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The role of adiponectin in cholesterol efflux and HDL biogenesis and metabolism

Anouar Hafiane [1], Karina Gasbarrino [1], Stella S. Daskalopoulou *

Department of Medicine, Faculty of Medicine, Research Institute of the McGill University Health Centre, McGill University, Montreal, Quebec, Canada

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ABSTRACT

Cholesterol efflux is the initial step in the reverse cholesterol transport pathway by which excess cholesterol in peripheral cells is exported and subsequently packaged into high-density lipoprotein (HDL) particles. Adiponectin is the most abundantly secreted adipokine that possesses anti-inflammatory and vasculoprotective properties *via* interaction with transmembrane receptors, AdipoR1 and AdipoR2. Evidence suggests that low levels of adiponectin may be a useful marker for atherosclerotic disease. A proposed anti-atherogenic mechanism of adiponectin involves its ability to promote cholesterol efflux. We performed a systematic review of the role of adiponectin in cholesterol efflux and HDL biogenesis, and of the proteins and receptors believed to be implicated in this process. Nineteen eligible studies (7 clinical, 11 fundamental, 1 clinical + fundamental) were identified through Ovid Medline, Ovid Embase, and Pubmed, that support the notion that adiponectin plays a key role in promoting ABCA1-dependent cholesterol efflux and in modulating HDL biogenesis *via* activation of the PPAR-γ/LXR-α signalling pathways in macrophages. AdipoR1 and AdipoR2 are suggested to also be implicated in this process, however the data are conflicting/insufficient to establish any firm conclusions. Once the exact mechanisms are unravelled, adiponectin may be critical in defining future treatment strategies directed towards increasing HDL functionality and ultimately reducing atherosclerotic disease.

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Contents

Abbreviations: RCT, reverse cholesterol transport; ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; HDL-C, high-density lipoprotein cholesterol; apoA-I, apolipoprotein A-I; CVD, cardiovascular disease; CAD, coronary artery disease; LXR, liver X receptor; SR-B1, scavenger receptor class B type 1; PPAR, peroxisome proliferator–activated receptor; HMW, high molecular-weight; NO, nitric oxide; FCR, fractional catabolic rate.

* Corresponding author at: Department of Medicine, Division of Internal Medicine, McGill University, 1001 Decarie Blvd, EM1.2230, Montreal, Quebec H4A 3J1, Canada.
E-mail addresses: anouar.hafiane@mail.mcgill.ca (A. Hafiane), karina.gasbarrino@mail.mcgill.ca (K. Gasbarrino), stella.daskalopoulou@mcgill.ca (S.S. Daskalopoulou).
[1] These authors share co-first authorship.

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1. Introduction

Under physiological conditions, cellular cholesterol uptake, synthesis, and release are in active equilibrium. An imbalance in this process can lead to an increase in cellular lipid accumulation and initiate the process of macrophage-foam cell transformation in the arterial wall contributing to the development and progression of atherosclerosis [1,2]. The reverse cholesterol transport (RCT) pathway, which involves the removal of excess cholesterol from peripheral tissues through the action of various transporters, such as ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1), is a multi-step process that is regulated by various mechanisms [3]. RCT is considered as the sum of high-density lipoprotein (HDL)-mediated cellular cholesterol efflux and transport capacities from peripheral tissues to the liver for excretion in the bile [4,5]. RCT, particularly from macrophages, is an important protective mechanism against atherosclerosis development [6,7]. In this pathway lipid-free apolipoprotein A-I (apoA-I) is the primary acceptor of cholesterol and the preferred substrate of ABCA1, and is essential for HDL biogenesis [8]. However, many factors present in the plasma can influence the cholesterol efflux process, including albumin [4,9], apoE [10], adiponectin [11], and exosomes [12].

Adiponectin, an anti-inflammatory and anti-atherogenic adipokine secreted from adipose tissue, acts as a key mediator of the crosstalk between adipose tissue, the immune system, and the vascular wall [13]. Compelling evidence suggests that adiponectin plays a significant role in promoting cellular cholesterol efflux and HDL biogenesis. These studies hypothesized that ABCA1 activity and adiponectin receptors, AdipoR1 and AdipoR2, play a critical role in adiponectin-mediated cholesterol efflux [14]. Further understanding of the molecular mechanisms underlining the adiponectin-cholesterol efflux process may be essential for treating the epidemic of cardiovascular disease (CVD), and for developing novel HDL biogenesis enhancers. This is particularly important after the failure of multiple pharmacological tools to increase the levels of HDL cholesterol (HDL—C) and reduce the risk of cardiac events and stroke [15]. Several reviews related to adiponectin and metabolic and CVDs have been published [16–18]; however, there are few articles that focus on the cholesterol efflux properties of adiponectin. Herein, we performed a systematic review of both clinical and fundamental evidence on the role of adiponectin in cholesterol efflux and HDL biogenesis and metabolism.

2. Methodology

2.1. General search strategy

A search strategy was developed by the authors for Ovid Medline (1946 to Present), Ovid Embase (1947 to Present), and PubMed. The search strategy was designed to answer a specific research question assessing the role of adiponectin in cholesterol efflux and HDL biogenesis and metabolism ([adiponectin OR Acrp30 OR Acrp 30 OR AdipoQ OR Apm1 OR Apm 1 OR GBP28 OR GBP 28 OR gelatin-binding protein OR gelatin binding protein] AND [cholesterol efflux OR cholesterol efflux capacity OR reverse cholesterol transport OR ATP-binding cassette transporter OR ABC transporter OR ABCA1 OR ABCG1 OR SRB-1 OR scavenger receptor class B type 1 OR apolipoprotein A1 OR ApoA1 OR high-density lipoprotein cholesterol OR high density lipoprotein cholesterol OR HDL OR cholesterol homeostasis]). The search was run independently by AH and KG on October 5th, 2017 and then re-run on May 31, 2019 to retrieve new studies published since the initial search. Reference lists of eligible studies were hand-searched, from which no additional studies were identified, thus validating our search strategy.

2.2. Eligibility criteria

Original human, animal, or *in vitro* studies reporting evidence on the role of adiponectin in cholesterol efflux and HDL homeostasis were considered eligible. Conference abstracts or abstracts resulting in published work were excluded. Although the search strategy did not restrict articles based on language, at the screening level of potentially eligible articles, we included only English or French records.

