Although statin therapy successfully reduces the risk of occlusive vascular events in individuals at high risk of cardiovascular disease, atherosclerotic cardiovascular disease is still the major cause of morbidity and mortality worldwide [1]. One of the earliest events in atherosclerosis is the adherence of monocytes to the endothelium and subsequent transmigration into the arterial intima where they differentiate into macrophages. Upon differentiation, macrophages begin to accumulate large amounts of lipids by the uptake of (modified and/or aggregated) lipoproteins, including oxidized LDLs (ox-LDLs), leading to the formation of foam cells [2–4]. Of particular importance in the initiation of atherosclerosis is the balance between cholesterol influx and efflux in macrophages. This balance is maintained by scavenger receptors and ATP-binding cassette (ABC) transporters, which are key mediators for macrophage cholesterol homeostasis as they facilitate the influx and efflux of lipids [5]. Macrophages are incapable of limiting the uptake of cholesterol by scavenger receptors, including scavenger receptor class A (SR-A) and CD36 [5,6]. Therefore, prevention of lipid accumulation in macrophages largely depends on cholesterol efflux pathways, which are mainly mediated by ABC transporters. Since excessive accumulation of cholesterol by macrophage-derived foam cells is one of the characteristic features of atherosclerotic lesion development, gaining more knowledge on the different efflux pathways is of prime importance for the development of new therapeutic strategies. This review highlights important aspects of macrophage cholesterol homeostasis mediated by the ABC transporters ABCA1 and ABCG1.

ABC transporters: drug efflux pumps & lipid transporters

The ABC transporter genes represent one of the largest family of transmembrane proteins. ABC transporters utilize the energy of ATP hydrolysis to pump a wide variety of substrates, including sugars, amino acids, metal ions, peptides, proteins and a large number of hydrophobic compounds and metabolites, across extra- and intra-cellular membranes [7]. To date, 52 members of the ABC transporter family have been identified in humans, which are divided into seven distinct subfamilies (ABCA–G) based on organization of domains and amino acid homology [8]. ABC transporters are organized as either full transporters or as half transporters. Prototype full transporters contain two identical transmembrane (TM) domains and two nucleotide binding folds (NBFs), which are key mediators for macrophage cholesterol homeostasis as they facilitate the influx and efflux of lipids [5]. Macrophages are incapable of limiting the uptake of cholesterol by scavenger receptors, including scavenger receptor class A (SR-A) and CD36 [5,6]. Therefore, prevention of lipid accumulation in macrophages largely depends on cholesterol efflux pathways, which are mainly mediated by ABC transporters. Since excessive accumulation of cholesterol by macrophage-derived foam cells is one of the characteristic features of atherosclerotic lesion development, gaining more knowledge on the different efflux pathways is of prime importance for the development of new therapeutic strategies. This review highlights important aspects of macrophage cholesterol homeostasis mediated by the ABC transporters ABCA1 and ABCG1.
Figure 1. A full ATP-binding cassette transporter. Full ATP-binding cassette transporters consist of two integral transmembrane domains, each consisting of six hydrophobic membrane-spanning α-helices, followed by the ATP-binding cassettes with characteristic Walker motifs A and B.

Amino-terminal end, which results in a total of 17 membrane-spanning α-helices [9]. The NBFs, also known as ATP-binding domains, bind ATP and act as an energy source, since the transport of substrates by ABC transporters is based on active transport. All ABC proteins contain three highly conserved domains within each NBF: Walker A and B domains, found in all ATP-binding proteins, and a signature (C) motif located just upstream of the Walker B site [10].

The first ABC protein identified in eukaryotes was class I P-glycoprotein, also known as multidrug transporter (MDR1) and ABCB1 [11,12]. Class I P-glycoproteins are integral membrane proteins that are abundant in multidrug-resistant tumor cells and function to reduce the intracellular concentrations of structurally diverse chemotherapeutic drugs [13]. Therefore, ABC transporters were originally identified as drug-efflux pumps involved in the multidrug resistance of cancer cells. Recent findings, however, have demonstrated the physiological importance of ABC transporters in cellular lipid transport and homeostasis [14].

The first ABC protein suggested to have a role in lipid homeostasis was the ABC transporter ABCB4 (MDR2), a second type of P-glycoprotein with structural similarity to the drug-transporting P-glycoprotein ABCB1 (MDR1) [15]. ABCB4 is a lipid flippase that has an essential role in the transport of phosphatidylethanolamine across the canalicular membrane during bile formation by the liver [16]. Recently, we also identified an essential role for ABCB4 in macrophage cholesterol homeostasis and prevention of atherosclerotic lesion development [17]. The importance of ABC proteins in lipid homeostasis was further confirmed by the identification of the role of ABCA4 (ABCR) in the transport of retinaldehyde and phosphatidylethanolamine in the retina of the eye [18]. Other discoveries indicating the involvement of ABC transporters in lipid transport were the findings that the half-size peroxisomal ABC proteins (ABCD1, ABCD2, ABCD3 and ABCD4) mediate the transport of long-chain and very long-chain fatty acids into peroxisomes [19,20] and that ABCB11 (BSEP) mediates the efflux of bile salt into the bile [21]. A major breakthrough, however, was the revolutionary discovery in 1999 that Tangier disease (TD), a rare inherited disorder, is caused by mutations in the gene of the ABC transporter ABCA1 [22–25]. Dysfunctional ABCA1 in TD individuals leads to the absence of HDL in the circulation and deposition of cholesterol esters in various tissues, including tonsils and lymph nodes [26]. In addition, Tangier cells were almost completely defective in both cholesterol and phospholipid efflux to ApoA-I and HDL [27,28], thereby explaining the massive lipid accumulation in the cells of these subjects.

