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

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Chapter 7

Summary, Discussion and Perspectives

INTRODUCTION

The skin represents the interface between the body and the environment. In this strategic position, this organ acts as a physical and immunological barrier protecting the organism against the excessive loss of water and nutrients and against the entry of chemicals and pathogens[1]. A key player in this protective role is the outermost layer of the skin - the stratum corneum (SC). The SC is composed of corneocytes (dead skin cells) embedded in a well-structured lipid matrix primarily composed of cholesterol, ceramides (CERs), and free fatty acids (FFAs) present in an equimolar ratio[2]. The functionality of the skin barrier relies on the organization of the lipid matrix as well as on the composition of the SC lipids, mostly synthesized by keratinocytes during their differentiation process to become corneocytes[3–8]. Extracutaneous lipids (*e.g.* lipoproteins) can also be found in the skin and may contribute to the formation of the SC lipid pool[9–13]. However, this crossroad between the local skin lipid synthesis and the uptake of extracutaneous lipids remains poorly understood.

In the plasma, lipids are mostly transported to and from peripheral tissues inside the core of four main groups of lipoproteins: chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Impaired metabolism of these lipoproteins leads to dyslipidemia, namely abnormal levels of lipids in the plasma. The most common form of dyslipidemia is hyperlipidemia, a condition characterized by the increased levels of circulating lipoproteins and lipids in the plasma that arises from genetic mutations (*e.g.* familial hypercholesterolemia; FH) or an alternate underlying etiology (*e.g.* diabetes, poor life style, and unhealthy diet)[14]. Hyperlipidemia is an important risk factor for the development of atherosclerosis, a major cause of cardiovascular disease[14]. Atherosclerosis is a pathology marked by the formation of lipid plaque(s) inside the arterial wall caused by a chronic immune reaction in response to disturbed cholesterol metabolism. In time, these lipid plaques can harden and narrow the arteries, disturbing the blood flow and oxygen supply and posing a risk to the formation of thrombus and the occurrence of strokes, myocardial infarction, among others.

Liver X receptor (LXR) is a nuclear receptor that plays relevant roles in lipid metabolism and inflammation[15–17]. In the context of atherosclerosis, modulation of LXR by synthetic agonists (*e.g.* GW3965) can impact the plaque development by (1) promoting reverse cholesterol transport from lipid-laden macrophages (foam cells) and (2) by general inhibition of pro-inflammatory genes[16]. However, systemic administration of LXR agonists as free drug directly impacts hepatic lipid synthesis and may induce hypertriglyceridemia[18,19]. Hence, research has focused on encapsulation of these

molecules in delivery systems, which improves biodistribution and introduces the possibility of targeted delivery.

In addition to the increased risk of developing atherosclerosis, individuals with hyperlipidemia (familial or acquired) may develop in time yellow, cholesterol-rich deposits (xanthomas) in the skin around their eyes or joints[20–22]. Xanthomas are known dermatological signs for underlying lipid disorders[21]. Despite the crucial role of lipids in the skin, the link between dyslipidemia and skin lipid homeostasis remains unclear. In this thesis, we explored this knowledge gap to assess whether and how the skin can reflect changes in plasma cholesterol levels at early age prior to the development of xanthomas (or other dermatological disorders) and atherosclerosis. Lastly, we explored the interaction of synthetic peptide Lyp-1 with p32 receptor expressed on lipid-laden macrophages in atherosclerotic plaques[23,24]. We focused on the therapeutic value of Lyp-1 as a targeting peptide on liposomes to deliver the LXR agonist GW3965 to the atherosclerotic plaques in hypercholesterolemic mice.

