to study CVD, either spontaneous mutated or genetically created, with or without a dietary trigger and all with their specific characteristics [140-142].

LESION REGRESSION MODELS

The concept that existing atherosclerotic lesions are capable to regress dates back ~60 years [143]. Since then, several rodent and non-rodent models have been developed to prove this concept. Many rodent atherosclerosis regression models are based on the ApoE knockout or LDLr knockout progression models [144]. The general strategy to induction of lesion regression in mouse models with established atherosclerotic lesions is lowering of the proatherogenic ApoB-containing lipoprotein in the circulation. This can be achieved by reintroduction ApoE in ApoE knockout mice via e.g. transplantation with ApoE containing bone marrow from wildtype mice [152] or via transduction with ApoE-encoding viral vectors [150, 151, 153]. A more invasive approach is the aortic arch transplantation model where a lesion-containing aortic arch segment of a hypercholesterolemic ApoE knockout mouse is transplanted into a normolipidemic recipient [154]. Subsequent regression has been achieved with either early and advanced lesions within respectively 3 days or 9 weeks post-transplantation [155-157].
The development of the Reversa mouse by Feig et a.l. (LDLr(-/-) ApoB (100/100) Mttp (flox/flox) Mx1-Cre), resulted in a model where a diet-induced hypercholesterolemia can be reversed by the expression of the MX1-Cre transgene that inactivates the gene encoding for MTTP [158,159]. The associated disruption of hepatic VLDL production resulted in a decrease of the plasma non-HDL fraction, a reduction in the plaque lipid content and egression of CD68-positive macrophages from the plaque.

More recently, a less technically demanding or time-consuming atherosclerosis regression model has been developed using an antisense oligonucleotide (ASO) that is targeted to the LDLr mRNA, inducing hypercholesterolemia and subsequent lesion formation in C57BL/6 wild type mice. By subsequently applying sense oligonucleotides (SO's) targeted to the antisense region of the targeted LDLr mRNA the hypercholesterolemia can be reversed, inducing regression of established atherosclerotic lesions [160].

A second theme in the lesion regression strategy is to increase the efficacy of the anti-atherogenic RCT route via augmenting ApoA1 and subsequently HDL levels. These increased ApoA1 levels significantly reduced the presence of early lesions in ApoE and LDLr knockout mice [145, 146] and decreased foam cell content of existing lesions in LDLr knockout mice [147]. The potential role of HDL in lesion regression was further underlined by the infusion of ApoA1/Milano/PC complex. Increasing the HDL levels in ApoE knockout mice reduced the foam cell content of existing plaques within 48 hours [148,149].

Table 3: A selection of atherosclerotic regression mouse models summarized by their characteristics. ApoE / B / A-I: Apolipoprotein E / B / A-I, ASO: antigen sense oligonucleotides, SO: sense oligonucleotides, FC: free cholesterol, HDL: high-density lipoprotein, LDLr: Low-density lipoprotein receptor, MQ: macrophage, MTTP: Microsomal triglyceride transfer protein, mRNA: messenger ribonucleic acid, RCT: reverse cholesterol transport, TC: total cholesterol, WT: wild type.

Mouse	Model	Progression	Regression method	Lipoprotein profile	Lesion	Reference
ApoE	Reintroduction of ApoE via bone marrow transplantation	Spontaneous or diet induced	Reintroduction of ApoE containing bone marrow	Normalisation of ApoB containing lipoproteins	Lesion size ↓ MQ content ↓ Collagen (advanced) =	152
ApoE	Reintroduction of ApoE via viral vectors	Spontaneous or diet induced	Transduction with ApoE-encoding viral vectors	Normalisation of ApoB containing lipoproteins	Lesion size ↓ MQ content = Collagen ↑	150, 151, 153
ApoE	Reintroduction of ApoE via aortic arch transplantation	Spontaneous or diet induced	Transplantation of an aortic segment containing plaque into a wild type mouse	Lipid levels according to the WT mice	Lesion size ↓ MQ content ↓ Collagen ↓	154-157
LDLr	LDLr(-/-) ApoB (100/100) Mttp (flox/flox) Mx1-Cre) "Riversa mouse"	Diet-induced hypercholesterolemia driven atherosclerosis	Inactivation of MTTP by activating MX1-Cre transgene	Normalisation of ApoB containing lipoproteins after inactivation of MTTP gene	Lesion size ↓ MQ content ↓ Collagen↑	158, 159
C57BL/6 wild type mice	LDLr mRNA targeting oligonucleotide model	Antisense oligonucleotide targeting the LDLr mRNA & diet, induce hypercholesterolemia driving lesion formation	Sense oligonucleotides (SO's) reverse the hypercholesterolemia	ASO's induced increase in ApoB containing lipoproteins, SO's induced reverse in ApoB containing lipoproteins	Lesion size = MQ content ↓ Collagen↑	160
LDLr / ApoE	Adenovirus gene transfer of helper-dependent adenovirus containing human ApoA-I gene targeting the liver	Diet induced	Helper-dependent adenovirus containing human ApoA-I gene induced long-term overexpression of hepatic ApoA-I	ApoB containing lipoproteins remain; efficacy of RCT is increased by increase of HDL	Lesion size ↓ MQ content ↓	145-147
ApoE	ApoA1/Milano/PC	Spontaneous or diet induced	Administration of single high / multiple dosses of recombinant ApoA1/ Milano/PC increase RCT	TC/FC conc. rise; efficacy of RCT is improved by increase of HDL	Lesion size ↓ MQ content ↓	148, 149

