Dyslipidemia at the crossroad of the skin barrier and the arterial wall

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Chapter 1

General Introduction

SKIN FUNCTION AND STRUCTURE

The skin is the largest organ in mammals and it acts as an interface between the body and the external environment[1]. It naturally functions as a barrier protecting the body from excessive loss of water and electrolytes (inside-outside barrier), and against the permeation of harmful agents and pathogens (outside-inside barrier)[1]. In addition to being a physicochemical barrier, the skin is densely populated with antigen presenting cells; thus, a highly immunogenic organ[2–4].

The skin is morphologically divided into three main layers from the inside to the outside: hypodermis, dermis and epidermis[5,6]. The hypodermis comprises the subcutaneous fat tissue involved in *e.g.* the storage of energy (fat), mechanical protection, and thermoregulation[6]. The dermis is mainly composed of fibroblasts surrounded by an extracellular matrix enriched with collagen and elastin[7,8]. The dermis accommodates all skin appendages (hair follicles, sebaceous glands and sweat glands) as well as nerve endings, blood- and lymphatic vessels. The presence and density distribution of the dermal components varies depending on the mammalian species and on body site[9–12].

The epidermis is the outer layer of the skin composed of melanocytes, Langerhans cells, Merkel cells, and keratinocytes, the latter comprising the predominant epidermal cell type[1,13]. From inside-out the epidermis can be divided into four sub-layers (strata): stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC) (Figure 1). In contrast to the human epidermis with multiple keratinocyte layers, murine epidermis is one quarter thinner and contains merely 2-3 layers of keratinocytes[14,15]. Epidermal keratinocytes proliferate in the SB and migrate upwards to the skin surface in a differentiation process to become flat enucleated dead cells called corneocytes[16,17]. As part of the differentiation process in the SS (early differentiation) and SG (late differentiation), the metabolism of the keratinocyte shifts to produce lamellar bodies, that are intracellular vesicles enriched with lipid processing enzymes and lipids[18]. At the terminal differentiation stage, the load of the lamellar bodies is extruded to form a well-structured lamellar lipid matrix surrounding the corneocytes leading to the formation of the SC layer[19].

STRATUM CORNEUM (SC)

The SC is the outermost layer of the epidermis and is primarily responsible for the barrier function of the skin. The SC is mainly composed of corneocytes embedded in an lipid matrix similarly to a “brick and mortar” structure. Corneocytes are dead cells that

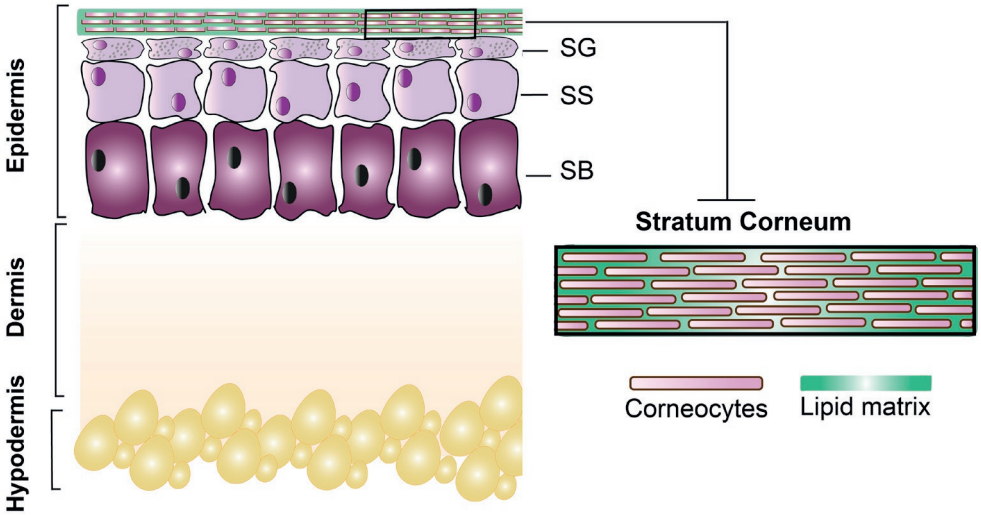

Figure 1. Structure of murine skin. The skin is divided into three main layers: hypodermis, dermis, and epidermis. The hypodermis is the deepest skin layer comprising the subcutaneous fat tissue. The dermis is the intermediate skin layer mostly composed of fibroblasts and collagen and where skin appendages, blood vessels, and nerve endings are found. The epidermis is the most superficial layer of the skin and is subdivided into four strata from inside to outside: stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC). In the SC, fully differentiated dead keratinocytes, now called corneocytes, are surrounded by a lipid matrix and undergo desquamation over time.

lost their organelles and replaced their cellular membranes by a highly impermeable crosslinked protein structure chemically attached to a lipid layer, namely the cornified envelope[17,20,21]. This monolayer of lipids serves as a template for the orientation of the SC lipid matrix (the mortar), a well-structured stack of lipid layers filling the intercorneocyte space; hence, the only continuous pathway in the SC. In time, corneocytes at the surface shed off, a process defined as desquamation, leading to SC renewal over time[17]. A complete turnover of keratinocytes in murine skin takes about 3 weeks while in human skin this process takes up to 5 weeks[22]. The thickness of the SC also differs between mouse (approximately 5 μm) and human (10–20 μm) skin[15,23].