Since ABC transporters play a role in lipid transport and a vast majority are highly expressed in macrophages [29,30], it was evident that ABC transporters are involved in macrophage lipid homeostasis and play critical roles in foam cell formation and atherogenesis.

ABC transporters: prevention of foam cell formation

A pathological hallmark of atherosclerosis is the accumulation of excess cholesteryl ester by macrophages in the arterial intima [2]. Cholesterol is taken up by macrophages via several different receptors, including the scavenger receptors CD36, SR-A and lectin-like ox-LDL receptor-1 (LOX-1), and to a lesser extent the LDL receptor (LDLr), LDLr-related protein, very-LDLr (VLDLr) and SR-BI [6]. Macrophages are incapable of limiting the uptake of lipids by scavenger receptors and, therefore, cellular lipid homeostasis depends largely on efflux mechanisms.

ABCA1, a 2261 amino acid, 240-kDa protein, full transporter, is a key transporter involved in the efflux of cholesterol and phospholipids from macrophages [7,34]. ABCA1 mRNA is widely distributed among multiple tissues, including placenta, liver, brain, lung, kidney and intestine [32]. Although ABCA1 is expressed in different cell types of many tissues, ABCA1 is highly expressed
in resident macrophages, where it plays a critical role in cholesterol transport [32]. The mechanism by which ABCA1 promotes lipid efflux to ApoA-I is not well established, despite extensive studies. Different mechanisms, however, have been proposed: lipid-poor ApoA-I interacts directly with ABCA1 or indirectly with lipid raft membrane domains created by ABCA1 activity [33–35], subsequently, cellular phospholipid and cholesterol are transported simultaneously or phospholipid efflux precedes cholesterol efflux [36,37]. Lipidation of ApoA-I results in a reduction in its affinity for ABCA1, leading to the release of the newly formed nascent HDL particle [35–37]. HDL particles subsequently transport the excess cholesterol from macrophages in the periphery back to the liver for catabolism and excretion in bile and feces, a process termed reverse cholesterol transport (RCT) [38,39]. The selective uptake of HDL-C by the liver is mediated by SR-BI, which has a high affinity for lipid-rich HDLs but low affinity for lipid-poor apolipoproteins [40].

The finding that mutations in ABCA1 cause TD has stimulated studies of ABCA1 function in vivo and in vitro. Abca1-deficient (Abca1-/-) mice exhibited low plasma levels of HDL and accumulation of sterols in tissues, similar to human TD [41]. Both studies of TD patients and animal models demonstrated that a severely impaired ability to lipidate apolipoproteins via the ABCA1 pathway leads to a rapid catabolism of lipid-poor ApoA-I and accumulation of sterols in tissue macrophages, intestinal cells and hepatocytes [41,42]. Several studies have reported that plasma HDL levels are dependent upon expression of Abca1 in the liver and intestine, while macrophage Abca1 has only a minor effect [43–45]. As ABCA1 is a key modulator of serum HDL levels, it was suggested to play a major role in the prevention of atherosclerosis.

Atherosclerotic determinations in TD patients are rare owing to the small number of TD patients. Studies using a large cohort of individuals with one functional ABCA1 gene, however, showed an enhanced risk for developing atherosclerosis [46]. Lower HDL-C concentrations in these ABCA1 heterozygotes were associated with an increased mean intima–media complex thickness, a surrogate marker for atherosclerosis [46]. In addition, Frickke-Schmidt et al. demonstrated that heterozygotes for four ABCA1 mutations (P106S, G121V, N1800H and R2144X) displayed a significant reduction in HDL-C levels versus noncarriers (41 vs 58 mg/dl, respectively) [47]. This reduction in HDL-C was associated with an increased hazard ratio of 1.7 for ischemic heart disease. However, the hazard ratio for ischemic heart disease in heterozygotes versus noncarriers was 0.67–0.93. These findings indicate that lower plasma levels of HDL-C due to heterozygosity for ABCA1 mutations are not persistently associated with an increased risk for ischemic heart disease.