SUMMARY

The impact of dyslipidemia on the skin

Familial hypercholesterolemia (FH) is a particular type of genetic lipoprotein disorder with an overall reported prevalence of 1:311, among the most common genetic disorders in the general population with similar distribution around the world[25]. This condition arises from mutations in low-density lipoprotein receptor (LDLR) gene and less common in other genes such as apolipoprotein B (APOB), proprotein convertase subtilisin/kexin type 9, apolipoprotein E (APOE) genes[26–28]. Although high cholesterol levels normally do not cause specific complaints early in life, FH patients often develop xanthomas around the eyelids, joints, tendons, and even in the margin of the iris in the long-term[29–31]. These dermatological signs are often the first indication of hypercholesterolemia, an important risk factor for atherosclerosis as described previously.

In **Chapter 2**, we examined the skin of young adult (16-18 weeks old) LDLR knockout (*LDLR*$^{-/-}$) and *APOE*$^{-/-}$ mice in search for early cutaneous disturbances related to hypercholesterolemia. Both *LDLR*$^{-/-}$ and *APOE*$^{-/-}$ mice are well-established hypercholesterolemic mouse models, and their skin is described to develop xanthomas upon prolonged high fat/high-cholesterol diet feeding or upon ageing. We showed that at young age the morphology of skin was preserved without signs of xanthomas, inflammation or hyperproliferation in both mouse models. Further analysis of the epidermis revealed that the barrier lipid composition was different between these two

models. The lipid composition in epidermis of the mild hypercholesterolemic *LDLR*$^{-/-}$ mice strongly resembled the lipid profile reported for normolipidemic wild-type control mice. In contrast, the epidermis of *APOE*$^{-/-}$ mice was enriched with unsaturated FFA species and short chain FFAs with less than 24 carbons atoms in their structure. Remarkably, the CER profile and cholesterol content were hardly impacted in the epidermis of *APOE*$^{-/-}$ mice. CERs and FFAs are known to share a common synthetic pathway in the skin with changes in the chain length and degree of unsaturation of FFA translating into similar trends in the CER profile[5,32]. As the changes observed for the epidermal FFA species were not accompanied by a similar profile in the CER group, we hypothesized that the short and unsaturated FFA species were likely of extracutaneous origin. In the plasma of *APOE*$^{-/-}$ mice cholesteryl esters (CE) are enriched with fatty acid C18:1-moiety[33]. Similarly, we reported increased levels of FFA 18:1 in the epidermis of the *APOE*$^{-/-}$ mice, which was accompanied by strong downregulation of mRNA of genes involved in both cholesterol (HMG-CoA reductase - *HMGCR*) and FFA (acetyl-Coenzyme A carboxylase alpha- *ACACA* or *ACC*) synthesis in the skin of these mice. This genetic profile indicates a compensatory downregulation of genes involved in the synthesis of cholesterol and fatty acids in response to the increased local levels of cholesterol and FFAs. Additionally, a reduction in mRNA levels of *LDLR* showed that the pathway to mediate uptake of lipids from lipoproteins was downregulated. Finally, the altered FFA pool in the epidermis of *APOE*$^{-/-}$ mice resulted in a reduced skin barrier function as shown by increased transepidermal water loss. In short, this study suggests that the severity of the hypercholesterolemia in mice, in particular the levels of CE in plasma, may contribute to the flux of extracutaneous lipids into the skin; thus, impacting the epidermal lipid composition and the functionality of the skin barrier already at young age. From the perspective of FH patients, our findings showed that the skin of hypercholesterolemic individuals may already be impacted on lipid composition and barrier function prior to the development of xanthomas or cardiovascular complications.

Hypercholesterolemic profiles may also emerge from accumulation of apolipoprotein A (APOA) carrying lipoproteins leading to hyperalphalipoproteinemia. Genetic factors such as deficiency of cholesteryl ester transfer protein (CETP) or rare mutations in scavenger receptor class B member I (SR-BI) are described as causes of hyperalphalipoproteinemia with striking increase in HDL-cholesterol levels in humans[34–36]. In contrast with FH patients, hyperalphalipoproteinemic patients are not reported to develop xanthomas[37]. However, the skin is one of the largest reservoirs of HDL in the body; thus, hyperalphalipoproteinemia may have an impact on the skin lipid homeostasis[38].