The lipids in the SC adopt both a lamellar and a lateral organization (Figure 2). The stacked lipid lamellae are oriented approximately parallel to the skin surface. Two distinct lamellar phases can be identified using small-angle X-ray diffraction (SAXD); a short periodicity phase (SPP) and a long periodicity phase (LPP) with repeated distances of approximately 6 nm and 13 nm in human SC, respectively[24,25]. The presence of a LPP highly correlates with a functional skin barrier[26]. Perpendicular to the skin surface, the lipids can adopt three types of lateral organization based on the distances between the lipid lattice planes: a dense orthorhombic phase (0.375 and 0.416 nm), a

less dense hexagonal phase (0.412 nm), and a liquid disordered phase (approximately 0.46 nm)[27]. In healthy human and murine SC, both orthorhombic and hexagonal phases co-exist. However, most lipids form a dense orthorhombic lipid packing, most favorable for the barrier function of the skin[28–32].

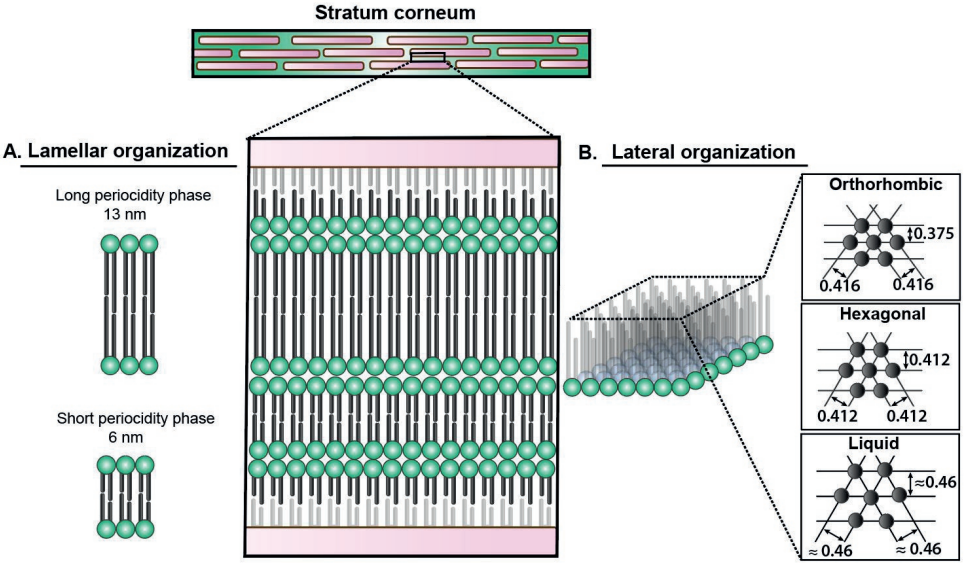

Figure 2. Organization of the lipids composing the stratum corneum (SC) lipid matrix. In the SC, corneocytes and lipid matrix are ordered similarly to a "brick and mortar" structure. In the lipid matrix, the lipids form a lamellar organization and a lateral organization. For the **(A)** lamellar organization two distinct phases are identified: the long periodicity phase and the short periodicity phase with 13 nm and 6 nm repeated distances, respectively. For the **(B)** lateral organization, the lipids can adopt a dense orthorhombic phase, a less dense hexagonal phase, and a liquid disordered phase based on the distance between lipid lattice planes (nm).

The composition of the SC lipids has major impact on the organization of the lipid matrix[33–35]. The main lipid classes composing the SC lipid matrix are cholesterol, ceramides (CERs), and free fatty acids (FFAs), which are present in the matrix in an approximately equimolar ratio (Figure 3)[36]. Other lipids have also been described as part of the skin surface lipids (*e.g.* wax-esters, triglycerides - TG) but at minor fractions and strongly associated with sebum lipids[37–39]. Cholesterol is the most abundant sterol in the SC, constituting 20-25% of the SC lipid mass[40,41]. At much lower levels (2-5% SC lipid mass), cholesterol sulfate is another sterol present in the SC[42,43]. Cholesterol sulfate is particularly expressed in the superficial layer in SC, where it participates as a signaling molecule in the desquamation of corneocytes[42,44].