Studies in different mouse models have led to conflicting results regarding the antiatherosclerotic role of Abca1 expression. Total body Abca1 deficiency led to reduced plasma cholesterol levels, mainly caused by a reduction in VLDL-C, but did not affect atherogenesis in wild-type apoE-/- and LDLr-/- mice fed a chow or atherogenic diet [41,44]. Interestingly, Singaraja et al. reported that overexpression of human ABCA1 in apoE-/- mice did result in dramatically smaller, less complex atherosclerotic lesions as compared with controls, which was associated with a small increase in HDL-C levels [48]. These findings suggest that raising ABCA1 activity in vivo results in significant protection against atherosclerosis. By contrast, Joyce et al. demonstrated that overexpression of ABCA1 in apoE-/- mice led to increased atherosclerotic lesion development [49]. The major difference between the studies, however, is that Joyce et al. overexpressed ABCA1 cDNA using the ApoE promoter, which may have resulted in nonphysiological cellular and subcellular expression of ABCA1, while Singaraja et al. overexpressed ABCA1 using a construct containing endogenous regulatory elements, which led to ABCA1 expression in a physiological manner [48,49].

Furthermore, marked expression of ABCA1 was found in atherosclerotic lesions, where it colocalizes with lipid-laden macrophages [50], indicating that ABCA1 can also locally affect atherosclerotic lesion development. Macrophage ABCA1 is highly upregulated upon lipid loading as a result of activation of the nuclear receptors liver X receptor (LXRα and/or β) and retinoid X receptor (RXR) by endogenous oxysterols accumulating within the cells [51,52]. To assess the critical role of macrophage Abca1 in lesion formation and progression, bone marrow transplantation studies were used to selectively disrupt Abca1 in hematopoietic cells. Reconstitution of LDLr-/- and apoE-/- mice with Abca1-/- bone marrow cells resulted in a marked increase in atherosclerotic lesion formation [53,54]. Conversely, bone marrow transplantation of LDLr-/- mice with Abca1-overexpressing bone marrow cells resulted in inhibition of atherosclerotic lesion progression [55]. Macrophage ABCA1 is, thus, a key mediator in the prevention of excessive cholesterol accumulation in macrophages of the arterial wall and, thereby, protects against atherogenesis. Strikingly, hepatic
overexpression of ABCA1 leads to accumulation of proatherogenic lipoproteins and enhanced atherosclerosis [54]. These findings indicate that ABCA1 overexpression in macrophages has an atheroprotective effect, while overexpression of ABCA1 in liver modulates plasma cholesterol levels of both HDL as well as non-HDL, which accounts for a proatherogenic effect.

In addition to ABCA1, ABCG1 has also been implicated in cholesterol efflux from macrophages [55]. ABCG1, also known as ABC8 or human white gene, is a half transporter with a single, six-helix TM domain and a single nucleotide-binding fold that needs to form a dimer with another ABC transporter to be functional [14,56]. Studies have shown that overexpression of ABCG1 alone induces transport activity, which suggests that ABCG1 can function as a homodimer. These findings, however, do not exclude the possibility that ABCG1 may dimerize with other ABC half transporters [57,58]. Expression analysis in a variety of human tissues demonstrated expression of ABCG1 in numerous tissues [59]. Unlike ABCA1 [32], however, ABCG1 showed the highest expression in adrenal glands, lung, heart and spleen, and appeared to be less abundantly expressed in fetal tissues. Similar to ABCA1, ABCG1 transcription is highly induced upon cholesterol loading and activation by the nuclear receptors LXR and RXR [51,52,59–61].

Interestingly, ABCG1 plays a critical role in lipid homeostasis by actively effluxing cellular cholesterol to mature HDL and other extracellular phospholipid-containing acceptors. By contrast, ABCA1 facilitates cholesterol and phospholipid efflux to lipid-poor apolipoproteins, including ApoA-I, ApoC-II or ApoE [36,62]. Recently, it has been suggested that ABCA1 and ABCG1 act in concert to mediate cellular phospholipid and cholesterol efflux from macrophages [63,64]. ABCA1 lipophilates lipid-poor/free ApoA-I, generating nascent or discoidal HDL. This newly formed nascent or discoidal HDL particle can subsequently function as an acceptor for ABCG1-mediated cholesterol efflux. Thus, efflux to ApoA-I via ABCG1 requires prior conversion of ApoA-I into a phospholipid-containing acceptor. These findings indicate a synergistic relationship between ABCA1 and ABCG1 in peripheral tissues [63,64].