THESIS OUTLINE

The first part of this thesis focuses on the role of lipid metabolism, and especially HDL, in the development and regression of atherosclerotic lesions. Low plasma HDL levels are associated with increased risk for CVD events. Furthermore, the anti-atherogenic properties of HDL play a central role in the current hypothesis that HDL is essential for lesion regression. SR-BI is a key player in HDL metabolism, facilitating the bi-directional flux of cholesterol between the particle and (hepatic) tissue. Functional loss of SR-BI in mice results in accumulation of abnormally large HDL-particles and, interestingly, increased susceptibility for atherosclerotic lesion development. The increased susceptibility can theoretically be driven by the functional loss of anti-atherogenic properties of the HDL particle. A key modulator in HDL maturation is the phospholipid transfer protein (PLTP). We hypothesized that the normalization of the particle size could recover HDL's anti-atherogenic function and decrease the susceptibility for lesion development. To validate this hypothesis, we generated SR-BI x PLTP double knockout mice that genetically lack both functional SR-BI and PLTP proteins. In **Chapter 2** the effects of functional ablation of the PLTP gene in SR-BI knockout mice on HDL particle size and functionality and atherosclerotic lesion formation are described.

The standard approach to reduce the risk for a CVD event in patients is statin-induced lowering of specifically the non-HDL fraction. Previous studies in animals and patient cohorts revealed that normalizing hyperlipidemia equals diminishing of the substrate driving lesion progression, resulting in a halted lesion development [144]. The golden bullet in the CVD field, inducing regression of existing lesions, is not yet established in humans. Animal models showed that by extensively lowering lipid levels, regression of early and advanced lesions is possible [143]. We recently introduced a new model for regression of existing lesions by bone marrow-derived reconstruction of ApoE in hyperlipidemic ApoE knockout mice [152]. In **Chapter 3** we monitored over time the changes in lesion size and morphology upon normalizing cholesterol levels in ApoE knockout mice using this technique. In addition, we studied the importance of the presence of HDL during regression of existing lesions in this model.

The second part of the thesis concentrates around the role of ApoE in adrenal glucocorticoid synthesis. Given that (1) high expression levels of ApoE are found within the adrenal gland [161,162] and (2) ApoE can modulate cholesterol metabolism at both the systemic and cellular level [162,163] the hypothesis is that systemic or local ApoE could influence steroidogenesis differently. The current hypothesis is that

CE transported by HDL form the primary substrate for steroidogenesis upon stress activation in mice and that non-HDL fraction is irrelevant. However, previous in vivo studies in mice showed that the different lipoprotein fractions (non-HDL versus HDL) independently affect either the adrenal cholesterol pool or GC output [164,165]. In this context it is of interest that hypercholesterolemic ApoE knockout mice display not only increases in plasma non-HDL levels but also elevated GC levels [108]. In **Chapter 4** we validated the hypothesis that the abundantly present ApoB-containing lipoproteins could act as a source for steroidogenesis in ApoE knockout mice. We therefore drastically lowered the non-HDL levels in ApoE knockout mice via a bone marrow transplantation-mediated reconstruction of ApoE functionality and assessed the outcome on adrenal GC production.

In **Chapter 5** we focus on a possible role for ApoE in cholesterol transport locally within the adrenals. We tested the hypothesis that this local cholesterol transport function can significantly influence the synthesis of GC by the adrenals. Hereto we used our recently established in vivo "whole adrenal transplantation" model to modulate exclusively the adrenal ApoE genotype in C57BL/6 wild-type mice.