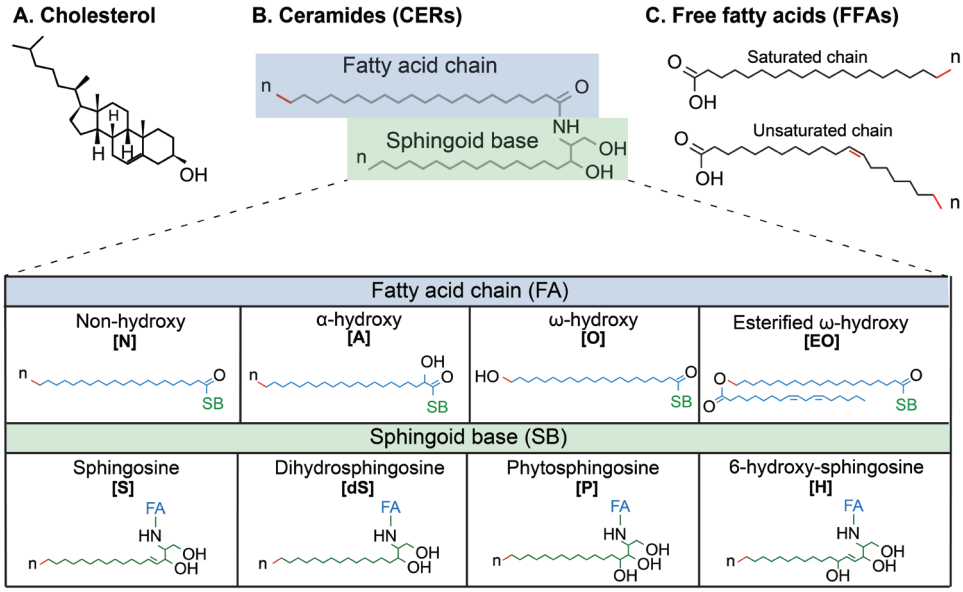

Figure 3. Schematic structure of the three main lipid classes present in the SC lipids matrix. (A) Cholesterol, (B) ceramides (CERs) and (C) free fatty acids (FFAs). CERs are named according to Motta *et. al.* (1993) based on their sphingoid bases (green marked area) and acyl chains (blue marked area)[45]. FFA chains can be (un)saturated and vary in number of carbon atoms (n).

CERs are sphingolipids composed of a sphingoid base linked to an acyl chain and they contribute to nearly 50-60% of lipid mass in the SC[40,41]. In this thesis, CER nomenclature is based on the type of acyl chain and the architecture of the headgroup in the sphingoid base present in their structures (Figure 3)[45]. The acyl chains present in CERs can be non-hydroxylated (N); α-hydroxylated (A); ω-hydroxylated (O) or esterified ω-hydroxylated (EO). The sphingoid bases can be classified as sphingosine (S), dihydrosphingosine (dS), phytosphingosine (P) or 6-hydroxy-sphingosine (H). Other CER groups are present in the SC in lower amounts, including the newly identified sphingoid base dihydroxy sphinganine (CER[T]) and the recently described acyl chain 1-O-acylceramindes (CER [1-O-E])[46,47]. In addition to their structural classification, a wide range of carbon chain length has been described for both the sphingoid base and the acyl chain, yielding CERs with a total chain length between 32-78 carbon atoms[48]. Although less abundant, unsaturated CERs can also be present in the SC[34,47,49].

FFAs are fatty acids in non-esterified form. In contrast to CERs, FFAs have a simpler structure and represent only 15-20% of the SC lipid weight[40,41]. FFA species in the SC vary in carbon chain length (12-36 carbon atoms), unsaturation (saturated or mono/polyunsaturated), and hydroxylation (non- or hydroxylated) (Figure 3)[31,50,51]. However,

in both murine and human skin, saturated long chain FFAs with 24 and 26 carbon atoms are most abundant[50,52]. Both unsaturated and hydroxylated FFAs are present at very low levels in healthy human SC[51]. In contrast, unsaturated FFA species can account for nearly 15% of the FFA content in murine skin[52].

SYNTHESIS OF SC BARRIER LIPIDS

The lipids present in the SC lipid matrix are primarily synthesized by keratinocytes during their differentiation process in the viable epidermis[19,41,53].

Cholesterol

Cholesterol is synthesized in the endoplasmic reticulum and peroxisomes of keratinocytes starting with condensation of three molecules of acetyl-co-enzyme-A (CoA) to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)[54]. The reduction of HMG-CoA to mevalonate by the enzyme HMG-CoA reductase (HMGCR) is the rate-limiting step in cholesterol synthesis[54,55]. Next, in a series of enzyme mediated steps, mevalonate is converted to lanosterol, which can be finally converted into cholesterol.

Ceramides (CERs)

The synthesis of CERs also occurs in the endoplasmic reticulum starting with the condensation of serine with palmitoyl-CoA by the enzyme serine-palmitoyl transferase to produce 3-keto-dihydrosphingosine[41,56,57]. Multiple steps involving the ceramide synthase family result in the acetylation of a FA to form a ceramide with a dihydrosphingosine base (CER[dS]). Next, the CER[dS] can be converted into other CER subclasses [S] and [P] by activity of dihydroceramide desaturase 1 (DESG1) and 2 (DESG2), respectively[41]. The mechanisms underlying the hydroxylation step involved in the conversion of CER [dS] into CER[H] remain unknown. The enzymes patatin like phospholipase domain containing 1 and CYP4F22 have been implicated in the esterification of linoleic acid (FA C18:2) to form the CER[EO] subclass; yet, the contribution of other mediators cannot be excluded[41,58]. At the final step, the CERs are transported to the Golgi complex for the addition of a glucose (glucosyl-CERs) or phosphocholine (sphingomyelin) followed by their storage in the LB as CER precursor lipids. Once extruded at the interface SG-SC, glucosyl-CERs and sphingomyelin are converted by β-glucocerebrosidase (GBA) and acid sphingomyelinase, respectively, into CERs for the lipid matrix[59,60].