Studies using genetically engineered mice have established the physiological importance of ABCG1. Targeted disruption of Abcg1 in mice resulted in age-related progressive pulmonary lipidosis when fed a regular chow diet containing no added cholesterol [65–67]. At 3 weeks of age, Abcg1−/− mice accumulated lipid at the edges of the lung, whereas the lungs of Abcg1−/− mice of 8 months of age exhibited an increase in lipids in macrophages and type 2 pneumocytes, and accumulation of excessive levels of surfactant phospholipid in the bronchoalveolar space [66,67]. Before onset of pulmonary lipidosis, macrophages of Abcg1−/− mice of 7 weeks of age showed increased inflammatory activity, resulting in increased numbers of inflammatory cytokines (IL-1α, -6, -12 and the keratinocyte chemoattractant [KC]) in the circulation and infiltration of neutrophils, eosinophils, dendritic cells, T- and B cells into the lungs [67]. Furthermore, freshly isolated alveolar macrophages from 6 month old Abcg1−/− mice showed decreased expression of genes involved in lipid synthesis (FAS, GPAT, HMGCR and SREBP-2), whereas LXR target genes (LXRA, ABCA1 and SREBP-1c) were induced, indicating disturbed lipid homeostasis in alveolar macrophages [66]. Bone marrow transplantation studies indicated that bone marrow-derived cells are responsible for the onset of lipidosis [67]. Since ABCG1 is highly expressed in macrophages, it was macrophages and not pneumocyte type 2 or other nonhematopoietic cells in the lung that appeared to be the primary cell type involved in the onset of inflammation and subsequent pulmonary lipidosis [67]. These findings implicate a critical role for ABCG1 in maintaining normal lipid metabolism in the lung, thereby preventing inflammatory responses triggered by massive cholesterol and/or cholesterol metabolite accumulation [65–67]. Challenging Abcg1−/− mice with a high-fat, high-cholesterol (HF/HC) diet led not only to massive accumulation of neutral lipids in pulmonary macrophages, but also to an increase in lipids in hepatocytes and Kupffer cells [55]. Lungs of Abcg1−/− mice on a HF/HC diet were white in appearance as a result of extreme neutral lipid accumulation in the subpleural regions of the lung. This severe phenotype of the lung was characterized by massive deposition of cholesterol esters and cholesterol crystals within alveolar macrophages, correlating with the suggested impaired cholesterol efflux in the absence of ABCG1 [55]. In addition, overexpression of ABCG1 protected against diet-induced lipid deposition within multiple tissues [55].

Although the critical role of ABCG1 in lung lipid homeostasis is clearly established, the role of macrophage ABCG1 in the development of atherosclerosis remains uncertain. Independent studies have demonstrated that macrophage ABCG1 might be proatherogenic as well as...
antiatherogenic. Out et al. observed a moderately significant increase in atherosclerotic lesion development in both total body and macrophage Abcg1−/− mice, likely as a direct result of impaired cholesterol efflux from lipid-laden macrophages in the atherosclerotic lesion (Figure 2A) [68,69]. By contrast, two other independent studies by Baldán et al. and Ranalletta et al. reported decreased atherosclerosis in LDLr−/− mice transplanted with Abcg1−/− bone marrow cells, which was explained by accelerated apoptosis of Abcg1−/− macrophages or compensatory upregulation of Abca1 expression and ApoE secretion in Abcg1−/− macrophages (Figure 2B & 2C) [70,71]. Abcg1−/− macrophages are indeed more susceptible to oxLDL-induced apoptosis as compared with Abcg1-expressing cells [72]. Efflux of 7-ketocholesterol, the main oxysterol present in ox-LDL, was completely dependent on expression of ABCG1 and not on the expression of ABCA1 [72]. Therefore, ABCG1-deficient macrophages showed increased accumulation of 7-ketocholesterol upon ox-LDL loading, which is cytotoxic to the cell and induces accelerated apoptosis. Furthermore, ABCG1-deficient macrophages showed disturbed cellular cholesterol efflux of ApoA-I and HDL and increased proinflammatory status (Figure 2D).
homeostasis, such as oxysterol accumulation, which can lead to activation of the LXRs [60,61]. Subsequent induction of the LXR target genes ABCA1 and apoE enhances cholesterol efflux from macrophages [73]. These compensatory mechanisms, masking the primary protective function of ABCG1 in cholesterol efflux, might explain the observed decrease in atherosclerotic lesion development in LDLr−/− mice lacking Abcg1.

Strikingly, Basso et al. reported that overexpression of ABCG1 also led to increased atherosclerotic lesion development in LDLr−/− mice, despite its role in mediating cholesterol efflux from cells [74]. ABCG1 transgenic/LDLr−/− mice exhibited increased concentrations of non-HDL proatherogenic lipoproteins [74]. Furthermore, the levels of several proinflammatory cytokines, namely MCP-1 and TNF-α, were increased in the circulation [74]. Thus, ABCG1 in some way appears to act as a proinflammatory and proatherogenic factor. By contrast, Burgess et al. reported that overexpression of ABCG1 did not affect atherogenesis in fat-fed ApoE-deficient mice, although subtle changes in sterol biosynthetic intermediate levels did occur [75]. The effects of ABCG1 on atherosclerotic lesion development might therefore be dependent on the presence of functional ApoE. In addition to ApoA-I, ABCA1 also facilitates cholesterol and phospholipid efflux to lipid-poor ApoE. Lipidated ApoE can subsequently function as an acceptor for ABCG1-mediated cholesterol efflux [76,77]. Therefore, overexpression of ABCG1 might require increased levels of ApoE-phospholipid acceptor particles to affect cholesterol efflux, a process that is inhibited in mice lacking ApoE. The differences in the effect of whole body overexpression of human ABCG1 on atherosclerosis in mice between Basso et al. and Burgess et al. might be explained by differences in bacterial artificial chromosome copy numbers (1.2-fold increase vs sevenfold increase in ABCG1 protein in macrophages, respectively) and differences in atherosclerotic mouse model used (apoE−/− mice vs LDLr−/− mice, respectively) [74,75]. Human data on the effect of these mutations on defects in sterol transport are essential to evaluate the contribution of ABCG1 to atherogenesis in humans. Studies reporting human genetic data of ABCG1 defects, however, are currently not available, but most likely to be expected in the near future.