Next, in **Chapter 3** we assessed whether the hypercholesterolemia derived from

hyperalphalipoproteinemia could also impact the epidermal lipid pool. For this purpose we used young adult (16-18 weeks old) *SR-BI*$^{-/-}$ mice, a hypercholesterolemic mouse model of hyperalphalipoproteinemia, in which the clearance of HDL particles by the liver is impaired as well as their uptake by steroidogenic tissues[39–41]. Notably, the levels of total CE transported in lipoproteins in *SR-BI*$^{-/-}$ mice are significantly higher than the levels reported for *LDLR*$^{-/-}$ mice, but not as severe as seen for the *APOE*$^{-/-}$ mice (**Chapter 2**). Despite the protective role of HDL in reverse cholesterol transport, hyperalphalipoproteinemia had a negative impact on the epidermal FFA composition of the *SR-BI*$^{-/-}$ mice. Although to a lower extent as reported for the skin of *APOE*$^{-/-}$mice, the relative percentage of unsaturated and short carbon chain FFA species, including FFA C18:1, was augmented in the epidermis of *SR-BI*$^{-/-}$ mice in comparison with the normolipidemic wild-type controls. Similar to the epidermis of *APOE*$^{-/-}$ mice, the cholesterol content and CER profile were preserved in the epidermis of these mice. Thus, the altered FFA profile in the epidermal barrier of *SR-BI*$^{-/-}$ mice is likely not linked to the biosynthetic pathway shared with CERs but rather linked to lipids of extracutaneous origin[5,32]. This hypothesis is supported by the reduced mRNA levels of genes related to cholesterol (HMG-CoA synthase 1; *HMGCS1*) and FFA synthesis (*ACACA* and fatty acid synthase; *FAS*) as well as lower levels of *LDLR*. The accumulation of CE in the plasma of *SR-BI*$^{-/-}$ mice inhibits the activity of the enzyme lecithin-cholesterol acyl transferase (LCAT)[42]. Mice with reduced LCAT activity reportedly show increased circulating levels of CE containing fatty acid C16:0- and C18:1 moieties, supporting once more a direct link between fatty acid C18:1-containing CE in plasma and elevated epidermal FFA C18:1[33]. Synthetic stratum corneum lipid model membranes mimicking the epidermal composition of the *SR-BI*$^{-/-}$ mice showed a more permeable outside-inside lipid barrier. However, *in vivo* transepidermal water loss revealed a functional inside-outside skin barrier. Importantly, we showed that effects on epidermal lipids are closely correlated with plasma lipid levels and less dependent on the lipoprotein group involved in the lipid transport. Although dermatological signs (*e.g.* xanthomas) have not been described in patients with hyperalphalipoproteinemia, our data demonstrates that the skin is negatively affected by the changes in the plasma lipid profile. Hence, it is relevant to pay additional attention to the skin of these patients to prevent the development of dermatological abnormalities.

It is important to note that dyslipidemia may also refer to reduced levels of plasma lipids, namely hypolipidemia, caused by genetic abnormalities or other disorders. Mutations in APOAI can severely affect the metabolism of HDL leading to a virtual absence of these particles, which results in reduced cholesterol in the plasma (especially lower CE levels) and an increased risk of cardiovascular disease[43–48]. In **Chapter 4** we assessed the impact of HDL-driven hypolipidemia (hypoalphalipoproteinemia) on the skin

barrier lipid composition of *APOAI*$^{-/-}$ mice. Hypoalphalipoproteinemia in the *APOAI*$^{-/-}$ mice did not impact the general morphology of the skin or the epidermal lipid profile. However, the mRNA expression of various genes involved in lipid synthesis and uptake of lipoproteins was upregulated in the skin of these mice (*e.g. HMGCR, LDLR, FAS, SR-BI*). In line, the downregulation of *DGAT2* (diacylglycerol O-acyltransferase 2) mRNA levels in the skin of these mice suggests a reduction in the storage of fatty acids in the form of triglycerides. Altogether, our data indicates that (1) HDL particles are not crucial for the skin reversed cholesterol transport but (2) contribute to the delivery of essential lipids to the skin. Importantly, the epidermal lipid barrier can be maintained by a compensatory rise in local lipid synthesis and in the uptake of lipid from apolipoprotein B-containing lipoproteins in the virtual absence of HDL.