Free fatty acids (FFAs)

The synthesis of fatty acids starts in the cytosol with the condensation of one acetyl-CoA and seven malonyl-CoA molecules to form palmitic acid, a saturated fatty acid

with 16 carbon atoms in its chain (FFA C16:0). Palmitic acid is then elongated in the endoplasmic reticulum through a four steps membrane-associated elongation cycle: condensation, reduction, dehydration, and reduction, respectively. The first step, condensation, is mediated by a family of seven elongases (ELOVL1-7) and is the rate limiting step in the fatty acid synthesis[61,62]. In addition to elongation, fatty acids can be desaturated by stearoyl-CoA desaturase (SCD) and fatty acid desaturases (FADS)[62–64]. At the end, fatty acids can be used in the CER synthesis or converted into phospholipids for storage in the LB as FFA precursors for the SC lipids. In fact FFAs and CERs share a common biosynthetic pathway in the epidermis and alterations in the chain length or unsaturation of FFA translate into similar trends in the CER profile[35,41,65]. Finally, as described for the CERs, following extrusion at the interface SG-SC, these precursors can be converted by various phospholipases into FFAs to compose the lipid matrix.

Extracutaneous lipids

In addition to local lipid synthesis by keratinocytes, lipids of extracutaneous origin (*e.g.* FAs, precursors CERs, and cholesterol) enter the skin and can be detected in the epidermis[66–71]. Nearly 20% of circulating low-density lipoprotein (LDL) particles reach the skin, specifically, the cells in the SB[72]. In line, labelled cholesterol can be found in the epidermis following systemic administration[67]. The uptake of CER precursors from the plasma can also contribute to the epidermal lipid pool[66,73,74]. In mice, sphingomyelin and ceramide products can be found in SC after oral administration of radiolabeled sphingomyelin[66]. Furthermore, oral administration of sphingomyelin also improved the hydration of the SC and increased the CER content in the epidermis of mice[66]. Similar effects have been reported after supplementation of rice-derived glucosylceramides to mice and to human skin equivalent[74]. Essential FAs are not produced in the body and enter the circulation via the intake of food[68,70]. These diet-derived FAs are also essential components of the SC lipid matrix[71].

The uptake of cholesterol and FAs from the plasma and from circulating lipoproteins are likely mediated by receptors expressed in the keratinocytes (*e.g.* low-density lipoprotein receptor - LDLR, scavenger receptor class B member I -SR-BI, fatty acid binding protein-FABP, fatty acid transport protein - FATP, and cluster of differentiation 36 - CD36)[68,75–78]. These receptors are especially expressed in the basal layer of the epidermis with reduced expression towards the skin surface[75,78]. Inhibition of local cholesterol synthesis by topical application of pitavastatin leads to upregulation of LDLR in both cultured human keratinocytes and *in vivo* murine skin[75]. The levels of SR-BI and CD36 in the epidermis increase in response to acute barrier disruption, which can be normalized by occlusion of the disrupted barrier area[68,78]. CERs are also transported in the plasma in lipoproteins[79] and are likely delivered to the skin as part of these particles. However, in contrast, the pathways involved in the uptake of extracutaneous CERs remain unclear.

In summary, the importance of plasma lipids for the skin has been demonstrated both *in vitro* and *in vivo* and the contribution of these lipids to skin function becomes further relevant once the SC barrier has been compromised.

TRANSPORT AND METABOLISM OF PLASMA LIPIDS

Lipids are transported through the plasma compartment mainly in the core of 4 groups of lipoproteins: chylomicrons, very-low-density lipoproteins (VLDL), LDL, and high-density lipoproteins (HDL) (Figure 4). Each of these groups of lipoproteins have distinct physicochemical properties, lipid content, and protein composition (Table 1).