2.3. Selection process

A flow–chart illustrating the review process is presented in Fig. 1. From the initial 5186 studies retrieved (after duplicate removal), 43 studies were identified and evaluated in detail by the two first authors (AH and KG). Of those, 24 were excluded due to reasons outlined in Fig. 1, resulting in 19 eligible studies (7 clinical, 11 fundamental, 1 clinical + fundamental). All eligible studies were written in the English language. Data extraction was performed independently by AH and KG and summarized in supplemental Tables S1 and S2.

3. Cholesterol efflux concept

3.1. Cholesterol efflux capacity as a measure of HDL functionality

Whereas epidemiological studies have consistently demonstrated that low plasma HDL-C levels are strongly associated with increased CVD risk, Mendelian randomization studies have cast doubt on whether HDL-C is causally related to CVD [19–21]. Moreover, clinical trials aimed at raising HDL-C pharmacologically using niacin and cholesteryl ester transfer protein inhibitors, have failed to reduce the risk of cardiac events and stroke [15,22,23]. Hence, challenging the HDL-C hypothesis [3,24]. Thus, measures of HDL functionality rather than its absolute cholesterol mass, may be more accurate indicators of CVD risk as well as better targets of novel therapies [3,25].

Cholesterol efflux capacity as a measure of HDL functionality has been shown to have independent inverse associations with increased carotid artery stenosis and advanced plaque morphology, with coronary artery disease (CAD), and with incident cardiovascular events among apparently healthy individuals [26–28]. In addition to mediating cholesterol efflux, HDL particles have a variety of pleiotropic effects; they possess anti-oxidant properties and modulate vascular inflammation, vasomotor function, and thrombosis [3,19,29].

3.2. The gatekeepers of the cellular cholesterol efflux process and HDL biogenesis

Cellular cholesterol efflux is considered the major source of HDL in plasma [30]. It is the initial step in the RCT pathway by which excess free cholesterol in peripheral cells is exported and subsequently packaged into HDL particles [3,8]. ABCA1, ABCG1, and scavenger receptor

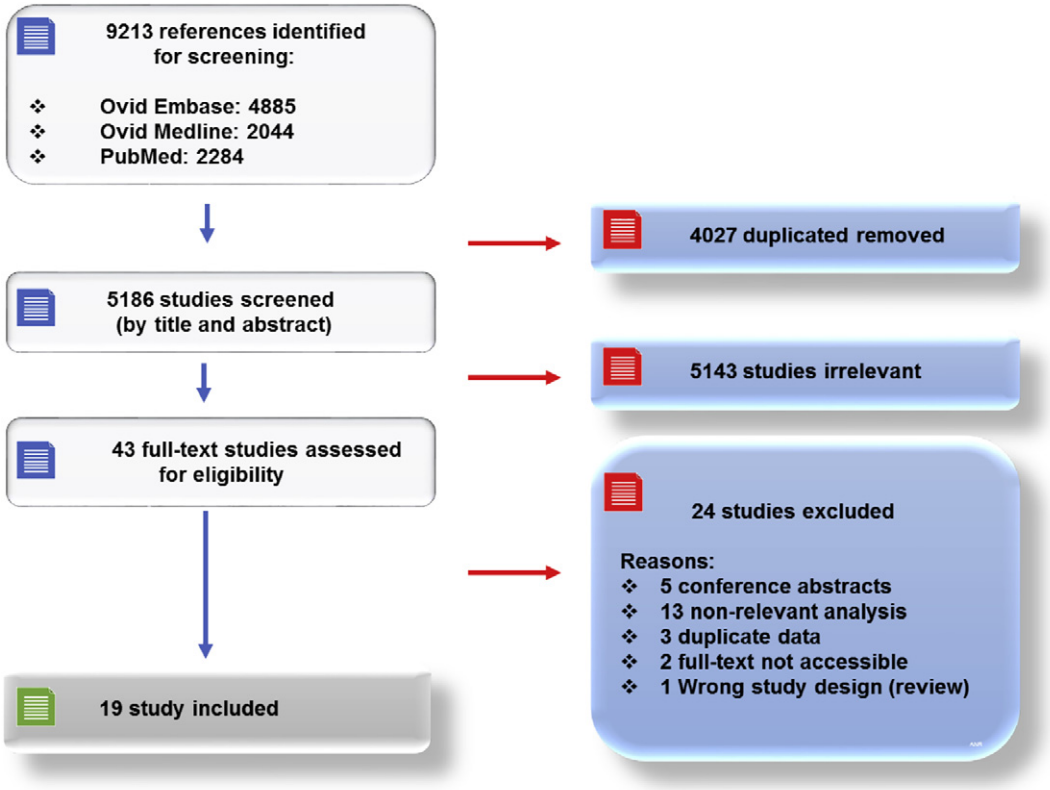

Fig. 1. Flow-chart of review process presenting the selection of eligible studies.

class B type 1 (SR-B1) are the three most important transporters implicated in cholesterol efflux and are all highly expressed in human and murine macrophages [3]. Studies of macrophage RCT have demonstrated that both apoA-I and ABCA1 play pivotal roles in initiating RCT [31–34]. The initial rate-limiting step of the RCT pathway is the formation of nascent HDL particles, which are made up primarily (approximately 65–70%) of apoA-I [4,31]. This step involves the interaction between monomeric lipid-poor or lipid-free apoA-I and ABCA1. The importance of ABCA1 in the lipidation of apoA-I has been emphasized by the fact that mutations in the ABCA1 gene (homozygous or compound heterozygous) cause Tangier disease and other familial HDL deficiencies [35]. Unlike ABCA1, ABCG1 is responsible for mediating the efflux of cellular cholesterol to lipidated (nascent or mature) HDL particles [4]. ABCA1 and ABCG1 gene expressions are regulated at the transcriptional level by members of the nuclear receptor superfamily, namely peroxisome proliferator–activated receptor (PPAR)-γ and/or liver X receptor (LXR)-α [36,37]. Similarly to ABCG1, SR-B1 in peripheral cells can also promote cholesterol efflux to mature HDL particles [38]. However, its role in the RCT pathway is more essential in the liver tissue where it modulates changes in the structure and composition of plasma HDL particles, by mediating the selective uptake of cholesteryl esters from HDL [39].