REFERENCES

1. Olson, R. E. Discovery of the Lipoproteins, Their Role in Fat Transport and Their Significance as Risk Factors. *J. Nutr.* 128, 439S-443S (1998).
2. Vance, D. E. & Van den Bosch, H. Cholesterol in the year 2000. *Biochim. Biophys. Acta* 1529, 1-8 (2000).
3. Bloch, K. Chapter 12 Cholesterol: evolution of structure and function, *Biochemistry* 363-381 (1991).
4. Simons, K. & Ikonen, E. How cells handle cholesterol. *Science* 290, 1721-6 (2000).
5. Björkhem, I. Do oxysterols control cholesterol homeostasis? *J. Clin. Invest.* 110, 725-30 (2002).
6. Tabas, I. Free cholesterol-induced cytotoxicity: A possible contributing factor to macrophage foam cell necrosis in advanced atherosclerotic lesions. *Trends Cardiovasc. Med.* 7, 256-263 (1997).
7. Tabas, I. Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. *J. Clin. Invest.* 110, 905-11 (2002).
8. Glomset, J. A. The plasma lecithins:cholesterol acyltransferase reaction. *J. Lipid Res.* 9, 155-67 (1968).
9. Brown, M. S. & Goldstein, J. L. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* 21, 505-17 (1980).
10. Engelking L. R.,*Textbook of Veterinary Physiological Chemistry (Third Edition)*, Chapter 63, Lipoprotein complexes 406-410 (2015) doi:10.1016/B978-0-12-391909-0.50063-3
11. Glass, C., Pittman, R. C., Civen, M. & Steinberg, D. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J. Biol. Chem.* 260, 744-50 (1985).
12. Duane, W. C. Effects of lovastatin and dietary cholesterol on bile acid kinetics and bile lipid composition in healthy male subjects. *J. Lipid Res.* 35, 501-9 (1994).
13. Goedeke, L. & Fernández-Hernando, C. Regulation of cholesterol homeostasis. *Cell. Mol. Life Sci.* 69, 915-30 (2012).
14. Fernández, C., Martín, M., Gómez-Coronado, D. & Lasunción, M. A. Effects of distal cholesterol biosynthesis inhibitors on cell proliferation and cell cycle progression. *J. Lipid Res.* 46, 920-9 (2005).
15. Behrman, E. J. & Gopalan, V. Cholesterol and Plants. *J. Chem. Educ.* 82, 1791 (2005).
16. Tricarico, P. et al. Mevalonate Pathway Blockade, Mitochondrial Dysfunction and Autophagy: A Possible Link. *Int. J. Mol. Sci.* 16, 16067-16084 (2015).
17. Dietschy, J. M. & Turley, S. D. Thematic review series: Brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J. Lipid Res.* 45, 1375-1397 (2004).
18. Xie, C., Lund, E. G., Turley, S. D., Russell, D. W. & Dietschy, J. M. Quantitation of two pathways for cholesterol excretion from the brain in normal mice and mice with neurodegeneration. *J. Lipid Res.* 44, 1780-1789 (2003).
19. Quan, G., Xie, C., Dietschy, J. M. & Turley, S. D. Ontogenesis and regulation of cholesterol metabolism in the central nervous system of the mouse. *Dev. Brain Res.* 146, 87-98 (2003).
20. Dietschy, J. M. & Turley, S. D. Control of cholesterol turnover in the mouse. *J. Biol. Chem.* 277, 3801-4 (2002).
21. Dietschy, J. M., Turley, S. D. & Spady, D. K. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* 34, 1637-59 (1993).
22. Kraemer, F. B., Khor, V. K., Shen, W.-J. & Azhar, S. Cholesterol ester droplets and steroidogenesis. *Mol. Cell. Endocrinol.* 371, 15-19 (2013).

23. Farese, R. V. & Walther, T. C. Lipid Droplets Finally Get a Little R-E-S-P-E-C-T. *Cell* 139, 855-860 (2009).
24. Sakakura, Y. et al. Sterol regulatory element-binding proteins induce an entire pathway of cholesterol synthesis. *Biochem. Biophys. Res. Commun.* 286, 176-83 (2001).
25. Goldstein, J. L. & Brown, M. S. Regulation of the mevalonate pathway. *Nature* 343, 425-430 (1990).
26. Tall, A. R., Wang, N. & Mucksavage, P. Is it time to modify the reverse cholesterol transport model? *J. Clin. Invest.* 108, 1273-5 (2001).
27. Ginsberg, H. N. Lipoprotein physiology. *Endocrinol. Metab. Clin. North Am.* 27, 503-19 (1998).
28. Mahley, R. W. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622-30 (1988).
29. Cox RA, García-Palmieri MR. Cholesterol, Triglycerides, and Associated Lipoproteins. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd ed. Boston: Butterworths; 1990.
30. Kapourchali, F. R., Surendiran, G., Goulet, A. & Moghadasian, M. H. The Role of Dietary Cholesterol in Lipoprotein Metabolism and Related Metabolic Abnormalities: A Mini-review. *Crit. Rev. Food Sci. Nutr.* 56, 2408-2415 (2016).
31. Field, F. J. & Mathur, S. N. Regulation of acyl CoA:cholesterol acyltransferase by 25-hydroxycholesterol in rabbit intestinal microsomes and absorptive cells. *J. Lipid Res.* 24, 1049-59 (1983).
32. Clearfield, M. B. A novel therapeutic approach to dyslipidemia. *J. Am. Osteopath. Assoc.* 103, S16-20 (2003).
33. Sudhop, T. et al. Changes in cholesterol absorption and cholesterol synthesis caused by ezetimibe and/or simvastatin in men. *J. Lipid Res.* 50, 2117-23 (2009).
34. Crouse, J. R. & Grundy, S. M. Evaluation of a continuous isotope feeding method for measurement of cholesterol absorption in man. *J. Lipid Res.* 19, 967-71 (1978).
35. Goldberg, I. J. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J. Lipid Res.* 37, 693-707 (1996).
36. Cooper, A. D. Hepatic uptake of chylomicron remnants. *J. Lipid Res.* 38, 2173-92 (1997).
37. Ha, Y. C., Calvert, G. D., McIntosh, G. H. & Barter, P. J. A physiologic role for the esterified cholesterol transfer protein: in vivo studies in rabbits and pigs. *Metabolism.* 30, 380-3 (1981).
38. Jiao, S., Cole, T. G., Kitchens, R. T., Pfleger, B. & Schonfeld, G. Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. *Metabolism.* 39, 155-60 (1990).
39. Jiang, X. C. et al. Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J. Clin. Invest.* 103, 907-14 (1999).
40. Jiang, X.-C. et al. Apolipoprotein B secretion and atherosclerosis are decreased in mice with phospholipid-transfer protein deficiency. *Nat. Med.* 7, 847-852 (2001).
41. Gibbons, G. F. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* 268, 1-13 (1990).
42. Spring, D. J., Chen-Liu, L. W., Chatterton, J. E., Elovson, J. & Schumaker, V. N. Lipoprotein assembly. Apolipoprotein B size determines lipoprotein core circumference. *J. Biol. Chem.* 267, 14839-45 (1992).
43. Tiwari, S. & Siddiqi, S. A. Intracellular trafficking and secretion of VLDL. *Arterioscler. Thromb. Vasc. Biol.* 32, 1079-86 (2012).
44. Gibbons, G. F. Regulation of fatty acid and cholesterol synthesis: co-operation or competition? *Prog. Lipid Res.* 42, 479-497 (2003).
45. Wetterau *et al*. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. *Science (80-.).* 258, 999–1001 (1992)
46. Zhang, S. H., Reddick, R. L., Piedrahita, J. A. & Maeda, N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 258, 468-71 (1992).