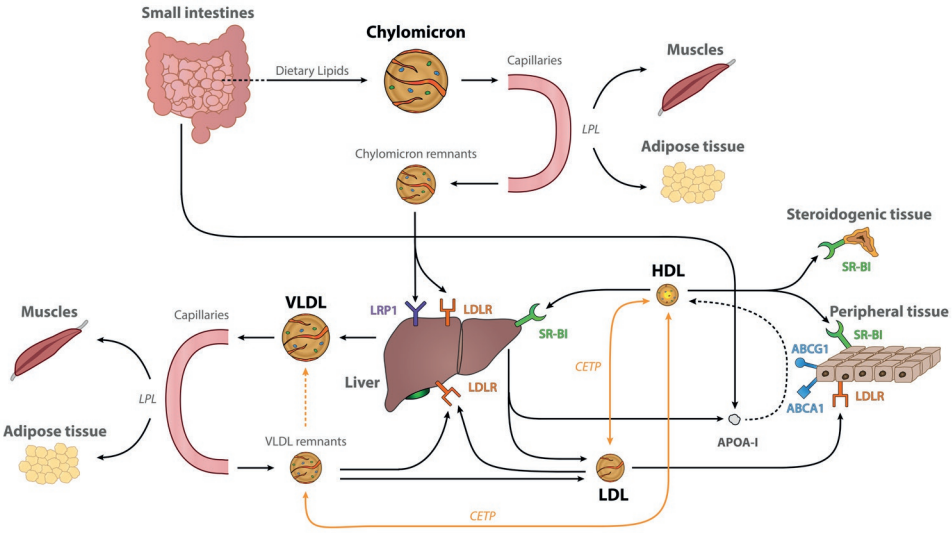

Figure 4. Metabolism of lipoproteins. The four major groups of lipoproteins are depicted: chylomicrons, very low-density lipoprotein (VLDL); low-density lipoprotein (LDL), and high-density lipoprotein (HDL). LPL is an extracellular enzyme present on the surface of the vascular endothelial involved in the metabolism of TG from VLDL and chylomicrons to generate free fatty acids that can be taken up by the muscles and adipose tissue. CETP is a lipid transfer factor present in humans but not in mice and it mediates the exchange of cholesteryl esters from HDL for triglycerides from VLDL/LDL (yellow arrows in the image). ABCA1 – ATP binding cassette A1; ABCG1; ATP binding cassette G1; APOAI-I – apolipoprotein A-I; CETP – cholesteryl ester transfer protein; LPL – lipoprotein lipase; LDLR – low-density lipoprotein receptor; LRP1 – low-density lipoprotein receptor related protein.

Chylomicrons are large particles produced by enterocytes and involved in the transport of lipids originated from the diet (exogenous source)[80,81]. The core of chylomicrons is primarily composed of TG but it also carries also cholesteryl esters (CE) and phospholipids. The main structural apolipoprotein (Apo) in chylomicrons is ApoB-48,

a truncated form of ApoB-100[82,83]. Once excreted by enterocytes into the lymphatic system and subsequently reaching the systemic circulation, the TG transported by chylomicrons are susceptible to the metabolic activity of lipoprotein lipase (LPL), leading to the removal of the FAs moieties. This process results in the formation of smaller cholesterol-rich chylomicron remnant particles that are rapidly taken up by the liver by interaction of ApoE with the LDLR-related protein 1 (LRP1) and LDLR.

VLDL are also TG-rich particles that carry a low amount cholesterol synthesized in the liver from acetyl-CoA molecules (endogenous source)[84]. The Apo profile in VLDL particles comprises ApoC-II and ApoE, and the main structural protein ApoB-100[85]. In rodents, ApoB-48 is also present in VLDL. VLDL is produced in the liver and released into the circulation where the TGs in the core of the particle undergo cleavage by LPL as described for chylomicrons[85]. TG-depleted VLDL remnants will lose ApoE and can be converted into LDL, CE-rich particles[85]. LDL delivers cholesterol to the tissues and is cleared from the circulation by the liver upon interaction with LDLR.

HDL represents another important group of lipoproteins involved in the transport of cholesterol throughout the body. In contrast to chylomicrons, VLDL and LDL that mainly participate in the delivery of lipids to the tissues, HDL especially mediates the transport of cholesterol from peripheral tissues to steroidogenic tissues and to the liver, the latter is part of a process defined as reverse cholesterol transport[86,87]. HDL particles contain ApoA-II, ApoC-II, ApoE, and ApoA-I, the latter comprising nearly 70% of the HDL protein content[88]. ApoA-I is synthesized by enterocytes and hepatocytes and secreted into the plasma in a lipid-poor form. ApoA-I develops into mature HDL particles by acquisition of lipids, in particular free cholesterol and phospholipids from other lipoproteins and via its interaction with the cellular cholesterol efflux pumps ATP-binding cassette (ABC)A1 and ABCG1[89]. Cholesterol is then stored in the core of the lipoprotein after esterification by the enzyme lecithin: cholesterol acyl transferase (LCAT)[90]. Interaction of HDL particles with SR-BI leads to the selective delivery of HDL-CE to steroidogenic tissues for hormone production or to the liver to be redistributed to the body or excreted via the bile, the last step in the reverse cholesterol transport process[91,92].

The distribution of TG and cholesterol within the lipoprotein groups varies according to the species[86,94]. In normolipidemic human subjects TG and CE are mainly transported in the core of VLDL and LDL, respectively. In normolipidemic rodents, VLDL is the main carrier of TG in the fasted state while almost all cholesterol circulates in HDL particles. These distinctions are crucial when translating the research performed in murine models to the human situation.

Table 1. Characteristics of the four main lipoprotein classes involved in the transport of lipids throughout the body: their density, size, lipid, and apolipoprotein composition[95,96] For each lipoprotein class the most representative lipids and apolipoproteins are described.

Lipoprotein	Density (g/ml)	Size (nm)	Lipid composition	Apolipoprotein composition
Chylomicrons	<0.930	75-1200	Triglycerides	ApoB-48, ApoC-II, ApoC-III, ApoE, ApoA-I, ApoA-II
VLDL	0.930-1.006	30-80	Triglycerides	ApoB-100, ApoE, ApoC-II, ApoB-48 (in rodents)
LDL	1.019-1.063	18-25	Cholesterol	ApoB-100, ApoB-48 (in rodents)
HDL	1.063-1.210	5-12	Cholesterol Phospho;lipids	ApoA-I, ApoA-II, ApoC-I, ApoC-II, ApoE

DYSLIPIDEMIA

Abnormalities in the metabolism of lipoproteins lead to the development of dyslipidemia, often marked by a persistent raise in the plasma lipid concentration, especially cholesterol and TG. Dyslipidemic profiles are a common cause of morbidity worldwide and hypercholesterolemia accounts for the majority of the cases. To date, several mutations have been identified in Apo's, enzymes, and receptors involved in the metabolism of lipoproteins.