4. Adiponectin biology and pleiotropic actions

Adiponectin is an adipocyte-secreted protein that can oligomerize to form various multimeric complexes in the circulation [40,41]. Evidence strongly suggests that the high molecular-weight multimer (HMW, 12- to 18-mer) of adiponectin is the most metabolically active isoform [40]. Adiponectin acts as a "pleiotropic cytokine" linked not only to adipocyte metabolism and homeostasis, but also to a wide range of diverse effects in many different organs and tissues (Fig. 2). Specifically, it is known for its anti-diabetic, insulin-sensitizing, anti-atherogenic, and anti-inflammatory properties [13,17,18,42–44]. These effects are mediated at least through two transmembrane receptors, AdipoR1 and AdipoR2 [45]. AdipoR1 is mainly implicated in the metabolic functions of adiponectin, whereas AdipoR2 is also involved in anti-inflammatory and anti-oxidative activities [46]. Another key receptor of adiponectin is T-cadherin, which plays an important role in mediating the cardioprotective actions of adiponectin as established in mice studies [47].

Reduced circulating adiponectin levels have been observed in patients with obesity, insulin resistance, and type 2 diabetes mellitus [48–51]. Thus, adiponectin has been thought to play a protective role in the development of type 2 diabetes and metabolic syndrome. Evidence also suggests that circulating adiponectin is an independent and inverse predictor of cardiovascular events. Individuals with adiponectin levels in the highest quintile have been shown to have reduced risk for myocardial infarction [52]. Furthermore, in the Framingham Offspring Study, elevated plasma adiponectin levels were associated with a decrease in future coronary heart events in men [53]. However, other studies have opposing results regarding the utility of adiponectin as a biomarker of cardiovascular risk [52,54–60]. Specifically, several meta-analyses have demonstrated that circulating adiponectin is positively associated with ischemic stroke risk, as well as with increased all-cause and cardiovascular mortality rates [56,57]. The pathophysiology underlying this paradoxical association with adiponectin remains unclear. Plausible mechanisms have been proposed: high adiponectin levels may reflect the phenomenon of adiponectin resistance (decreased signalling efficacy) in response to disease progression or may be attributed to progressive compensatory response to underlying vascular inflammation.

Adiponectin's atheroprotective effects have been recognized in cellular and animal models in all stages of atherosclerotic plaque development [42]. Several studies using animal models with genetic manipulations provided direct causal evidence highlighting the

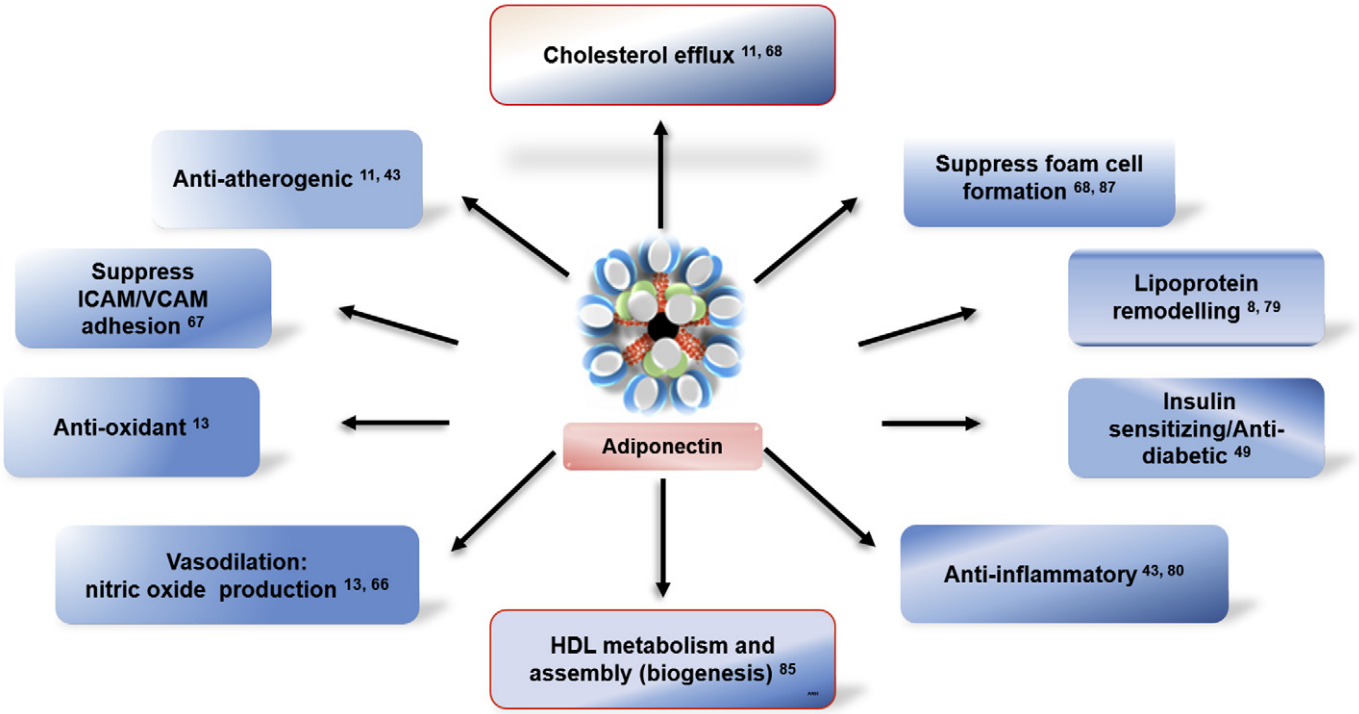

Fig. 2. Pleiotropic effects of adiponectin. In addition to its ability to stimulate cellular cholesterol efflux and modulate HDL metabolism and biogenesis, adiponectin also displays a variety of pleiotropic effects on a multitude of different cell types. Specific interest in adiponectin's role in cholesterol efflux and HDL metabolism is outlined in a red box. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

atheroprotective role of adiponectin in inhibiting the buildup of atherosclerotic lesions [61–64]. On the other hand, a study by Nawrocki et al. revealed the lack of an association between adiponectin levels and atherosclerosis in a low-density lipoprotein receptor knockout mouse model [65]. This study demonstrates the potential limitations of using a mouse model in order to reproduce the reported associations for adiponectin with CVD in humans. *In vitro* evidence further supports adiponectin as an anti-atherogenic adipokine [11,43]. Data suggest that adiponectin can stimulate the production of nitric oxide (NO) in vascular endothelial cells, suppressing the endothelial inflammatory reaction and reducing the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 [66,67]. This leads to less inflammatory cell recruitment to atherosclerotic plaques. Furthermore, adiponectin can decrease cholesterol accumulation in macrophages,

Table 1
Antiatherogenic properties of adiponectin: summary of adiponectin's direct effects on cholesterol efflux, HDL biogenesis, and lipid metabolism.