ABCA1 and ABCG1 have been reported to act synergistically to remove cellular cholesterol from macrophages [63]. In addition, ABCA1 and ABCG1 are both targets of LXR activation [51,52,59–61]. When one transporter is dysfunctional, the other is upregulated as a result of oxysterol accumulation and subsequent LXR activation, masking potential effects of the specific transporter on atherogenesis [70,71]. This compensatory response was also observed in macrophages from patients with TD, which showed an upregulation of ABCG1 expression in response to the impaired ABCA1-mediated efflux [78]. Therefore, the effect of ABCA1 and ABCG1 deficiency on atherogenesis may be best determined in the absence of both ABCG1 and ABCA1. Abca1/Abcg1 double-knockout (Abca1−/−/Abcg1−/−) mice were recently generated by two independent groups to analyze the potential synergistic relationship between ABCA1 and ABCG1 in mediating cellular cholesterol homeostasis. On a regular chow diet, combined deficiency of Abca1 and Abcg1 already led to extreme lipid accumulation and foam cell formation in tissue macrophages of the lung, liver, spleen, Peyer’s patches and lymph nodes, despite severe plasma hypocholesterolemia [79]. Furthermore, Abca1−/−/Abcg1−/− mice exhibited severe hepatosplenomegaly and enlargement of lymph nodes and Peyer’s patches, whereas no such phenotype was evident in the Abca1 and Abcg1 single-knockout or wild-type animals. The extreme phenotype in Abca1−/−/Abcg1−/− mice indicates the importance of both ABCA1 and ABCG1 in promoting lipid efflux from macrophages and their effect on inhibiting foam cell formation.

In addition to active transport of cholesterol mediated by ABCA1 and ABCG1, other potential efflux pathways from macrophages to HDL have been described, including passive or diffusional efflux and SR-BI-mediated efflux. Recent studies indicated that macrophages lacking Abca1 and Abcg1 have highly reduced cholesterol efflux to ApoA-I, HDL and serum [79–83]. Out et al. reported that combined Abca1 and Abcg1 deficiency resulted in a completely abolished cholesterol mass efflux to HDL [79], while [3H]-cholesterol label efflux was reduced by only 35% [80]. Similar studies by Yean-Charvet et al. observed a 30% reduction in cholesterol mass efflux from Abca1−/−/ Abcg1−/− macrophages to HDL [81]. Adorni et al. demonstrated that in unloaded macrophages, aqueous diffusion is the primary mechanism for cholesterol efflux, with a small contribution from ABCA1 [82]. Under cholesterol-loading conditions, however, ABCA1 and ABCG1 contribute approximately 80 and 20% to the total cholesterol efflux capacity of macrophages to human serum, respectively. In vivo lipid efflux studies using a macrophage-specific RCT assay demonstrated that cholesterol efflux from Abca1−/−/ Abcg1−/− macrophages to plasma, liver and feces was
highly impaired as compared with animals that received macrophages from wild-type, Abca1−/− or Abcg1−/− mice [79,83]. Together, these studies provide clear evidence of the major and additive function of macrophage ABCA1 and ABCG1 in the prevention of macrophage foam cell formation. The observed massive accumulation of lipid in tissue macrophages of Abca1−/−Abcg1−/− mice is, thus, most likely a direct result of impaired efflux capacity of macrophages lacking both ABCA1 and ABCG1.

The effect of combined deletion of ABCA1 and ABCG1 in macrophages on the susceptibility to atherosclerotic lesion development was subsequently studied by transplantation of bone marrow cells from Abca1−/−Abcg1−/− mice to LDLr−/− animals. Interestingly, LDLr−/− mice transplanted with Abca1−/−Abcg1−/− bone marrow showed increased secretion of inflammatory cytokines and chemokines and profoundly increased apoptotic responses, suggesting an increased inflammatory status [81]. Single Abcg1−/− transplanted mice showed a similar, although less severe, increase in the secretion of inflammatory cytokines and chemokines, while Abca1 deficiency had no effect, suggesting that ABCG1 has a specific role in decreasing inflammatory and chemokine response [81]. Together, these recent studies demonstrating highly impaired cholesterol efflux and a more proinflammatory condition in Abat−/−Abcg1−/− mice would suggest a dramatic enhancement of the atherosclerosis susceptibility of these animals.