The relevance of the skin site to research – a barrier lipid assessment

The studies described in the previous sections (**Chapters 2-4**) were performed using the back skin of the mice as it comprised a large area for sampling and analysis. However, skin of both ear and back are commonly used for research in mouse models and these sites strongly differ in the density of hair follicles and the number of cell layers in the SC. Furthermore, these skin sites may yield different effects/phenotypes regarding for instance drug treatment and tissues regeneration studies[49–51]. Despite these differences little information is available regarding the composition of their lipid barrier, especially regarding the ear epidermis.

In **Chapter 5**, we provide evidence that the epidermal lipid composition and the mRNA gene expression profile are fundamentally different between the ear and the back skin in a normolipidemic background. Compared to the back skin, the epidermis of the ears revealed stronger prevalence of sphingosine base (CER[S]) in the overall CER pool, including short chain CERs (33-34 carbons). Additionally, the ear epidermis was enriched with shorter and unsaturated FFA species, leading to a reduction in the mean chain length of the FFA pool. Both unsaturated- and short chains lipids in the SC are associated with reduced barrier function of the skin. For instance, the skin of atopic dermatitis patients shows a higher percentage of CER [S], 34 carbon chain CERs, short FFAs, and unsaturated FFAs[5]. This profile in atopic dermatitis accounts for a reduced barrier function of the skin marked by an increased transepidermal water loss in these patients[5]. However, in the case of the murine model, the ear skin may compensate for this unfavorable lipid profile with an increased number of corneocyte layers in the epidermis. In addition to the reported differences in the epidermal lipid profile, the ear skin showed a reduction in the mRNA expression of genes involved in the lipid synthesis (*e.g. FAS, elongases, HMGCS1*) and late differentiation markers for keratinocytes (keratin

10 and involucrin), indicating a lower metabolic activity in this skin site. In agreement, the network of blood- and lymphatic vessels is less prominent in the ear[52,53].

Further in **Chapter 5**, we described that the skin of the ear marginally responded to hypercholesterolemia as compared to the back skin in *APOE*$^{-/-}$ mice[54]. The FFA epidermal profile of the ears of normolipidemic and hypercholesterolemic mice was fairly comparable, with only a minimal increase in the overall percentage of the short chain FFA species. Further, we showed that changes in the FFA component of sebaceous lipids do not account for the striking changes reported in the back skin of *APOE*$^{-/-}$ mice. These findings indicate that the back skin is likely a more active metabolic site compared to the ear skin and these sites respond differently to specific treatments leading to distinct outcomes as previously reported[49–51]. Thus, the selection of the skin site can have an impact on the outcome of a study and should be carefully considered to match the goal of the study. For instance, studies to assess the penetration of topically applied compounds could be performed on the ears due to their larger interfollicular area and higher number of corneocyte layers; which more closely resembles human skin.

Lyp-1: small peptide to target liposomes to atherosclerotic lesion macrophages

In addition to its effects on the skin, hypercholesterolemia is an important risk factor for atherosclerosis, a disease characterized by the pathologic narrowing of large and medium arteries[55]. The onset and development of this pathology is primarily driven by lipid (LDL and other lipoprotein remnants) buildup in macrophages in the intima of these vessels, leading to the formation of atherosclerotic plaques[55–57]. In **Chapter 6**, we described our efforts to design a liposome formulation to deliver LXR agonist GW3965 to plaque macrophages stimulating reverse cholesterol transport and ultimately promoting plaque stabilization[58–60]. Atherosclerotic plaques show high expression of C1q-binding protein, also known as p32, in particular in lipid-rich macrophages (foam cells)[23,24]. Lyp-1 is a small cyclic peptide (CGNKRTRGC) known to bind to p32[23]. To date, the potential of Lyp-1 as targeting molecule has been mostly explored for plaque imaging purposes, which also benefits from macrophages propensity to take up nanoparticles[61,62].