Antiatherogenic properties of adiponectin	References
Modulates cholesterol efflux pathways	
Increases ABCA1 expression (ABCA1 mRNA and ABCA1 protein)	[11,14,81]
Increases ABCA1-mediated cholesterol efflux to apoA-I/HDL	[14,48,74]
Might not enhance SR-B1-mediated cholesterol efflux	[11]
Increases ABCG1 cholesterol efflux to HDL	[68]
Activates PPAR-γ and LXR-α	[88]
Generates significantly higher efflux *via* AdipoR1/AdipoR2 in cells treated with adiponectin	[14,42]
Increases apoA-I synthesis in liver, and increases pre-β1 HDL particles	[85,87,90]
Down-regulates foam cell formation	
Reduces expression of adhesion molecules (ICAM-1/VCAM-1) in endothelial cells	[66,67]
Suppresses foam cell formation	[68,83]
Reduces lipid accumulation (free cholesterol, cholesterol ester) in foam cells	[68,88]
Down-regulates the expression of ACAT-1 as well as SR-A1 in human macrophages	[96]
Reduces lipid droplet size	[63]
Modulates hormone-sensitive lipase activity	[95]
Reduces atherosclerosis by 30–40% *in vivo*	[88]
Regulates HDL metabolism	
Is associated with higher HDL-C levels	[89]
Promotes triglyceride-rich lipoprotein catabolism, and lower triglyceride levels	[72,77]
Is a determinant of HDL/apoA-I catabolism	[77]
Inhibits hepatic lipase, and activates lipoprotein lipase	[79,80]
Increases HDL assembly in liver	[87]

ABCA1 indicates ATP-binding cassette transporter A1; PPAR-γ, peroxisome proliferator-activated receptor gamma; LXR-α, liver X receptor alpha; ABCG1, ATP-binding cassette transporter G1; HDL—C, high-density lipoprotein cholesterol; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; ACAT-1, Acetyl-CoA acetyltransferase; SR-A1, scavenger receptor class A type 1; SR-B1, scavenger receptor class B type 1; apoA-I, apolipoprotein A-I.

thereby suppressing macrophage-to-foam cell transformation [68]. A proposed anti-atherogenic mechanism by which adiponectin achieves lower intracellular cholesterol levels is by promoting an increase in cholesterol efflux capacity (Table 1). Herein, we present the clinical and fundamental evidence examining the role adiponectin plays in cholesterol efflux and HDL biogenesis and metabolism, and we highlight the proteins and receptors believed to be implicated in this process.

5. Clinical evidence of adiponectin's effects on cholesterol efflux and HDL biogenesis and metabolism

The link between adiponectin and CVD could be partly mediated by adiponectin's effects on lipid metabolism. Several studies indicate that the inverse association between adiponectin and CAD is significantly attenuated after adjustments for lipids, particularly HDL-C [52,69]. In fact, a strong correlation between plasma HDL-C and circulating adiponectin concentrations exists in both healthy and diseased populations, *i.e.*, with obesity, metabolic syndrome, type 2 diabetes, or CVD, where HDL-C and adiponectin levels are observed to be significantly lower compared to healthy individuals [70,71]. Despite this well-documented association, the mechanisms involved have been poorly investigated. Following the literature search, only 8 clinical studies aimed to explain the association between circulating adiponectin levels and HDL—C, by identifying a relationship between adiponectin and 1) HDL biogenesis (*via* cholesterol efflux), or 2) HDL catabolism (Table S1) [68,72–78].

The majority of the studies demonstrated circulating adiponectin levels to be an independent predictor of cellular cholesterol efflux capacity in humans [68,74,76,78]. One study showed plasma adiponectin levels to be strongly associated with efflux in healthy adults older than 18 years, independently of age, sex, body mass index, glucose, blood pressure, and markers of inflammation and liver function [74]. Moreover, low serum adiponectin levels were also highly correlated with impaired cholesterol efflux capacity in diabetic subjects, and positively associated with low cholesterol efflux to total isolated HDL in subjects with established coronary heart disease [68,76]. Stepwise regression analyses demonstrated that adiponectin accounted for 10.7% of the variance in cholesterol efflux, a value higher than that for apoA-I, which accounted for only 3.9% of the variance [76]. It is largely hypothesized that adiponectin can modulate HDL biogenesis, by increasing the generation of nascent HDL particles during the apoA-I/ABCA1 interaction. *In vivo* evidence demonstrated that low circulating adiponectin levels were significantly and independently associated with reduced ABCA1 expression on monocytes of overweight and obese subjects, but not with ABCG1 or SR-B1 expression. This finding suggests that adiponectin may be an important regulator of ABCA1 expression [78].

Clinical evidence also demonstrated that adiponectin may play a direct role in HDL catabolism. A strong and negative correlation was observed between adiponectin levels and the HDL-apoA-I fractional catabolic rate (FCR), in various populations, such as in obese subjects, subjects with metabolic syndrome, or in healthy individuals [72,75,77]. Furthermore, these studies showed that adiponectin was a significant predictor of HDL-apoA-I FCR, independent of important variables including but not limited to age, sex, body mass index, insulin resistance, and triglyceride levels. Verges et al. determined that plasma adiponectin, on its own, can explain 43% of the variance of HDL-apoA-I FCR [77]. In addition, plasma adiponectin concentrations were also significantly and positively associated with triglyceride-rich lipoprotein (VLDL-apoB) catabolism, and negatively with triglyceride levels [72,77]. Thus, low circulating adiponectin levels may not only enhance the catabolism of HDL-apoA-I but also reduce the clearance rate of VLDL, apoB-related particles, and triglycerides. Although the mechanisms linking adiponectin and lipoprotein metabolism have not been fully explored, adiponectin's above-mentioned effects may be partly mediated through its inhibition of hepatic lipase and activation of lipoprotein lipase [79,80].

6. Fundamental evidence of adiponectin's effects on cholesterol efflux and HDL biogenesis

Fundamental evidence also supports the notion that adiponectin plays a key role in promoting HDL/apoA-I cholesterol efflux at various steps throughout the RCT pathway [11,14,68,81–89] (Table S2), in which adiponectin has been shown to act mainly at the level of the liver and macrophage.