47. Piedrahita, J. A., Zhang, S. H., Hagaman, J. R., Oliver, P. M. & Maeda, N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc. Natl. Acad. Sci. U. S. A.* 89, 4471-5 (1992).
48. Véniant, M. M., Withycombe, S. & Young, S. G. Lipoprotein size and atherosclerosis susceptibility in ApoE(-/-) and Ldlr(-/-) mice. *Arterioscler. Thromb. Vasc. Biol.* 21, 1567-70 (2001).
49. Zannis, V. I., Cole, F. S., Jackson, C. L., Kurnit, D. M. & Karathanasis, S. K. Distribution of apolipoprotein A-I, C-II, C-III, and E mRNA in fetal human tissues. Time-dependent induction of apolipoprotein E mRNA by cultures of human monocyte-macrophages. *Biochemistry* 24, 4450-5 (1985).
50. Brunham, L. R. et al. Intestinal ABCA1 directly contributes to HDL biogenesis in vivo. *J. Clin. Invest.* 116, 1052–1062 (2006).
51. Hoekstra M., Van Eck M. Mouse Models of Disturbed HDL Metabolism. In: von Eckardstein A., Kardassis D. (eds) High Density Lipoproteins. *Handbook of Experimental Pharmacology,* vol 224. Springer, Cham (2015)
52. Zannis, V. I., Chroni, A. & Krieger, M. Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. *J. Mol. Med.* 84, 276-294 (2006).
53. Bale, P. M., Clifton-Bligh, P., Benjamin, B. N. & Whyte, H. M. Pathology of Tangier disease. *J. Clin. Pathol.* 24, 609-16 (1971).
54. McNeish, J. et al. High density lipoprotein deficiency and foam cell accumulation in mice with targeted disruption of ATP-binding cassette transporter-1. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4245-50 (2000).
55. Xu, S. et al. Apolipoproteins of HDL can directly mediate binding to the scavenger receptor SR-BI, an HDL receptor that mediates selective lipid uptake. *J. Lipid Res.* 38, 1289-98 (1997).
56. Yancey, P. G. et al. High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. *J. Biol. Chem.* 275, 36596-604 (2000).
57. Kellner-Weibel, G. et al. Expression of Scavenger Receptor BI in COS-7 Cells Alters Cholesterol Content and Distribution. *Biochemistry* 39, 221-229 (2000).
58. Out, R. et al. Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice. *J. Lipid Res.* 45, 2088-95 (2004).
59. Oldoni, F. et al. Naturally Occurring Variants in LRP1 (Low-Density Lipoprotein Receptor-Related Protein 1) Affect HDL (High-Density Lipoprotein) Metabolism Through ABCA1 (ATP-Binding Cassette A1) and SR-B1 (Scavenger Receptor Class B Type 1) in Humans. *Arterioscler. Thromb. Vasc. Biol.* 38, 1440-1453 (2018).
60. Kwan, B. C. H., Kronenberg, F., Beddhu, S. & Cheung, A. K. Lipoprotein metabolism and lipid management in chronic kidney disease. *J. Am. Soc. Nephrol.* 18, 1246-61 (2007).
61. Hu, J., Zhang, Z., Shen, W. J., Azhar, S. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutr Metab (Lond).* 1;7:47 (2010)
62. Sevilla L., Pérez, P. Roles of the Glucocorticoid and Mineralocorticoid Receptors in Skin Pathophysiology *Int J Mol Sci.* 19(7): 1906. (2018)
63. Fuller, P. J. et al Specificity in mineralocorticoid versus glucocorticoid action *Kidney Int.*, 57 1256–1264 (2000)
64. Kadmiel, M. & Cidlowski, J. A. Glucocorticoid receptor signaling in health and disease. *Trends Pharmacol. Sci.* 34, 518 (2013).
65. Buttgereit, F. A fresh look at glucocorticoids how to use an old ally more effectively. *Bull. NYU Hosp. Jt. Dis.* 70 Suppl 1, 26-9 (2012).
66. Adcock, I. M. & Mumby, S. in *Handbook of experimental pharmacology* 237, 171-196 (2016).
67. Biddie, S. C., Conway-Campbell, B. L. & Lightman, S. L. Dynamic regulation of glucocorticoid signalling in health and disease. *Rheumatology* 51, 403-412 (2012).