In our study, liposome functionalization with Lyp-1 was the key strategy in our formulation design to increase target and retention time of the liposomes in the plaque. Despite their inherited properties towards particle uptake, lipid-rich murine macrophages displayed uptake preference towards Lyp-1-containing liposomes compared to the Lyp-1-free particles in the in vitro setting. In agreement, *LDLR*$^{-/-}$ mice on Western type diet treated with GW3965-loaded liposomes showed increased retention

for the particles functionalized with Lyp-1 in the aorta. In line with the *in vitro* data, the percentage of aortic macrophages "positive" for Lyp-1-targeting liposomes was superior to the Lyp-1-free liposomes. Although no impact in plaque size was observed in this study, the group treated with GW3965-loaded Lyp-1 liposomes had reduced plaque macrophage content and enhanced plaque stability as evidenced by increased collagen content. Additionally, treatment with the functionalized GW3965-loaded liposomes did not impact liver and serum lipid content. Hence, liposomes functionalized with Lyp-1 are a valuable tool to target lipid-rich macrophages in the atherosclerotic lesions and may increase efficacy of a moderate dose of GW3965, paving the way to the development of better therapies for atherosclerosis.

DISCUSSION AND PERSPECTIVES

The research described in this thesis broadens our perspective of how dyslipidemia impacts the quality of life of the individuals beyond cardiovascular- and metabolic diseases. It highlights the relevance of unraveling the governance of the skin lipid homeostasis in response to dyslipidemic profiles to properly advice and to treat these patients on dermatological disorders they may develop. In turn, this knowledge may facilitate the future development of therapies that could tackle both sides of this spectrum.

Some dermatological disorders (*e.g.* psoriasis) with chronic and systemic inflammatory components have been associated with plasma lipid abnormalities[22]. Also, individuals suffering from dyslipidemia are generally unaware of their condition unless in case of a severe profile. As a consequence, skin signs like xanthomas may be the first indication of an imbalanced metabolic lipid profile[21,22]. For instance, patients with FH present increased plasma cholesterol levels from birth; however, the development of skin xanthomas can occur at different stages of life and likely dependent on the severity of the dyslipidemic profile[28]. Here, we report that skin lipid composition can be negatively impacted prior to the development of macroscopic dermatological abnormalities in dyslipidemic mice fed a regular chow diet (low-fat diet).

It is important to note that the dyslipidemic murine models used in this thesis are a result of total body gene deletion; hence, the deletion affects all organs in the body in which the gene of interest is normally expressed. The deletion of *SR-BI* and *LDLR* in the liver impacts the metabolism of lipoproteins strongly contributing to the dyslipidemic profiles seen in the mice. However, *SR-BI*, *LDLR*, and *APOE* are also expressed in the skin, especially in keratinocytes in the basal layer[63–65]. In our studies, the consequences of the local gene deletion could not be evaluated with our full body knockout models;

thus, a potential local effect cannot be discarded. The roles of SR-BI and APOE in the skin remain unclear. To differentiate local (skin) effect from systemic effects (plasma-derived), the generation of skin-specific knockout models by Cre-LoxP system can be a valuable tool to assess the role of these genes and their respective protein/receptor exclusively in the skin[66,67]. In line with previous studies, we showed that different results may be obtained depending on the skin site used in the research[49–51,68]. Also for this topic, skin specific knockout of genes may help shed some light on the diversity of responses of the skin sites, in particular between areas where differences in metabolic activity are anticipated.