A co-culture model of adipocytes and hepatocytes revealed a direct involvement of adipose tissue in hepatic cholesterol metabolism, mainly *via* adiponectin [82]. In liver hepatoma G2 (HepG2) cells, adiponectin treatment (1, 5, 10 μg/mL) increased the synthesis and secretion of apoA-I in a dose-dependent manner and enhanced the cellular expression of ABCA1, however had no effect on ABCG1 and SR-B1 expression [85]. This suggests that in the liver adiponectin might increase HDL assembly specifically through ABCA1. Animal studies, using an adiponectin knock-out mouse model, also demonstrated the importance of adiponectin in regulating apoA-I levels in plasma and ABCA1 expression in the liver. Adiponectin deficiency in mice caused an impairment in HDL assembly in the liver [87]. Upon daily treatment of adiponectin knock-out mice with adiponectin (50 to 250 μg/kg per day) for four weeks, HDL-C levels in the serum and ABCA1 expression in the liver were increased in a dose-dependent manner [89]. In accordance, adiponectin-transgenic mice had significantly higher plasma HDL levels compared to wild-type mice and also had altered expression of key liver genes involved in lipid metabolism [84].

While the liver is central in the regulation of cholesterol levels in the body, RCT from macrophages is defined as the critical step in protecting against atherosclerotic plaque development. *In vitro* studies indicate that adiponectin treatment might protect against atherosclerosis by significantly enhancing apoA-I-mediated cholesterol efflux from macrophages through an ABCA1-dependent pathway [11,81]. Adiponectin is believed to indirectly modulate cellular cholesterol efflux at various doses (1, 5, 10 μg/mL) in murine and human macrophages by positively affecting ABCA1 mRNA and protein expression [11,81,88]. Furthermore, human macrophage-derived foam cells, designed to express the adiponectin gene, showed a significant reduction in intracellular cholesterol accumulation, upregulation in ABCA1 and SR-B1 protein expression, and increased HDL-mediated cholesterol efflux, compared to control macrophage foam cells not expressing adiponectin [88]. Interestingly, adiponectin multimers differentially affected macrophage cholesterol metabolism, with HMW adiponectin being more effective than the low molecular-weight one in reducing cholesterol accumulation in the macrophage foam cells [88]. This suggests that HMW adiponectin may be more atheroprotective than the smaller-sized isoforms. Furthermore, *in vivo* studies suggest that adiponectin may play a critical atheroprotective role by altering macrophage lipid metabolism. Adiponectin knock-out mice showed decreased macrophage ABCA1 expression and apoA-I-mediated cholesterol efflux compared to wild-type mice, and they exhibited apparent aortic atherosclerotic lesions and large lipid deposition in vessel walls [11,89]. In mice lacking ABCA1, specifically in adipocytes, a significant increase in cholesterol accumulation and a reduction in adiponectin expression were noted, that is likely a result of decreased ABCA1-mediated cholesterol efflux [33]. However, further studies are needed to assess the direct effects of adiponectin on the RCT pathway in knockout animal models of ABCA1.

While most reports focused on studying ABCA1-mediated cholesterol efflux and have demonstrated compelling evidence that adiponectin increases the rate of cholesterol removal *via* the ABCA1 pathway, few studies have investigated the effect of adiponectin on ABCG1 and SR-B1. Little evidence suggests that adiponectin may also affect cholesterol efflux through ABCG1. Specifically, Wang et al. reported that in monocyte-derived macrophages obtained from patients with type 2 diabetes mellitus, adiponectin treatment can significantly increase cholesterol efflux to mature HDL, partly by mediating an upregulation in the mRNA and protein expression of ABCG1 [68]. Moreover, ABCG1 siRNA in murine

macrophages, but not ABCA1 siRNA, resulted in a diminishment of adiponectin's effects on the lipidation of HDL particles [68]. However, the precise contribution of adiponectin in mediating cholesterol efflux through the ABCG1 transporter remains to be elucidated.

The impact of adiponectin on HDL subspecies remains elusive. Our own preliminary data demonstrated that adiponectin promotes cholesterol efflux by increasing the generation of both preβ- and α-HDL species [90]. Preβ-HDL is an immature form of HDL, which acts as both a product and substrate in the ABCA1-mediated cholesterol efflux process [91]. On the other hand, α-HDL species represent mature forms of HDL and high levels of the larger α-species are associated with decreased CVD risk [34]. Our findings suggest that adiponectin is efficient in maintaining cholesterol efflux by promoting the generation of a continuous pool of HDL particles acting as acceptors. Moreover, adiponectin also promotes HDL maturation. However, the functional properties of these generated HDL particles remain unclear.

Table 1 summarizes adiponectin's direct effects on cholesterol efflux, HDL biogenesis and lipid metabolism.

7. Cholesterol efflux assay methodologies

Based on our search strategy, all identified studies were limited to using radiolabeled cholesterol in their cholesterol efflux assays. Radiolabeling was performed either *in vitro*, using distinct cell lines such as THP-1 [42,83,88,90], human fibroblasts [73], HEK293T [14], HepG2 [85,86], J774 [50,74], Fu5AH [76], RAW 264.7 macrophages [81], and primary human and murine macrophages [11,68] or *in vivo*, where animals were injected with radiolabeled cholesterol [89]. Various acceptors were used in combination with adiponectin, such as apoA-I [11,90], HDL [11,76,88], reconstituted HDL [14], plasma- [73] or serum-depleted apoB [68,74]. However, as reviewed extensively by our group [3,24,92] and others [93,94], several other methodologies have been proposed to measure cholesterol efflux *in vitro* and *in vivo*, and in the future it may be important to determine how adiponectin modulates cholesterol efflux activity using such assays. Advantages and limitations of various cholesterol efflux assay methodologies are summarized in Table 2.

8. Potential mechanisms of adiponectin's effects on cholesterol efflux

ApoA-I lipidation and HDL maturation by ABCA1 and/or ABCG1, in the presence of adiponectin, is a complex process that likely involves indirect interactions between adiponectin and these transporters. To date, the underlying mechanisms have not been fully clarified. Importantly, various proteins have been suggested to be implicated in adiponectin's effects on the RCT system, namely PPAR-γ and LXR-α [11,68,81,88] (Fig. 3); however, it is unclear how adiponectin regulates their expression. Adiponectin's effects may also be mediated by the same receptors (AdipoR1 and/or AdipoR2) that transduce its other cellular signals (Fig. 3) [14,68].