Interestingly, total body Abca1−/−Abcg1−/− mice showed no lipid accumulation in the arterial wall, despite extreme foam cell formation in tissue macrophages [79]. The severe hypcholesterolemic conditions observed in these total body Abat−/−Abcg1−/− mice might be unable to provide the stimulus to attract macrophages to the arterial wall, thereby preventing atherosclerotic lesion development. LDLr−/− mice transplanted with Abat−/−Abcg1−/− bone marrow cells fed a Western-type diet (0.15% cholesterol and 15% fat) exhibited a dramatic reduction in VLDL-C and LDL-C levels compared with wild-type, Abat−/− and Abcg1−/− mice [80]. Nevertheless, atherosclerotic lesion development was significantly increased in Abat−/−Abcg1−/− mice relative to the level of plasma cholesterol (Figure 2D) [80]. In another study, Yvan-Charvet et al. reported that on a HF/HC bile salt diet (1.25% cholesterol, 7.5% cocoa butter and 0.5% cholic acid), reconstitution of LDLr−/− mice with Abat−/−Abcg1−/− bone marrow cells led to a marked increase in atherosclerosis, while macrophage Abat−/−Abcg1−/− deficiency did not affect VLDL and LDL-C levels [81]. Differences in the levels of ApoB-containing lipoproteins between the studies of Out et al. and Yvan-Charvet et al. might be explained by differences in genetic background of mice (LDLr−/− vs LDLr−/−, respectively) and differences in diet (0.15% cholesterol and 15% fat vs 1.25% cholesterol, 7.5% cocoa butter and 0.5% cholic acid, respectively) used in these studies [80,81]. Interestingly, similar to total body combined Abca1 and Abcg1 deficiency, both studies reported that combined deletion of ABCA1 and ABCG1 specifically in macrophages resulted in prominent accumulation of foam cells in various organs, including heart, spleen, intestine, lung, liver and Peyer’s patches [80,81]. Together, these studies indicate a pivotal and additive effect of ABCA1 and ABCG1 on macrophage lipid homeostasis and an important role in the prevention of atherosclerotic lesion development. Therefore, new therapeutic strategies aimed at increasing cholesterol efflux by enhancing macrophage ABCA1 and ABCG1 expression are likely to be beneficial for the treatment of atherosclerosis.

**ABC transporters & their regulation**

Liver X receptors, divided into the subtypes LXRα and LXRβ, are nuclear receptors that regulate the metabolism of several important lipids, including cholesterol and bile acids [84,85]. Following the discovery that ABCA1 and ABCG1 are sterol-sensitive genes [32,59], ABCA1 and ABCG1 have been identified as LXR α and LXRβ target genes [51,52,59–61]. LXR requires heterodimerization with the RXR to be functional [86]. This complex drives LXR-dependent transactivation of multiple genes involved in sterol transport (ABCA1, ABCG1, ABCG5 and ABCG8), cholesterol efflux and HDL metabolism (ABCA1, ABCG1, apoE, CETP and PLTP), and sterol catabolism (CYP7A1) [87,88]. A critical role for the LXRs in cholesterol homeostasis was established by combined deletion of both LXRα and LXRβ in mice, which induced significant foam cell accumulation in lung, spleen and the arterial wall [89]. The oxysterols 24,25-epoxycholesterol (24,25-EC) and 22(R)-hydroxycholesterol (22-HC) are the most potent physiological activators of LXR [90]. The ability of oxysterols to induce LXR target genes involved in cholesterol efflux is expected to have a beneficial effect on the prevention of macrophage foam cell formation. Recent studies indeed reported that 22-HC induced cholesterol efflux from both unloaded and acetylated (ac)LDL-loaded macrophages by induction of ABCA1 and ABCG1 expression [91]. 24,25-EC, however, induced cholesterol
efflux only in unloaded macrophages and not in loaded macrophages. In fact, increased levels of 24,25-EC correlated with a decrease in cholesterol efflux to ApoA-I and HDL in acLDL-loaded macrophages [91]. Interestingly, although 24,25-EC treatment in acLDL-loaded macrophages led to a marked induction of ABCA1 and ABCG1 expression, it was not associated with enhanced ABCA1- and ABCG1-mediated cholesterol efflux [91,92]. Apparently, 24,25-EC promotes the accumulation of esterified cholesterol by inhibition of cholesteryl ester hydrolysis, resulting in decreased availability of free cholesterol. As free cholesterol acts as the substrate for ABCA1-mediated efflux to ApoA-I and ABCG1-mediated efflux to HDL, decreased availability of free cholesterol results in an overall decrease in cholesterol efflux to ApoA-I and HDL. Thus, despite inducing a marked increase in ABCA1 and ABCG1 expression, 24,25-EC impaired cholesteryl ester hydrolysis in lipid droplets of macrophage foam cells, resulting in reduced efflux to ApoA-I and HDL and enhanced cholesteryl ester accumulation in macrophages. 7-ketocholesterol has also been suggested to impair cholesterol efflux to ApoA-I from macrophage foam cells [63,93]. Gaus et al. demonstrated that 7-ketocholesterol selectively depletes plasma membrane lipid raft cholesterol and, subsequently, blocks the binding of ApoA-I to these domains, thereby inhibiting cholesterol efflux [93]. These findings indicate that, in addition to 24,25-EC, more oxysterols might inhibit RCT. Therefore, potential LXR agonists for therapeutic LXR activation have to be selected with extreme caution.