Mutations in, but not limited to, LDLR and ApoE lead to the development of familial hypercholesterolemia (FH) and familial combined hyperlipidemia, respectively[97–99]. The loss of a functional LDLR hinders the clearance of ApoB- and ApoE-containing lipoproteins from the plasma[100]. These lipoproteins are then only catabolized via LRP1. In contrast, the lack of functional ApoE impacts the clearance chylomicrons remnants and VLDL via both the LDLR and LRP1, as this Apo is their main ligand[100]. Nonetheless, FH is marked by an increased retention time of LDL in the circulation, and thus, a higher concentration of cholesterol in the plasma. FH patients display an increased risk of pre-mature development of cardiovascular diseases (*e.g.* as a result of enhanced atherosclerosis)[98]. In addition, FH patients often present cholesterol deposits (xanthomas) in tendons, cornea, and in various skin sites. Interestingly, the composition of the xanthomas is similar to that described for atherosclerotic plaques in these patients[101].

Accumulation of HDL particles, namely hyperalphalipoproteinemia, can also cause hypercholesterolemia. Among others, mutations in cholesteryl ester transfer protein (CETP) and SR-BI have been identified as underlying causes of hyperalphalipoproteinemia[102–105]. Absence of CETP limits the transfer of CE from

HDL to ApoB-containing lipoproteins, hence, increasing the plasma fraction of HDL-cholesterol[106,107]. Loss of functionality or absence of SR-BI impairs the clearance of plasma HDL leading to a rise in the HDL-cholesterol fraction mainly carried by larger and abnormal HDL particles[104,108,109]. Although HDL is involved in reversed cholesterol transport, higher levels of HDL are not always associated with a reduced risk of cardiovascular diseases[86,110,111]. In addition, accumulation of HDL particles has been described to impact platelet function and steroidogenesis, among others[112,113].

As illustrated in the previous paragraphs, cardiovascular diseases are highly associated with hypercholesterolemia. The epidemiology of this disease can be related to genetic predisposition (as reported for FH patients) but other factors such as age, gender, hypertension, diabetes, obesity, and lifestyle may be contributors to the onset and development of this disease. Interestingly, the skin and nails of individuals with cardiovascular problems can be the first sites to show warning signs for heart conditions[101,114,115]. These individuals may, amongst others, develop yellow deposits under the skin, skin coloring (blue/purple net pattern), and non-itchy rashes.

ATHEROSCLEROSIS

Atherosclerosis is marked by the accumulation of cholesterol in the wall of medium-sized and large arteries leading to a chronic narrowing and hardening disease in these vessels (Figure 5a)[116,117]. This process initiates at early age and can remain asymptomatic through a lifetime or culminate in complications such as myocardial infarction.

According to the response-to-injury hypothesis, the development of atherosclerosis starts with endothelial dysfunction and activation, characterized by enhanced expression of adhesion molecules. LDL can pass the endothelial layer of the arterial wall by passive diffusion or through active transcytosis. Once in the sub-endothelial space, the LDL particles are trapped and undergo extensive modifications (lipolysis, oxidation)[116]. The buildup of oxidized LDL (oxLDL) in the intima of the arterial wall activates the endothelial cells to enhance the production of adhesion molecules and to secrete cytokines and growth factors. Interaction with adhesion molecules leads to an increase in monocyte infiltration from the blood stream into the arterial wall[117]. In the arterial wall, monocytes differentiate into macrophages and phagocyte oxLDL becoming lipid-rich foam cells with reduced mobility and insufficient cholesterol efflux capacity (Figure 5b)[117].

The development of the atherosclerotic plaque progresses as a local chronic inflammatory reaction with further recruitment of monocytes, mast cells, and T-cells[117–119]. Eventually, the overgrown lipid-laden foam cells die releasing their debris and lipid content into the plaque, which in turn aggravate the local inflammatory

response. At this late stage, the core of the atherosclerotic plaque consists of cellular debris, infiltrated immune cells and cholesterol, and is covered by a collagen-rich fibrous cap produced by smooth muscle cells[120]. The thickness of this cap correlates with the stability of the plaque and can ultimately hinder plaque rupture; hence, reducing the likelihood of a thrombotic event[120].

Reducing risk factors is pivotal in lowering atherosclerosis-related risks[117,121]. Thus, patients are advised to switch to a healthy diet, do physical exercise, stop tobacco abuse, and control hypertension and diabetes mellitus[117]. The main benefits of these treatments are to stabilize and to slow down the development of atherosclerotic plaques. Most treatments for atherosclerosis focus on lowering LDL cholesterol (gold standard prevention method)[121–123]. HMG-CoA reductase inhibitors (statins) are the main molecules employed to reduce plasma LDL levels. Interestingly, statins also impact the cholesterol synthesis in the skin and have been associated with increased risk of skin infection[124]. Other approaches to lower cholesterol levels include the use of cholesterol absorption inhibitors, PCSK9 inhibitors, and bile acid sequestrants[117,121]. Lowering TG levels is also important in atherosclerotic patients. For that purpose, statins, fibrates and n-3 fatty acids are the most common therapies[125–127]. As HDL is a key lipoprotein in the reverse cholesterol transport, increasing HDL has also been investigated as a possible therapeutic approach, mostly with unsatisfactory results[128]. CETP inhibitors and ApoA-I mimetics have also been explored as intervention therapies, although late development of these compounds have been complicated when translating to the clinic, with some CETP inhibitors failing at phase III trials[129,130]. As the efficacy of these interventions can vary between individuals and residual risk of coronary event remains over 50%, new therapeutic approaches are required[131–134]. In view of the pivotal role of macrophages in of atherosclerosis, therapies that can reduce local lipid content and inhibit inflammation remain interesting in the treatment of this pathophysiology[135].