In addition to genetic predisposition, dietary habits affect the composition and the metabolism of plasma lipids. In both mice and humans, hypercholesterolemia may also derive from dietary habits such as the regular intake of lipid-rich diet as commonly observed in Western countries. In our studies, the use of young adult mice fed a regular low-fat diet allowed us to assess the impact of "naturally occurring" (genetically) imbalanced lipid metabolism on the skin without the contribution of ageing and unhealthy diets. Several studies in murine models have reported the negative impact of lipid-rich diets on the skin[69–73]. For instance, *LDLR*$^{-/-}$ mice fed a diet containing 1.25% cholesterol, 7.5% cocoa butter, 7.5% casein and 0.5% cholate for 5 to 7 months developed strong skin xanthoma, a phenotype not observed upon challenge with Western-type diet, containing 0.15% cholesterol and 21% fat for 3 to 5 months[74,75]. These studies demonstrate that the composition of the diet and its duration determine development of dermatological abnormalities in mice. However, it is important to note that these extreme hyperlipidemic models do not develop human-like lipoproteins profiles. APOE*3-Leiden.huCETP mice have reduced clearance of apolipoprotein B containing lipoproteins and express human CETP, a protein that facilitates the exchange of triglycerides between HDL and apolipoprotein B containing lipoproteins[76,77]. As a result, these mice develop a human-like lipoprotein profile when challenged with a cholesterol-rich Western type diet[78,79]. Hence, analysis of the skin of APOE*3-Leiden.huCETP with and without the challenge of lipid rich diets is important to facilitate the translation of these dermatological findings to the human situation.

Hypercholesterolemia is also a well-defined risk factor for the development of atherosclerosis, a pathology marked by impaired cholesterol metabolism. To expand our knowledge on the plasma lipid changes underlying the development of atherosclerosis metabolomics (in particular lipidomics) could be employed. In *SR-BI*$^{-/-}$ mice, plasma metabolomics identified potential biomarkers for cardiovascular disease[80]. Similarly, a wide-ranging plasma metabolomics analyses of *APOE*$^{-/-}$ mice revealed distinct glycerophospholipid and sphingolipid metabolites profiles associated with different

stages of atherosclerosis development in these mice detectable from 5-15 weeks of age[81]. Importantly, glycerophospholipids and sphingolipids have also been reported to be altered in the plasma of psoriatic patients[82,83]. In addition, literature reports that FH patients with xanthomas have a higher risk of developing cardiovascular disease compared to those without these dermatological signs[84]. Although much remains to be understood regarding the plasma-skin interaction as described in the previous paragraphs, these findings bring forward the opportunity to explore the connection between atherosclerosis development and early dermatological disorders. This knowledge (metabolomics) may in the future ratify epidermal lipid screening as a marker for dyslipidemia and other metabolic disorders to bring the opportunity of early detection and treatment to improve the quality of life of the patients.