8.1. PPAR-γ/LXR-α signalling pathway

The activation of PPAR-γ and LXR-α/ß is the major mechanism through which ABCA1 and ABCG1 expression can be upregulated [36,37]. *In vitro* experiments and animal studies have suggested that

Table 2
Cholesterol efflux assay methodologies: advantages and limitations.

Cholesterol efflux assay methodologies	Advantages	Limitations
Radioactive assay		
Using 3[H]-FC, 14C	- Gold standard for determination of efflux at cellular level [92,93] - Better CVD marker than HDL-C and apoA-I concentration - Key metric for determining the anti-atherosclerotic function of HDL - Inverse association with CVD development [28,111] and carotid atherosclerosis [112] - Valuable in studying extracellular lipid vesicles [12,101]	- Represents only a small fraction of macrophage RCT - Lack of standardization - Controversial association with myocardial infarction [9] - Failure of measuring phospholipid efflux by ABCA1 - Low throughput [24,92] - Difficult in assessing the terminal components of the RCT pathway
Non-radioactive assay		
Cholesterol exchange onto lipid poor apoA-I	- Use of radioisotope avoided - Quantification of HDL apoA-I exchange in blood plasma [113]	- Confirmation in large clinical studies is needed
Fluorescence assay		
BODIPY-cholesterol	- Efflux rates approximately 3 times greater than with 3[H]-cholesterol [114] - Safe, sensitive, and reproducible alternative, and great potential as a valuable tool when incorporated into a drug discovery program [115,116]	- Probable generation of nonspecific cholesterol removal [92,116]. - Validation in large-scale clinical studies is needed - Discrepancy between studies regarding correlation with 3[H]-cholesterol [114]
Pennsylvania Green/N-alkyl-3β-cholesterylamine-derived molecular probe (F-Ch)	- Comparable kinetics with 3[H]-cholesterol [115] - Sensitive and high-throughput to quantify cholesterol in complex matrices, such as cells [117]	- Limited use
Enzymatic method		
Immobilized liposome-bound gel beads	- Culture of cells not required [118]	- Additional validation is needed - No available data
In vivo assays		
13[C2]-cholesterol approach	- Measurement of free cholesterol exchange with red blood cells, esterification of plasma free cholesterol, and clearance of cholesterol ester [119]	- Need to determine if efflux correlates with *in vitro* assays
3[H]-cholesterol nanoparticles approach	- Measurement of the rate of macrophage RCT *in vivo* in humans [120]	- No clinical data are available - Determination of best targets to optimize *in vivo* cholesterol efflux is needed and whether this translates into CVD risk

FC indicates free cholesterol; HDL—C, high-density lipoprotein cholesterol; apoA-I, apolipoprotein A-I; RCT, reverse cholesterol transport; ABCA1, ATP-binding cassette transporter A1; CVD, cardiovascular disease