Another interesting LXR signaling pathway has recently been discovered in T cells [94]. Expression of ABCA1 and ABCG1 was rapidly downregulated upon T-cell activation and proliferation [94]. During T-cell activation, the intracellular availability of oxysterols that can activate the LXR pathway was significantly reduced owing to induction of the oxysterol-metabolizing enzyme SULT2B1 [94]. ABCA1 and ABCG1 expression in T cells might therefore be regulated by modulators of oxysterol levels.

ATP-binding cassette transporter protein expression levels are determined by the balance between synthesis as well as degradation. Fatty acids (FAs), such as arachidonic and linoleic acid, can regulate ABCA1 expression and function by enhancing protein degradation and serine phosphorylation [95,96]. Recently, Nagelin et al. reported that eicosanoids, synthesized by 12/15-lipoxygenase (12/15-LO) from FAs, induce ABCG1 serine phosphorylation in macrophages, leading to increased degradation and reduced expression of ABCG1 [97]. Furthermore, it has been previously shown that unsaturated FA downregulates ABCA1 mRNA and protein [98]. Unsaturated FAs do not suppress ABCA1 via transcriptional regulation of LXRα, but inhibit the activation of LXRβ through competition with LXR ligands for the ligand-binding domain of LXR [98]. Recently, Uehara et al. demonstrated that ABCG1 expression, similar to ABCA1, is suppressed by unsaturated FA [99]. Unsaturated FA decreases ABCG1 mRNA and protein expression, as well as the activity of the ABCG1 promoter. Downregulation of ABCA1 and ABCG1 by unsaturated FA indicates that elevated levels of unsaturated FA inhibit both ApoA-I and HDL-induced cholesterol efflux from macrophages. This impaired cholesterol efflux may contribute to low HDL-C and increased cardiovascular risk observed in patients with diabetic mellitus [99]. These findings indicate that FAs and/or FA metabolites can act as regulators of ABCA1 and ABCG1 degradation as well as transcription.

Type 2 diabetes mellitus is associated with enhanced foam cell formation and an increased risk for atherosclerosis [100,101]. Interestingly, the 12/15-LO pathway is upregulated by glucose and its activity is highly increased in patients with diabetes, suggesting that 12/15-LO has a critical role in diabetes-associated cardiovascular diseases [102]. Peritoneal macrophages from mice with diabetes showed an increase in the expression of CD36 and decreased expression of ABCG1, resulting in a twofold increase in esterified cholesterol within the cell. Glucose-induced downregulation of ABCG1 was confirmed by culturing C57BL/6 macrophages in the presence of elevated glucose levels [103]. Furthermore, macrophages from patients with diabetes showed a 30% reduction in cholesterol efflux to HDL [104]. The reduced efflux coincided with decreased expression of ABCG1 in macrophages of diabetic patients, whereas ABCA1 expression in macrophages was similar in both control subjects and patients with diabetes. The reduction in ABCG1 expression and consequently cellular efflux was associated with increased intracellular cholesterol accumulation. Glucose-induced downregulation of macrophage ABCG1 expression might thus contribute to the observed enhanced foam cell formation in diabetic patients. Interestingly, upregulation of LXR was able to rescue the dramatic foam cell formation in macrophages of diabetes patients, most likely as a result of induction of the LXR target gene apoE [104]. Zhou et al. recently reported that the reduced expression of ABCG1
in monocytes from Type 2 diabetic patients is partly related to the increased exposure to serum advanced glycation end products (AGEs) [105]. Previous studies indeed demonstrated that AGEs or their precursors can downregulate ABCG1 and ABCA1 expression [106,107]. Although, ABCG1 expression was reduced in macrophages of patients with Type 2 diabetes mellitus, ABCA1 expression was not affected. The effect of AGEs on ABCG1 expression is mediated by the receptor for AGE (RAGE) through a LXR-independent pathway. However, the exact mechanism remains unknown [107]. These findings suggest that glucose affects ABCG1 expression both via the enhanced 12/15-LO/FA pathway and/or via enhanced AGE/RAGE pathway. In addition to the ability of glucose to reduce cholesterol-eflux pathways, it has been suggested to induce lipid-uptake pathways by increasing the expression levels of SR-A, LOX-1 and CD36 [108–111]. As decreased ABCG1 expression in Type 2 diabetics is most likely related to decreased cholesterol efflux and increased cholesterol ester accumulation, strategies to induce ABCG1 expression might have therapeutic potential in the prevention of atherosclerosis in patients with Type 2 diabetes mellitus.