As atherosclerosis remains a leading cause of death worldwide, identifying novel therapeutic targets and strategies to treat this pathology is crucial, in particular at an early stage of disease development[85]. In this battle against atherosclerosis, synthetic LXR agonists have extensively been investigated to stimulate reversed cholesterol transport from atherosclerotic plaques[60]. It is clear that systemic administration of LXR agonists as free drugs can lead to an unwanted rise in hepatic- and serum lipids, thereby limiting their anti-atherogenic effects[86–88]. For instance, oral administration of free GW3965 at 10 mg/kg for 12 weeks strongly reduced atherosclerotic lesion size[87]. However, this effect was accompanied by hypertriglyceridemia in mice. Thus, encapsulation of these small molecules in drug carriers is an useful tool to modify the biodistribution of these compounds[89]. The use of drug delivery systems also brings the opportunity to employ targeting molecules to increase delivery to the atherosclerotic plaque. The synthetic cyclic peptide Lyp-1 interacts with p32, a receptor highly expressed in macrophage foam cells in atherosclerotic lesions[24]. Hence, functionalization of GW3965-loaded liposomes with this small peptide can enhance targeting of plaque macrophages. The targeting properties of Lyp-1 increased the efficacy of a moderate dose of GW3965 (6.5 mg/kg) yielding a plaque with reduced macrophage content and higher collagen content. The atherosclerotic lesion size was not impacted by treatment with free-GW3965 or GW3065-loaded-lyp-1 liposomes, which can be driven by the moderate dose used in our studies. To achieve plaque size reduction by treatment with Lyp-1 liposomes loaded with a higher dose of GW3965 (*e.g.* 10 mg/kg), it is crucial to concurrently explore strategies to minimize hepatic uptake of these particles. In our study we used poly-ethylene glycol (PEG) to create a stealthy particle surface and reduce removal of the liposomes by the reticuloendothelial system (RES). Yet, we observed a significant hepatic accumulation of PEG-coated-Lyp-1 liposomes. Other strategies to increse circulation time and/or evade the immune system can be evaluated to improve the performance of the Lyp-1 liposomes such as polyvinyl pyrrolidone, polyoxazolines, CD47-mimicking peptide, PEG-

based copolymers[90,91]. Another interesting strategy to evade the RES system comprises "RES blockage" in which pre-administration of empty liposomes to occupy the RES system enables a longer circulation time of subsequently administered nanoparticles[92]. However, the effectiveness of this approach is controversial and the biodistribution of the subsequent dose could be affected in other ways that aimed for. The delivery of other drug classes (*e.g.* microRNAs, atorvastatin) could also be considered as macrophages are also involved in the local inflammatory response in the atherosclerotic plaque[93–96]. Different delivery systems yield different particle biodistribution. In line, other drug delivery systems could be functionalized with Lyp-1 as this small peptide has been reported to deliver supramagnetic iron oxide nanoworm-based magnetic resonance imaging probes and dendrimers to the atherosclerotic plaque[23,62]. For instance, (co) Polymeric systems such as poly(lactic-co-glycolic acid) (PLGA) may also be investigated as they are easy to functionalize and PLGA particles have already been successfully employed to deliver GW3965 to atherosclerotic plaques[97]. In summary, we showed that Lyp-1 is a valuable tool to target atherosclerotic lesions and it enhanced the therapeutic window of a moderate dose of LXR agonist. Future studies could explore functionalization of Lyp-1 (1) on other types of delivery systems, (2) in combination with different surface stealth materials, and (3) even evaluate the impact of targeted delivery of other drugs (*e.g.* anti-inflammatory drugs).

In view of the potential value of LXR agonists in atherosclerosis, it is important to note that LXR is also expressed in the skin of both humans (LXRα and LXRβ) and mice (LXRβ). In human skin, LXRα is the predominant isoform and its expression can be impacted in diseased skin as previously reported for psoriasis[98]. Lesional psoriatic skin shows reduced expression of LXRα compared to non-lesional and control skin[98]. In contrast, in murine skin LXRβ is the predominant isoform and the deletion of LXRα in normolipidemic mice did not affect keratinocyte differentiation or skin morphology[99]. The deletion of LXRβ in normolipidemic mice resulted in thinner epidermis and mild reduction in the expression of differentiation markers[99]. In the skin, naturally occurring LXR agonists (*e.g.* 22(R)-hydroxy-cholesterol) drive keratinocyte differentiation and improve barrier function[99–101]. Synthetic LXR agonists (*e.g.* T0901317 and GW3965) have been shown to reduce skin inflammation, inhibit fibrosis, prevent photo- and chronological aging, and induce cholesterol efflux in both human and murine skin by upregulation of ABCA1 in normolipidemic backgrounds[102–104]. However, to date the effect of LXR activation on the skin in hypercholesterolemic backgrounds remains to be investigated. One of the main downstream targets of LXR is stearoyl-CoA desaturase-1, a desaturase with crucial role in the formation of unsaturated fatty acids[105]. As previously mentioned, rises in the fraction of unsaturated lipids have a negative impact in the barrier organization and function. Human skin equivalents are *in vitro* 3D models of skin cultured with