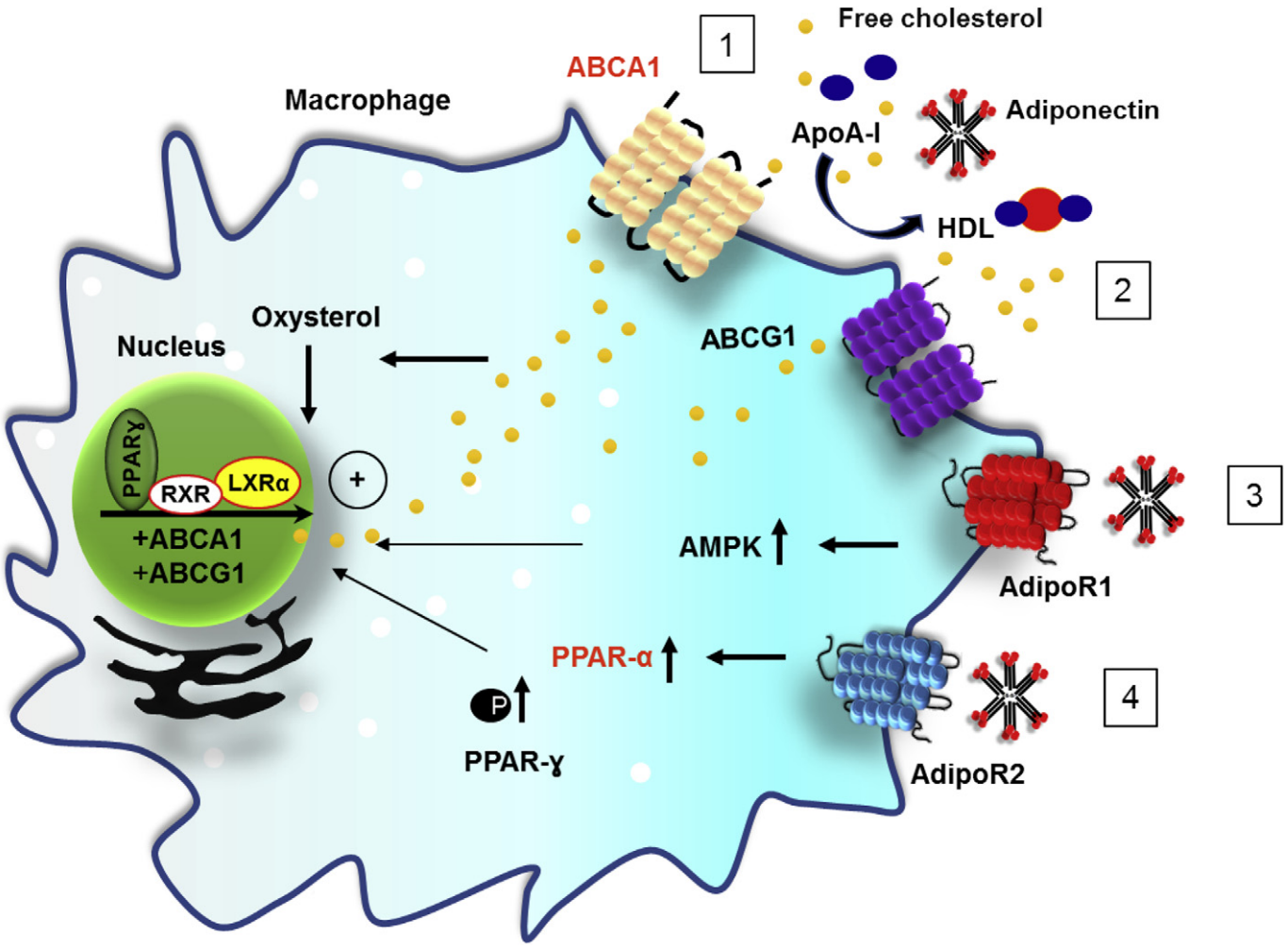

Fig. 3. Adiponectin modulates multiple pathways of lipid metabolism in macrophages. Summary of proteins and receptors implicated in adiponectin's role in macrophage cholesterol efflux and HDL biogenesis and metabolism. Various proteins have been hypothesized to be implicated in adiponectin's effects on ABCA1- and ABCG1-mediated cholesterol efflux (boxes 1 and 2, respectively), namely PPAR-ɣ and LXR-α. PPAR-ɣ and LXR-α induce a series of genes that are involved in cholesterol efflux, absorption, transport, and excretion. Moreover, adiponectin's effects may also be mediated by the same receptors (AdipoR1 [box 3] and AdipoR2 [box 4]) that transduce its other cellular signals. Activation of these receptors induces a cascade of signalling pathways involving the following molecules: PPAR-α, AMPK, MAPK, ERK, AKT, and P38, which in turn activates PPAR-γ, LXR-α, ABCA1, and ABCG. Oxysterols are directly involved in cellular lipid transport. In this process, the nuclear receptors, LXR, PPAR, and RXR, are prerequisite for nuclear transduction of oxysterol signalling. Particularly, LXR receptors form heterodimers with the RXR receptor that binds to LXR/RXR response elements within target genes. The transactivation of the ABCA1 promoter by LXR/RXR ligands results in expression of the ABCA1 within the plasma membrane. This pathway triggers efflux of lipids to apoA-I, which initiates the RCT process to eliminate excess cellular lipids. Free cholesterol molecules are illustrated by the yellow circles. ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; PPAR-ɣ, peroxisome proliferator-activated receptor gamma; LXR-α, liver X receptor alpha; AdipoR1/R2, adiponectin receptor R1/R2; AMPK, adenosine monophosphate-activated protein kinase; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; RCT, reverse cholesterol transport. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adiponectin can increase cholesterol efflux from macrophages through an ABCA1- (or ABCG1-) dependent pathway by activation of PPAR-ɣ and/or LXR-α (Table 1). Tian et al. was the first to report that activation of the PPAR-ɣ/LXR-α signalling pathways may be necessary for adiponectin's attenuation of lipid accumulation in macrophage-derived foam cells. In this study, macrophage-derived foam cells expressing the adiponectin gene had 30–40% reduction in cholesterol accumulation when compared to control foam cells [88]. The mechanism proposed is activation of LXR-α and PPAR-ɣ by adiponectin, resulting in an increase in ABCA1 expression. Other studies demonstrated that treatment of macrophages with adiponectin increased LXR-α expression and activation, while LXR-α siRNA completely abolished the promotion effects of adiponectin on ABCA1 expression and cholesterol efflux [11,68,81]. Alternative mechanisms of adiponectin's anti-atherogenic effect have been proposed; adiponectin can affect multiple pathways of lipid metabolism in macrophages by modulating hormone-sensitive lipase activity [95] or down-regulating the expression of acetyl-CoA acetyltransferase-1 as well as scavenger receptor class A, that are involved in foam cell formation and cholesterol ester accumulation [88,96,97].

8.2. AdipoR1 and AdipoR2

The binding of adiponectin to its receptors, AdipoR1 and AdipoR2, and the subsequent signalling events are responsible for the physiological effects induced by adiponectin [45]. It is believed that these receptors play a key role in mediating adiponectin's effects on lipid metabolism by inducing a cascade of signalling pathways involving the following molecules: PPAR-α, AMPK, PI3K/Akt, p38 MAPK, and Rab5 [98], that may in turn activate PPAR-γ, LXR-α, ABCA1, and ABCG1. Overexpression and downregulation of AdipoR1 and AdipoR2 in HEK293T human kidney cells provided the first evidence that both receptors are positively involved in the cholesterol efflux process *via* up-regulation of ABCA1 expression and activity [14]. However, there is conflicting evidence regarding the importance of each receptor in

macrophage lipid metabolism. In macrophages isolated from diabetic patients, adiponectin increased macrophage cholesterol efflux and upregulated ABCG1 and LXR-α expression in the presence of intact AdipoR signalling [68]. However, these effects were blunted in macrophages lacking an AdipoR1 signalling pathway but not AdipoR2 [68]. Instead, Li et al., found that knockdown of AdipoR2 in human THP-1 macrophages resulted in reduced cellular lipid accumulation, enhanced apoA-I- and HDL-mediated cholesterol efflux, and increased expression of PPAR-γ, LXR-α, ABCA1, and ABCG1 [83], while the contribution of the AdipoR1 pathway was not investigated. However, Li's efflux experiments were performed in the absence of adiponectin [83]. Therefore, a firm conclusion regarding the direct impact of AdipoR2 gene silencing on adiponectin's effects on cholesterol efflux cannot be established. In fact, the upregulation observed in cholesterol efflux may be attributed to off-target effects of downregulating AdipoR2 on the expression of ABCA1, ABCG1, LXR-α, PPAR-γ. Conversely to Li et al., we have recently demonstrated that decreased AdipoR2 activity is associated with increased foam cell formation and greater atherosclerotic plaque instability [42]. Further investigation is needed to elucidate the distinct effects of AdipoR1 and AdipoR2 in macrophage lipid metabolism and atherosclerosis development.

T-cadherin has been identified as an additional receptor for adiponectin, where its interaction with adiponectin in the vasculature has been suggested to play a protective role against neointimal and atherosclerotic plaque formation [99]. Therefore, T-cadherin may be another potential player involved in the effects of adiponectin on cholesterol efflux. However, there is no evidence of studies investigating the involvement of T-cadherin in the RCT pathway and HDL metabolism.