68. Webb, T. R. & Clark, A. J. L. Minireview: The Melanocortin 2 Receptor Accessory Proteins. *Mol. Endocrinol.* 24, 475-484 (2010).
69. Christenson, L. K. & Strauss, J. F. Steroidogenic acute regulatory protein (StAR) and the intramitochondrial translocation of cholesterol. *Biochim. Biophys. Acta* 1529, 175-87 (2000).
70. Stocco, D. M. StAR Protein and the Regulation of Steroid Hormone Biosynthesis. *Annu. Rev. Physiol.* 63, 193-213 (2001).
71. van der Sluis, R. J., Van Eck, M. & Hoekstra, M. Adrenocortical LDL receptor function negatively influences glucocorticoid output. *J. Endocrinol.* 226, 145–54 (2015).
72. Pihlajoki, M., DÃrner, J., Cochran, R. S., Heikinheimo, M. & Wilson, D. B. Adrenocortical Zonation, Renewal, and Remodeling. *Front. Endocrinol.* (Lausanne). 6, 27 (2015).
73. Payne, A. H. & Hales, D. B. Overview of Steroidogenic Enzymes in the Pathway from Cholesterol to Active Steroid Hormones. *Endocr. Rev.* 25, 947-970 (2004).
74. Miller, W. L. & Auchus, R. J. The Molecular Biology, Biochemistry, and Physiology of Human Steroidogenesis and Its Disorders. *Endocr. Rev.* 32, 81-151 (2011).
75. McEwen, B. S. Physiology and Neurobiology of Stress and Adaptation: Central Role of the Brain. *Physiol. Rev.* 87, 873-904 (2007).
76. Raber, J. Detrimental effects of chronic hypothalamic-pituitary-adrenal axis activation: From obesity to memory deficits. *Mol. Neurobiol.* 18, 1–22 (1998)
77. Vale, W., Spiess, J., Rivier, C. & Rivier, J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 213, 1394-7 (1981).
78. Bose, H. S., Sugawara, T., Strauss, J. F. & Miller, W. L. The Pathophysiology and Genetics of Congenital Lipoid Adrenal Hyperplasia. *N. Engl. J. Med.* 335, 1870-1879 (1996).
79. Utiger, R. D. Adrenal gland | Definition, Anatomy, & Function | Britannica.com. april (2018). Available at: https://www.britannica.com/science/adrenal-gland/media/6405/121578. (Accessed: 27th December 2018)
80. Ortsater, H., Sjoholm, A. & Rafacho, Chapter: Regulation of Glucocorticoid Receptor Signaling and the Diabetogenic Effects of Glucocorticoid Excess. Book: in State of the Art of Therapeutic Endocrinology DOI: 10.5772/51759 (2012)
81. Montero, J. et al. Mitochondrial Cholesterol Contributes to Chemotherapy Resistance in Hepatocellular Carcinoma. *Cancer Res.* 68, 5246-5256 (2008).
82. Vergès, B. Lipid modification in type 2 diabetes: the role of LDL and HDL. *Fundam. Clin. Pharmacol.* 23, 681-685 (2009).
83. Haffner, S. M., Lehto, S., Rönnemaa, T., Pyörälä, K. & Laakso, M. Mortality from Coronary Heart Disease in Subjects with Type 2 Diabetes and in Nondiabetic Subjects with and without Prior Myocardial Infarction. *N. Engl. J. Med.* 339, 229-234 (1998).
84. Arispe, N. & Doh, M. Plasma membrane cholesterol controls the cytotoxicity of Alzheimer's disease AβP (1-40) and (1-42) peptides. *FASEB J.* 16, 1526-1536 (2002).
85. Shobab, L. A., Hsiung, G.-Y. R. & Feldman, H. H. Cholesterol in Alzheimer's disease. *Lancet Neurol.* 4, 841-852 (2005).
86. McGovern, P. G. et al. Recent trends in acute coronary heart disease--mortality, morbidity, medical care, and risk factors. The Minnesota Heart Survey Investigators. *N. Engl. J. Med.* 334, 884-90 (1996).
87. Ross, R. Atherosclerosis — An Inflammatory Disease. *N. Engl. J. Med.* 340, 115-126 (1999).
88. Yoshida, H. & Kisugi, R. Mechanisms of LDL oxidation. *Clin. Chim. Acta.* 411, 1875-82 (2010).
89. Glass, C. K. & Witztum, J. L. Atherosclerosis. the road ahead. *Cell* 104, 503-16 (2001).
90. Mestas, J. & Ley, K. Monocyte-Endothelial Cell Interactions in the Development of Atherosclerosis. *Trends Cardiovasc. Med.* 18, 228-232 (2008).
91. Muller, W. A. Getting leukocytes to the site of inflammation. *Vet. Pathol.* 50, 7-22 (2013).
92. Babaev, V. R. et al. Reduced atherosclerotic lesions in mice deficient for total or macrophage-specific expression of scavenger receptor-A. *Arterioscler. Thromb. Vasc. Biol.* 20, 2593-9 (2000).