human skin cells (keratinocytes and fibroblasts)[106]. The SC lipid composition of these models is enriched with short and unsaturated CERs and FFAs species, sharing to some extend similarities with the *APOE*$^{-/-}$ epidermal lipid profile[106]. In these *in vitro* models, exposure to T0901317 was further detrimental to the SC lipid composition with further increase in the pool of unsaturated and short FFAs and CERs[106]. Another key point to be considered is that LXR agonists can also induce the expression of other members of the nuclear receptor family. LXR agonist T0901317 also induces the expression of farsenoid-X-receptor (FXR) and peroxisome-X-receptor (PXR), both expressed in the skin[107–109] . Activation of FXR (EC_{50}=5 μM) by small heterodimer partner (SHP) downregulates LXR and its target genes (*e.g. SREBP-1C* and *FAS*)[109]. In particular, activation of PXR has been associated with hepatic steatosis and impaired skin barrier function[110]. In contrast, the LXR agonist GW3965 is a strong activator of LXR but without inducing FXR or PXR expression to the same extent as described for T0901317[110]. This more selective profile of GW3965 favors this compound as a frontrunner in future investigations when aiming for concomitant tackling of dermatological and atherogenic disturbances. In addition to its effects in modulating cholesterol metabolism, LXR also plays a role in regulation of inflammation[111,112]. The skin of young adult *APOE*$^{-/-}$ mice does not present signs of inflammation but with aging accumulation of lipid-laden macrophages (foam cells) in the skin of these mice has been reported[113]. Activation of LXR leads to downregulation of diverse inflammatory mediators such as interleukin-1 and tumor necrosis factor-alpha in keratinocytes[111]. These anti-inflammatory properties derived from LXR activation have been reported to ameliorate irritant and allergic contact dermatitis and to reduce aging related photo damage in murine models. In short, it remains unclear whether LXR activation in the skin would be beneficial for the barrier lipid profile in hypercholesterolemia. Nonetheless, there is evidence on both sides that shows that it may be worthy to investigate it.

The findings described in this thesis focused on dyslipidemic mouse models and should not be directly applied to the human situation without further considerations and studies. Mice and humans will present similarities and differences in all research topics explored in this thesis (skin, lipid metabolism, atherosclerosis development)[114–117]. For instance, both plasma and skin lipidomics have identified differential lipid fingerprints between the two species but also strong similarities, in particular when compared to other animal research models (*e.g.*; non-human primates and rats)[114]. Additionally, it is important to mention that, although not explored in depth in this thesis, the skin is an active immunological site[118]. Also here, the two species present convergent and divergent immunologic profiles that should be carefully considered when translating findings in mouse models to the human situation[118]. Importantly, these differences should not discourage the use of mouse models as they have greatly contributed to

landmark discoveries in the research fields explored in this thesis. Instead, awareness of these differences is important to help scientists better design their experiments and later appropriately translate their findings to the human situation. Lastly, the next step in this research should certainly focus on the evaluation of the skin lipid composition and barrier functionality in individuals suffering from FH. The data generated from such study would be the first step to understand the value of using dyslipidemic murine models to comprehend the crossroads between the skin, the plasma and dyslipidemic related pathologies for the human situation.

In summary, the research described in this thesis shows that hypercholesterolemia, a well-established risk factor for atherosclerosis, can impact skin lipid pool and barrier function already at young age. Elucidation of the mechanisms underlying this intercommunication between plasma and skin may also bring valuable opportunities to prevent and treat dermatological pathologies in dyslipidemic patients; perhaps in combination with anti-atherogenic therapies. Thus, by deepening our knowledge we may improve our advice to the patients and ultimately improve their quality of life.

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