**Future perspective**

Currently, the treatment of atherosclerosis is mainly based on reducing serum LDL levels by the use of statins, inhibitors of de novo cholesterol synthesis. Transgenic overexpression of ApoA-I in mice and infusion of ApoA-I/phospholipid complexes in humans cause reduced progression, or even regression, of atherosclerotic lesions [112]. These observations suggested that HDL-raising therapies might be an effective way to reduce atherosclerotic cardiovascular events in patients with sustained cardiovascular disease. Other potential strategies for protection against atherosclerosis might be modulation of the expression of members of the ABC transporter family. ABCA1 and ABCG1 play critical roles in the homeostasis of lipids and other metabolites in macrophages, making them important therapeutic targets. Combined deletion of ABCA1 and ABCG1 in macrophages resulted in massive foam cell formation and an increased susceptibility to lesion development. While macrophage ABCA1 overexpression has been demonstrated to inhibit the progression of advanced atherosclerosis, studies on the overexpression of ABCG1 have largely been disappointing until now. The generation of mice with a combined overexpression of ABCA1 and ABCG1 in macrophages is expected to shed further light on the therapeutic potential of combined upregulation of ABCA1 and ABCG1 in macrophages.

The next challenge is to identify therapeutic molecules that can specifically modulate the expression of ABCA1 and ABCG1 in macrophages. This review, however, showed that the regulation of ABCA1 and ABCG1 expression and function is complex. In addition to sterols and phospholipids, eicosanoids, fatty acids, glucose and AGEs have been shown to influence ABC transporter expression and, hence, modulate macrophage RCT. Oxysterols are beneficial for upregulating ABCA1 and ABCG1 expression in macrophages and enhancing cholesterol efflux [51,52,60], and are therefore considered potential therapeutic drugs for the prevention of atherosclerosis. However, LXR activation is often associated with hepatic steatosis and hypertriglyceridemia by activation of sterol regulatory binding protein 1c [113]. As LXR activation by 24,25-EC did not affect triglyceride levels, it was suggested to be a potential drug. 24,25-EC-induced activation, however, showed an unexpected decrease in cholesterol efflux due to reduced availability of free cholesterol for ABCA1-mediated and ABCG1-mediated efflux to ApoA-I and HDL, respectively. Owing to major unsolved problems with side effects upon the use of oxysterols, other strategies for regulating ABCA1 and ABCG1 expression are required.

To date, 52 members of the family of ABC transporters have been identified, of which a vast majority are expressed on macrophages and show cholesterol-responsive regulation [29,30]. Therefore, it is reasonable to assume that more ABC transporters are involved in macrophage lipid homeostasis and play critical roles in foam cell formation and atherogenesis. Animal models as well as clinical studies will further improve our understanding of the physiological roles of ABC proteins and their regulation. Conceivably, in the long term, modulation of ABC transporters might be a new therapeutic strategy to prevent atherosclerosis or to induce the regression of established atherosclerotic lesions.

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Executive summary

Introduction
- Excessive accumulation of cholesterol by macrophage-derived foam cells is one of the characteristic features in atherosclerosis.
- Current treatment strategies for preventing atherosclerosis are mainly based on reducing risk factors, such as reducing plasma cholesterol levels by the use of statins.
- Modulating macrophage cholesterol-efflux pathways might be a new approach in the treatment of atherosclerotic vascular disease.

ABC transporters as effective macrophage lipid transporters
- ABCG1 mediates efflux of cholesterol from macrophages to HDL, preventing macrophage foam cell formation.
- Although both ABCA1 and ABCG1 have critical roles in mediating cholesterol efflux, the role of ABCG1 in atherosclerosis is still unclear, as macrophage Abcg1 deficiency appeared to be proatherogenic as well as antiatherogenic.
- Different compensatory mechanisms in Abcg1-deficient mice might mask the primary important role of ABCG1 in cholesterol efflux.
- Combined deletion of Abca1 and Abcg1 in macrophages leads to extreme lipid accumulation in tissue macrophages and increased susceptibility to atherosclerosis, despite relatively low plasma cholesterol levels. The extreme phenotype in mice lacking Abca1 and Abcg1 in macrophages indicates the importance of both ABCA1 and ABCG1 in promoting lipid efflux from macrophages and their effect on inhibiting foam cell formation.

Complex regulatory mechanisms of ABC transporters
- Levels of macrophage ABCA1 and ABCG1 do not only depend on oxysterol-induced transcription, but also on compounds involved in the phosphorylation and subsequent degradation of these transporters.
- Beside sterols and phospholipids, eicosanoids, fatty acids, glucose and advanced glycation end products influence ABCA1 and ABCG1 expression and function.
- ABCG1 is regulated by both glucose and fatty acids, which has important clinical implications for diabetic atherosclerosis.

Future perspective
- Generation of mice with combined overexpression of ABCA1 and ABCG1 in macrophages is expected to shed further light on the therapeutic potential of combined upregulation of ABCA1 and ABCG1 in the prevention of atherosclerosis.

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- of considerable interest


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Role of the ABC transporters ABCA1 & ABCG1 in foam cell formation & atherosclerosis

**Demonstrates that 12/15-lipoxygenase activity induces ABCG1 serine phosphorylation in macrophages, leading to increased degradation of ABCG1.**

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