93. Endemann, G. et al. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* 268, 11811-6 (1993).
94. Annema, W. & Tietge, U. J. Regulation of reverse cholesterol transport - a comprehensive appraisal of available animal studies. *Nutr. Metab.* (Lond). 9, 25 (2012).
95. Hansson, G. K., Robertson, A.-K. L. & Söderberg-Nauclér, C. Inflammation and atherosclerosis. *Annu. Rev. Pathol. Mech.* Dis. 1, 297-329 (2006).
96. Bennett, M. R. Life and death in the atherosclerotic plaque. *Curr. Opin. Lipidol.* 21, 422-6 (2010).
97. Rudijanto, A. The Role of Vascular Smooth Muscle Cells on The Pathogenesis of Atherosclerosis. *Acta Med Indones.* 39(2):86-93. (2007)
98. Clarke, M. C. H. et al. Chronic apoptosis of vascular smooth muscle cells accelerates atherosclerosis and promotes calcification and medial degeneration. *Circ. Res.* 102, 1529-38 (2008).
99. Weissberg, P., Clesham, G. & Bennett, M. Is vascular smooth muscle cell proliferation beneficial? *Lancet* 347, 305-307 (1996).
100. de Jager, S. C. A. et al. Preeclampsia and coronary plaque erosion: Manifestations of endothelial dysfunction resulting in cardiovascular events in women. *Eur. J. Pharmacol.* 816, 129-137 (2017).
101. Vrijenhoek, J. E. P. et al. Sex Is Associated With the Presence of Atherosclerotic Plaque Hemorrhage and Modifies the Relation Between Plaque Hemorrhage and Cardiovascular Outcome. *Stroke* 44, 3318-3323 (2013).
102. Hellings, W. E. et al. Gender-associated differences in plaque phenotype of patients undergoing carotid endarterectomy. *J. Vasc. Surg.* 45, 289-296 (2007).
103. Budoff, M. et al. Effect of Vascepa (icosapent ethyl) on progression of coronary atherosclerosis in patients with elevated triglycerides (200-499 mg/dL) on statin therapy: Rationale and design of the EVAPORATE study. *Clin. Cardiol.* 41, 13-19 (2018).
104. Kapourchali, F. R. et al. Animal models of atherosclerosis. *World J. Clin.* cases 2, 126-32 (2014).
105. Gordon, S. M. et al. A comparison of the mouse and human lipoproteome: suitability of the mouse model for studies of human lipoproteins. *J. Proteome Res.* 14, 2686-95 (2015).
106. Hogarth, C. A., Roy, A. & Ebert, D. L. Genomic evidence for the absence of a functional cholesteryl ester transfer protein gene in mice and rats. Comp. Biochem. Physiol. *B. Biochem. Mol. Biol.* 135, 219-29 (2003).
107. Barter, P. J. et al. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 23, 160-7 (2003).
108. Plump, A. S. *et al.* Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* **71**, 343–53 (1992)
109. Blue, M. L., Williams, D. L., Zucker, S., Khan, S. A. & Blum, C. B. Apolipoprotein E synthesis in human kidney, adrenal gland, and liver. *Proc. Natl. Acad. Sci. U. S. A.* 80, 283-7 (1983).
110. Driscoll, D. M. & Getz, G. S. Extrahepatic synthesis of apolipoprotein E. *J. Lipid Res.* 25, 1368-79 (1984).
111. Williams, D. L., Dawson, P. A., Newman, T. C. & Rudel, L. L. Apolipoprotein E synthesis in peripheral tissues of nonhuman primates. *J. Biol. Chem.* 260, 2444-51 (1985).
112. Mahley, R. W. & Ji, Z. S. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J. Lipid Res.* 40, 1-16 (1999).
113. Mahley, R. W. Central Nervous System Lipoproteins. *Arterioscler. Thromb. Vasc. Biol.* 36, 1305-1315 (2016).
114. Thorngate, F. E., Strockbine, P. A., Erickson, S. K. & Williams, D. L. Altered adrenal gland cholesterol metabolism in the apoE-deficient mouse. *J. Lipid Res.* 43, 1920-6 (2002).
115. 't Hoen, P. A. C. et al. Aorta of ApoE-deficient mice responds to atherogenic stimuli by a prelesional increase and subsequent decrease in the expression of antioxidant enzymes. *Circ. Res.* 93, 262-9 (2003).