LIVER X RECEPTOR (LXR)

The liver X receptor (LXR) is a nuclear transcription factor that functions as an intracellular cholesterol sensor and a suppressor of inflammatory genes[136–138]. Increased intracellular (oxidized) cholesterol levels result in activation of LXR followed by induction of cholesterol efflux pathways by upregulation of ABCA1 and ABCG1[139,140]. In addition, LXR activation impacts cellular fatty acid metabolism by, among others, regulating the expression of genes involved in fatty acid synthesis[136,141]. LXR also has a role in regulation of inflammation by, for instance, repressing the expression of tumor necrosis factor alpha, cyclooxygenase 2, nitric oxide synthase, and matrix metalloprotease[137]. Two isoforms of LXR have been identified; LXRα and LXRβ. LXRα is mostly expressed in the liver, intestines, spleen, and adipose tissue, while LXRβ is ubiquitously expressed[142]. A group of small molecules defined

as synthetic LXR agonists (*e.g.* TO901317 and GW3965) has been described to activate LXR and its downstream targets involved in the regulation of lipid and inflammation pathways[143–145].

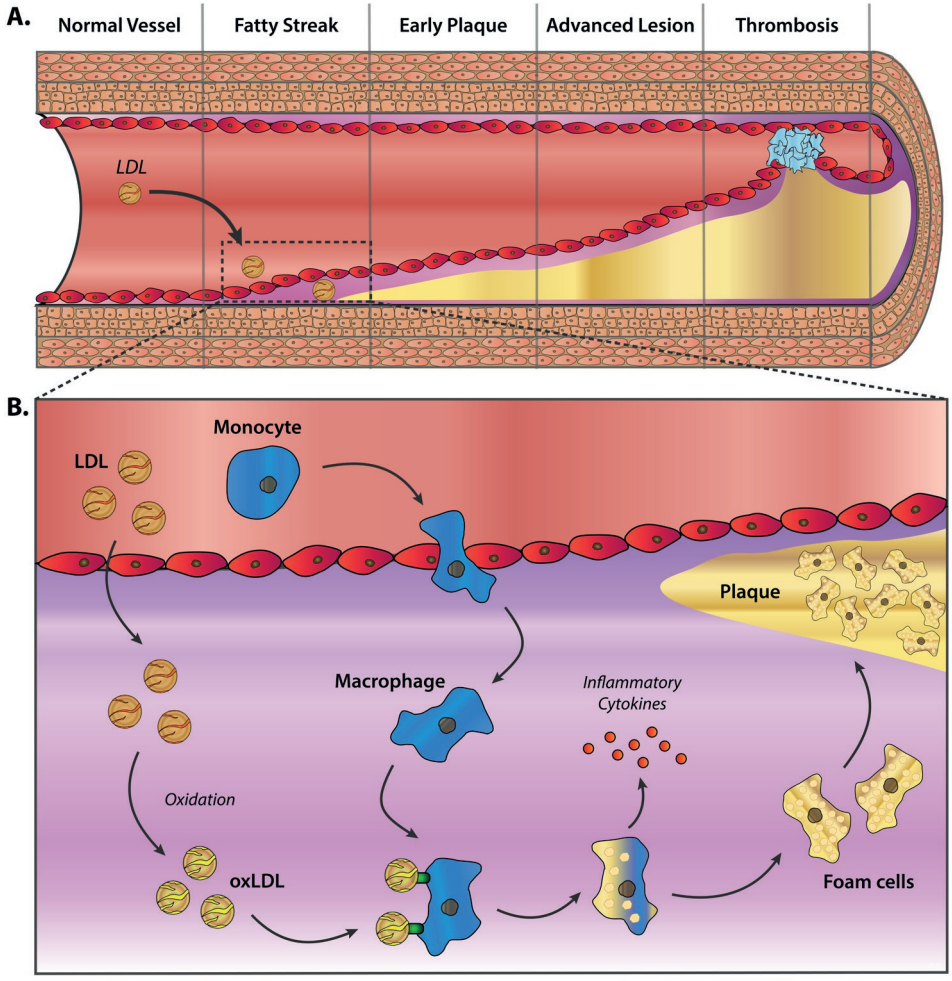

Figure 5. The pathogenesis of atherosclerosis. (A) The stages of atherosclerotic plaque development; from a healthy artery to an atherosclerotic artery with an advanced lesion and, in extreme cases, a thrombotic event. (B) The pivotal role of macrophages in pathogenesis of atherosclerosis. LDL particles can penetrate the intima of the arterial wall where it can undergo modifications (*e.g.* oxidation). Monocytes that penetrate the intima differentiate into macrophages. The oxidized LDL particles can be cleared by the macrophages via interaction with scavenger receptors expressed on the surface of these cells. Macrophages release inflammatory cytokines to recruit more monocytes and other immune cells to the site. The intracellular accumulation of lipids in the macrophages leads to the formation of lipid-rich cells (foam cells), a hallmark in the pathogenesis of atherosclerosis. LDL - low-density lipoprotein; oxLDL - oxidized low-density lipoprotein.