9. Adiponectin pathway as a potential therapeutic target for the modulation of atheroprotective HDL functionality

As previously discussed, randomized drug-intervention trials have failed to prove the benefit of increasing HDL-C levels for the prevention of atherosclerotic CVD [3]. Thus, there is a need to develop novel approaches that enhance HDL's ability to mediate cholesterol efflux. To date, a variety of HDL/apoA-I-based therapies as HDL biogenesis enhancers, are under investigation [3,100,101]. These apoA-I-containing compounds or apoA-I mimetic peptides may prove to be clinically useful for CVD prevention and treatment as they promote RCT [3]. Moreover, adiponectin alone or in combination with these HDL/apoA-I-based therapies, may also act as a promising target for future investigations in reducing the morbidity and mortality of atherosclerotic disease, as it can accelerate cholesterol efflux and HDL biogenesis and slow HDL-apoA-I catabolism. Given adiponectin's beneficial properties, its interaction with apoA-I may result in 'super-HDL' particles with greater functionality, than those generated from the therapies that are currently under investigation.

Interestingly, various currently used therapeutic interventions can modulate and increase circulating adiponectin levels [102–106]. These include, but not limited to, PPAR-α and PPAR-γ agonists, anti-diabetic and anti-hypertensive medications, as well as statins [102]. For example, a meta-analysis confirmed a significant increase in plasma adiponectin concentrations by 0.57 μg/mL following statin use [104]. Moreover, thiazolidinediones (PPAR-γ agonists), beyond improving insulin sensitivity, can also raise adiponectin levels and induce cholesterol efflux from human macrophages and macrophage-derived foam cells through stimulation of the ABCA1 pathway [105]. Along with increasing the cholesterol efflux process, apoA-I mimetic peptides (such as L-4F) have also been found to increase adiponectin concentrations [106]. The elevation in adiponectin levels associated with these compounds suggests that adiponectin may contribute to some beneficial cardiometabolic effects of these agents. While pharmacological elevation of circulating adiponectin may represent a promising therapeutic strategy to increase HDL functionality, another potential target is activation of the AdipoRs. AdipoRon, a new orally active synthetic agonist that binds to and activates both AdipoR1 and AdipoR2, has led to an improvement in insulin resistance, dyslipidemia, and glucose intolerance in db/db mice [107]. Furthermore, *in vitro* and *in vivo* studies have also demonstrated that AdipoRon has anti-oxidative and anti-apoptotic properties [108]. However, its beneficial effects on lipid metabolism and atherosclerosis prevention have yet to be investigated.

10. Knowledge gaps in understanding the role of adiponectin in atherosclerosis and RCT

Understanding the direct effects of adiponectin on atherosclerosis and the RCT pathway has been limited to the use of either cellular models, which do not capture the complexity of the disease or phenomenon, or murine pre-clinical models, whose lipid metabolism differs from that in humans and they do not develop human-like endpoints, such as stroke and sudden death. Thus, extrapolation of data from these model systems should be considered with caution. An interesting approach to address part of these limitations is to create an *in vitro* 3-dimensional human cell-based atherosclerotic plaque model that would replicate the cellular architecture and microenvironment of 'actual' human atherosclerotic plaques [109].

Although adiponectin has been demonstrated to have a clear and significant effect on promoting apoA-I-mediated cholesterol efflux, the exact mechanisms through which this process occurs remain unknown. Most of the down-stream signalling molecules involved in adiponectin's activation of the AdipoR1 and AdipoR2 receptors have yet to be identified, which has limited our understanding of the key players responsible for mediating adiponectin's effects on cholesterol efflux. For instance, several studies suggest that the adiponectin RCT effects may occur through increased expression of LXR and PPAR-γ. However, there is lack of evidence demonstrating the contribution of adiponectin on the production of endogenous sterol or lipid ligands that, in turn, would activate these transcription factors. Furthermore, while the biology of the AdipoR1 and AdipoR2 pathways appear quite distinct, their independent role and precise contribution in atherosclerotic disease and the RCT pathway remain to be elucidated. Studies involving selective activation of one receptor or the other may provide better insight into the potential involvement of AdipoR1 and AdipoR2 in mediating adiponectin's effects on cholesterol efflux and may serve as a potential therapeutic strategy in the context of RCT. To date, AdipoRon is the first orally-active synthetic small molecule that was identified to bind and activate both AdipoR1 and AdipoR2. No AdipoR-selective agonists currently exist. However, the recent crystallization of the AdipoR structure can help identify novel selective agonists of the AdipoR1 and AdipoR2 pathway [110]. Lastly, adiponectin's structural design alone seems unable to modulate the observed lipid activity, requiring an interaction with HDL/apoA-I that is complex and unclear. Thus, structural studies are also needed to understand the biochemical basis of adiponectin's role in the cholesterol efflux process. Currently, the field of adiponectin is still in its infancy and the mechanisms involved remain to be fully elucidated. Nonetheless, the anti-inflammatory and atheroprotective effects of this adipokine are a promising therapeutic target.

11. Conclusions

Adiponectin is a remarkable bioactive peptide that may beneficially affect atherosclerosis progression. The therapeutic potential of adiponectin in atherosclerosis has not been fully clarified yet. Based on existing evidence, adiponectin has the potential to modulate cholesterol efflux to HDL/apoA-I. Following recent re-evaluation of the "HDL-C hypothesis", the atheroprotective function of adiponectin should be considered within the HDL biogenesis pathway as a potential target for the modulation of HDL functionality. Clinical and fundamental evidence presented herein suggest that adiponectin may play a critical atheroprotective role in promoting ABCA1-dependent cholesterol efflux

and modulating HDL homeostasis (*i.e.*, by increasing HDL biogenesis and decreasing HDL catabolism) possibly *via* activation of the PPAR-γ/LXR-α signalling pathways. AdipoR1 and AdipoR2 could also be implicated in this process, however data are scarce. Increased cholesterol efflux results in a reduction in intracellular cholesterol accumulation and protection against the formation of macrophage foam cells. Therefore, adiponectin may direct future treatment strategies aimed at increasing HDL functionality, which may play a beneficial role in the prevention and treatment of atherosclerotic CVD.

Declaration of Competing Interest

None.

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Author contributions

AH and KG share equal contribution in the design and conduction of all aspects of this study. They performed review of the literature search, retrieval of included studies, data extraction and analysis, data interpretation, and manuscript drafting. SSD contributed to the design and conduction of the study, in addition to data interpretation, and manuscript revision. All authors have provided a final approval of the contents of the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2019.153953.

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