116. Zadelaar, S. et al. Mouse Models for Atherosclerosis and Pharmaceutical Modifiers. *Arterioscler. Thromb. Vasc. Biol.* 27, 1706-1721 (2007).
117. Reddick, R. L., Zhang, S. H. & Maeda, N. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. *Arterioscler. Thromb. a J. Vasc. Biol.* 14, 141-7 (1994).
118. Brown, M. S. & Goldstein, J. L. A receptor-mediated pathway for cholesterol homeostasis. *Science* 232, 34-47 (1986).
119. Ishibashi, S. et al. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J. Clin. Invest.* 92, 883-893 (1993).
120. Goldstein, J. L. & Brown, M. S. The LDL receptor. *Arterioscler. Thromb. Vasc. Biol.* 29, 431-8 (2009).
121. Hobbs, H. H., Brown, M. S. & Goldstein, J. L. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum. Mutat.* 1, 445-466 (1992).
122. Getz, G. S. & Reardon, C. A. Diet and Murine Atherosclerosis. *Arterioscler Thromb Vasc Biol.* 26:242-249 (2006)
123. Acton, S. et al. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 271, 518-20 (1996).
124. Nakagawa-Toyama, Y. et al. Human scavenger receptor class B type I is expressed with cell-specific fashion in both initial and terminal site of reverse cholesterol transport. *Atherosclerosis* 183, 75-83 (2005).
125. Wang, N., Weng, W., Breslow, J. L. & Tall, A. R. Scavenger receptor BI (SR-BI) is up-regulated in adrenal gland in apolipoprotein A-I and hepatic lipase knock-out mice as a response to depletion of cholesterol stores. In vivo evidence that SR-BI is a functional high density lipoprotein receptor under fe. *J. Biol. Chem.* 271, 21001-4 (1996).
126. Landschulz, K. T., Pathak, R. K., Rigotti, A., Krieger, M. & Hobbs, H. H. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J. Clin. Invest.* 98, 984-95 (1996).
127. Zanoni, P. et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. *Science* (80-.). 351, 1166-1171 (2016).
128. Chadwick, A. C. & Sahoo, D. Functional Characterization of Newly-Discovered Mutations in Human SR-BI. *PLoS One* 7(9):e45660 (2012).
129. Brunham, L. R. et al. Novel mutations in scavenger receptor BI associated with high HDL cholesterol in humans. *Clin. Genet.* 79, 575-581 (2011).
130. West, M. et al. Scavenger Receptor Class B Type I Protein as an Independent Predictor of High-Density Lipoprotein Cholesterol Levels in Subjects with Hyperalphalipoproteinemia. *J. Clin. Endocrinol. Metab.* 94, 1451-1457 (2009).
131. Hsu, L. A. et al. Association between a novel 11-base pair deletion mutation in the promoter region of the scavenger receptor class B type I gene and plasma HDL cholesterol levels in Taiwanese Chinese. *Arterioscler. Thromb. Vasc. Biol.* 23, 1869-1874 (2003).
132. Vergeer, M. et al. Genetic Variant of the Scavenger Receptor BI in Humans. *N Engl J Med* 364, 136-45 (2011).
133. Rigotti, A. et al. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc. Natl. Acad. Sci. U. S. A.* 94, 12610-5 (1997).
134. Meurs, I. et al. HDL cholesterol levels are an important factor for determining the lifespan of erythrocytes. *Exp. Hematol.* 33, 1309-1319 (2005).
135. Korporaal, S. J. A. et al. Deletion of the high-density lipoprotein receptor scavenger receptor BI in mice modulates thrombosis susceptibility and indirectly affects platelet function by elevation of plasma free cholesterol. *Arterioscler. Thromb. Vasc. Biol.* 31, 34-42 (2011).
136. Kaplan, Z. S. & Jackson, S. P. SR-BI and fatty platelets. *Blood* 116, 1827-1828 (2010).
137. Van Eck, M. et al. Increased oxidative stress in scavenger receptor BI knockout mice with dysfunctional HDL. *Arterioscler. Thromb. Vasc. Biol.* 27, 2413-9 (2007).