LXR has been extensively investigated as a potential therapeutic target in atherosclerosis as the deletion of LXR in mice is followed by a remarkable increase in susceptibility to atherosclerotic lesion development[143,146–148]. Activation of LXR by a synthetic agonist stimulates cholesterol efflux from foam cells in the arterial wall by upregulation of ABCA1 and ABCG1 expression[146,149,150]. However, severe undesired effects have been reported related to the administration of synthetic LXR agonists as free drug with remarkable impact on hepatic lipid metabolism[147,151]. Hence, various studies focus on the encapsulation of LXR agonist into (targeting) particles to increase the delivery to the atherosclerotic lesion while reducing unwanted effects[152,153].

THESIS AIM AND OUTLINE

The research reported in this thesis focuses on the impact of an imbalanced plasma cholesterol metabolism on skin lipid homeostasis (Figure 6). As patients with cardiovascular diseases are known to develop dermatological problems/signs, we hypothesized that the skin may have its own lipid profile impacted by one of the most important risk factors for atherosclerosis, namely increased cholesterol levels.

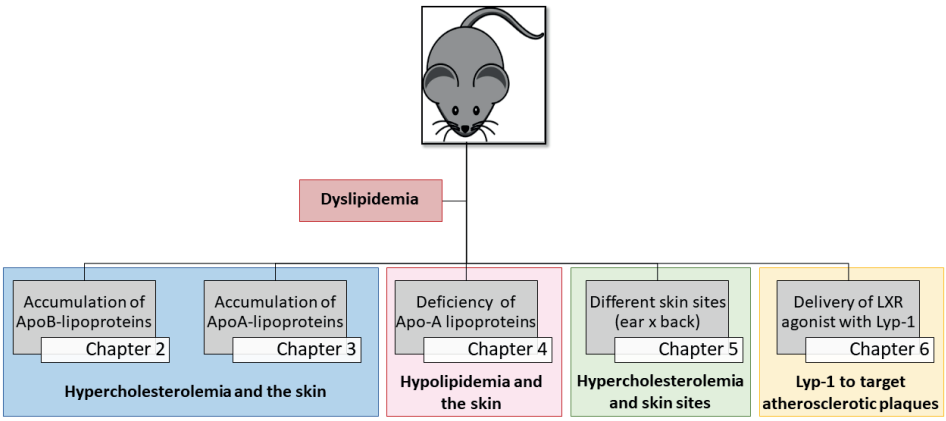

Figure 6. Research chapters presented in this thesis. Chapters 2-5 of this thesis focus on the impact of various dyslipidemic profiles on the skin lipid composition, organization and barrier function. Chapters 6 reports the use of a cyclic peptide (Lyp-1) to deliver LXR-loaded liposomes to atherosclerotic lesion macrophages to reduce hypercholesterolemia associated cardiovascular risk.

In our studies, young adult dyslipidemic mouse models not challenged with lipid rich-diets were used to investigate our hypothesis. In **Chapter 2** we show the impact of mild- and severe hypercholesterolemia associated with increased plasma levels of ApoB-containing lipoproteins (VLDL; LDL) on the skin lipid composition and barrier function of *LDLR*$^{-/-}$ and *APOE*$^{-/-}$ mice. In **Chapter 3** we report the effects of hypercholesterolemia

driven by the accumulation of ApoA-containing lipoproteins (HDL) on the skin lipid profile and lipid barier function in *SR-BI*$^{-/-}$ mice. In a new approach, in **Chapter 4**, we evaluate the changes in the skin lipid homeostasis as a result of the disrupted metabolism of ApoA-containing lipoproteins (HDL) in the hypolipidemic profile of *APOAI*$^{-/-}$ mice. In **Chapter 5**, we use both wild-type and *APOE*$^{-/-}$ mice to investigate the epidermal lipid profile of two commonly skin sites used in research as well as their responses to altered plasma lipid levels.

Hypercholesterolemia is highly associated with the risk of developing cardiovascular diseases. Although effective, nearly 65-70% of the cardiovascular events cannot be prevented with the current therapeutic strategies. Therefore, new strategies are necessary. Specific activation of LXR in atherosclerotic plaques offers the opportunity to lower the residual cardiovascular risk by modulating lesion lipid content and inflammation. Thus, in **Chapter 6** we explore a new peptide-targeting approach to deliver liposomes carrying an LXR agonist to foam cells as a treatment for atherosclerosis in *LDLR*$^{-/-}$ mice. Finally, in **Chapter 7** we present a summary of the findings described in this thesis and discuss the conclusions and implications of this research to the field and for patients.

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