138. Hoekstra, M., Van Berkel, T.-J. & Van Eck, M. Scavenger receptor BI: a multi-purpose player in cholesterol and steroid metabolism. *World J. Gastroenterol.* 16, 5916-24 (2010).
139. Van Eck, M. et al. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *J. Biol. Chem.* 278, 23699-23705 (2003).
140. Friedman, M., Byers, S. O. & Rosenman, R. H. Resolution of aortic atherosclerotic infiltration in the rabbit by phosphatide infusion. *Proc. Soc. Exp. Biol. Med.* 95, 586-8 (1957).
141. Russel J. C. Small Animal Models of Cardiovascular Disease: Tools for the Study of the Roles of Metabolic Syndrome, Dyslipidemia, and Atherosclerosis *Cardiovascular Pathology* 15 318–330; (2006).
142. Veseli B. E. et al Animal Models of Atherosclerosis *Eur J Pharmacol* 816:3-13. (2017).
143. Lee, Y. T., Lin, H. Y., Chan, Y. W. F. *et al.* Mouse models of atherosclerosis: a historical perspective and recent advances. *Lipids Health Dis* 16, 12 (2017).
144. Feig, J. E. Regression of atherosclerosis: insights from animal and clinical studies. *Ann. Glob. Heal.* 80, 13-23 (2014).
145. Tangirala, R. K. et al. Regression of Atherosclerosis Induced by Liver-Directed Gene Transfer of Apolipoprotein A-I in Mice. *Circulation 100*(17):1816-22. (1999).
146. Benoit P, et al. Somatic gene transfer of human ApoA-I inhibits atherosclerosis progression in mouse models. *Circulation. 99*: 105–110 (1999)
147. Tangirala RK, Tsukamoto K, Chun SH, et al. Regression of atherosclerosis induced by liver-directed gene transfer of apolipoprotein A-I in mice. *Circulation.100*: 1816–1822 (1999).
148. Shah, P. K. et al. High-Dose Recombinant Apolipoprotein A-IMilano Mobilizes Tissue Cholesterol and Rapidly Reduces Plaque Lipid and Macrophage Content in Apolipoprotein E-Deficient Mice. *Circulation* 103:3047–3050, (2001).
149. Shah, P. K. et al. Effects of Recombinant Apolipoprotein A-I(Milano) on Aortic Atherosclerosis in Apolipoprotein E-deficient Mice *Circulation* 97(8):780-5 (1998).
150. Tian, F. et al. Comparative Antiatherogenic Effects of Intravenous AAV8- and AAV2-Mediated ApoA-IMilano Gene Transfer in Hypercholesterolemic Mice. *J. Cardiovasc. Pharmacol. Ther.* 20, 66-75 (2015).
151. Belalcazar, L. M. et al. Long-Term Stable Expression of Human Apolipoprotein A-I Mediated by Helper-Dependent Adenovirus Gene Transfer Inhibits Atherosclerosis Progression and Remodels Atherosclerotic Plaques in a Mouse Model of Familial Hypercholesterolemia. *Circulation* 107, 2726-2732 (2003).
152. van der Stoep, M. et al. Elimination of macrophages drives LXR-induced regression both in initial and advanced stages of atherosclerotic lesion development. *Biochem. Pharmacol.* 86, 1594-602 (2013).
153. Potteaux, S. *et al.* Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe–/– mice during disease regression. *J. Clin. Invest.* 121, 2025–2036 (2011).
154. Reis, E. D. et al. Dramatic remodeling of advanced atherosclerotic plaques of the apolipoprotein E-deficient mouse in a novel transplantation model. *J. Vasc. Surg.* 34, 541-7 (2001).
155. Trogan, E. et al. Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 103, 3781-6 (2006).
156. Trogan, E. et al. Serial studies of mouse atherosclerosis by in vivo magnetic resonance imaging detect lesion regression after correction of dyslipidemia. *Arterioscler. Thromb. Vasc. Biol.* 24, 1714-9 (2004).
157. Llodra, J. et al. Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc. Natl. Acad. Sci.* 101, 11779-11784 (2004).
158. Feig, J. E. et al. Reversal of hyperlipidemia with a genetic switch favorably affects the content and inflammatory state of macrophages in atherosclerotic plaques. *Circulation* 123, 989-98 (2011).

159. Lieu, H. D. et al. Eliminating Atherogenesis in Mice by Switching Off Hepatic Lipoprotein Secretion. *Circulation* 107, 1315-1321 (2003).
160. Basu, D. et al. Novel Reversible Model of Atherosclerosis and Regression Using Oligonucleotide Regulation of the LDL Receptor. *Circ. Res.* 122, 560-567 (2018).
161. Nicosia, M., Prack, M. M. & Williams, D. L. Differential regulation of apolipoprotein-E messenger RNA in zona fasciculata cells of rat adrenal gland determined by in situ hybridization. *Mol. Endocrinol.* 6, 288-98 (1992).
162. Williams, D. L., Wong, J. S., Wissig, S. L. & Hamilton, R. L. Cell surface blanket of apolipoprotein E on rat adrenocortical cells. *J. Lipid Res.* 36, 745-58 (1995).
163. Weisgraber, K. H. Apolipoprotein E: Structure-Function Relationships. *Adv. Protein Chem.* 45, 249-302 (1994).
164. Hoekstra, M., Van Der Sluis, R. J., Van Eck, M. & Van Berkel, T. J. C. Adrenal-specific scavenger receptor BI deficiency induces glucocorticoid insufficiency and lowers plasma very-low-density and low-density lipoprotein levels in mice. *Arterioscler. Thromb. Vasc. Biol.* 33, e39-46 (2013).
165. van der Sluis, R. J., Van Eck, M. & Hoekstra, M. Adrenocortical LDL receptor function negatively influences glucocorticoid output. *J. Endocrinol.* 226, 145-154 (2015).

[1]Division of BioTherapeutics, Leiden Academic Centre for Drug Research, Leiden, The Netherlands.
[2]Leiden University Medical Center, Department of Medicine, Division of Endocrinology, Leiden, The Netherlands.
[3]Department of Pharmacology, Temple University, School of Medicine, Philadelphia, Penn.
[4]National Public Health Institute and FIMM, Institute for Molecular Medicine, Biomedicum, Helsinki, Finland.

Accepted at Arteriosclerosis, Thrombosis, and Vascular Biology (2020) as:

Disruption of PLTP (Phospholipid Transfer Protein)-Mediated HDL (High-Density Lipoprotein) Maturation Reduces SR-BI (Scavenger Receptor BI) Deficiency–Driven Atherosclerosis Susceptibility Despite Unexpected Metabolic Complications. *Arterioscler Thromb Vasc Biol.* 2020;40(3):611-623. doi:10.1161/ATVBAHA.119.313862