

Endocannabinoid Signalling in Atherosclerosis and Related Metabolic Complications

Raquel Guillaumat-Prats¹ Martina Rami¹ Stephan Herzig^{2,3,4} Sabine Steffens^{1,5}

¹Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University (LMU) Munich, Munich, Germany

²Institute for Diabetes and Cancer, Helmholtz Center Munich, Neuherberg, Germany

³Joint Heidelberg-IDC Translational Diabetes Program, Inner Medicine I, Heidelberg University Hospital, Heidelberg, Germany

⁴Molecular Metabolic Control, Technical University Munich, Munich, Germany

⁵German Centre for Cardiovascular Research (DZHK), Partner Site Munich Heart Alliance, Munich, Germany

Address for correspondence Sabine Steffens, PhD, Institute for Cardiovascular Prevention (IPEK), Ludwig-Maximilians-University (LMU), Pettenkoferstraße 9, 80336 Munich, Germany (e-mail: sabine.steffens@med.uni-muenchen.de).

Thromb Haemost 2019;119:567–575.

Abstract

Endocannabinoids are a group of arachidonic acid-derived lipid mediators binding to cannabinoid receptors CB1 and CB2. An overactivity of the endocannabinoid system plays a pathophysiological role in the development of visceral obesity and insulin resistance. Moreover, elevated circulating endocannabinoid levels are also prevalent in atherosclerosis. The pathophysiological increase of endocannabinoid levels is due to an altered expression of endocannabinoid synthesizing and degrading enzymes induced by inflammatory mediators such as cytokines or lipids. Emerging experimental evidence suggests that enhanced endocannabinoid signalling affects atherosclerosis via multiple effects, including a modulation of vascular inflammation, leukocyte recruitment, macrophage cholesterol metabolism and consequently atherosclerotic plaque stability. In addition, recent findings in various metabolic disease models highlight the relevance of peripheral CB1 cannabinoid receptors in adipose tissue, liver and pancreas, which crucially regulate lipid and glucose metabolism as well as macrophage properties in these organs. This suggests that targeting the endocannabinoid system in the vasculature and peripheral organs might have a therapeutic potential for atherosclerosis by inhibiting vascular inflammation and improving metabolic risk factors. This review will provide a brief update on the effects of endocannabinoid signalling in atherosclerosis and related metabolic complications.

Keywords

- ▶ atherosclerosis
- ▶ inflammation
- ▶ lipid mediators
- ▶ metabolic disorders
- ▶ obesity

Introduction

The prevalence of overweight and obesity has reached epidemic proportions in Western countries, but it is also rising in low- and middle-income countries. Globally, over 2 billion people are overweight, of which one-third suffer from obesity.^{1–5} Obesity has been related to several chronic diseases including atherosclerosis, hypertension, insulin resistance and

other inflammatory diseases.^{6,7} In particular, abdominal obesity is associated with increased incidence of cardiovascular risk factors, which are elevated triglycerides, low high-density lipoprotein (HDL) levels, increased blood pressure and hyperglycemia.⁶ Apart from these metabolic complications, an important process that links obesity to atherosclerosis and other cardiovascular diseases is chronic inflammation. The endocannabinoid system is involved in the regulation of energy

received

October 18, 2018

accepted after revision

January 3, 2019

© 2019 Georg Thieme Verlag KG
Stuttgart · New York

DOI <https://doi.org/>

10.1055/s-0039-1678738.

ISSN 0340-6245.

homeostasis and plays a pathophysiological role in the development of visceral obesity and insulin resistance.^{8,9} It has also arisen as a potential therapeutic target for cardiovascular diseases including atherosclerosis, restenosis and myocardial infarction.¹⁰ In addition to the well-established central and systemic effects of endocannabinoid signalling, there is emerging evidence for a peripheral control of metabolic functions in the liver, pancreas and adipose tissue based on cell-specific knockout models and selective peripheral antagonists. An exciting novel tool for non-invasive imaging of CB1 receptors has been recently reported, based on positron emission tomography/computer tomography imaging of selective radioligand binding. As a proof of concept, enhanced cardiac uptake of the radioactive CB1 ligand [¹¹C]-OMAR was shown in obese mice as well as in humans with advanced obesity compared with normal-weight subjects.¹¹ This technique may provide new insights into pathophysiological changes of CB1 signalling during cardiovascular disease development not only in animal models, but also in the clinical setting.

The role of the central endocannabinoid system in the regulation of metabolic homeostasis and pathophysiological conditions has been extensively reviewed by others.^{8,9,12,13} Here, we will focus on recent findings on peripheral endocannabinoid signalling, which might be relevant for therapeutic approaches targeting vascular, immune cell, liver as well as adipocyte cannabinoid receptors in atherosclerosis and related metabolic dysfunctions.

The Endocannabinoid System

The endocannabinoid system is an endogenous lipid signalling system that regulates several pathways in the central nervous system and peripheral tissues. It plays an essential role in the control of food intake and energy expenditure, energy homeostasis, insulin sensitivity, as well as glucose and lipid metabolism and fat accumulation.⁸ The endocannabinoid system comprises endogenous lipid mediators, the G protein-coupled receptors (GPCRs) cannabinoid receptors 1 and 2 (CB1 and CB2) and enzymes involved in endocannabinoid synthesis and inactivation.^{8,10,14,15} In addition, other GPCRs sensitive to endocannabinoids have been described in the last few years, which are GPR18, GPR55 and GPR119.¹⁶ Endocannabinoids are arachidonic acid-derived bioactive lipids including anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These molecules are produced on demand by tissues and circulating immune cells by the cleavage of membrane fatty acids (► Fig. 1). The main synthesizing enzymes are N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) for AEA and diacylglycerol lipase (DAGL) for 2-AG. AEA metabolism is mainly catalysed via fatty acid amide hydrolase (FAAH) and 2-AG via monoacylglycerol lipase (MAGL).^{8,10,14}

There is increasing clinical evidence that enhanced endocannabinoid system activation is associated with metabolic disorders, in particular abdominal obesity, dyslipidemia, insulin resistance, as well as atherosclerosis.¹⁴ Circulating endocannabinoid levels are elevated in patients with coronary artery disease¹⁷ and in obese individuals compared with

lean individuals,¹⁸ and positively correlate with parameters such as body fat and visceral fat mass, as well as with circulating triglyceride levels.^{18–20} There is also a positive correlation between circulating endocannabinoid levels in obese individuals and coronary endothelial and circulatory dysfunction.^{21,22} Moreover, CB1 expression is increased in the myocardium of obese individuals and epicardial adipose tissue from ischaemic subjects as well as in human atherosclerotic plaques of unstable versus stable angina patients.^{11,17,23} The CB1 inverse agonist rimonabant, which was tested and approved for the treatment of obesity, was withdrawn for clinical use due to the elevated incidence of central side effects such as depression.²⁴ Nevertheless, the available clinical data indicate that inhibition of CB1 signalling may improve cardiovascular risk factors in obese patients.^{13,25}

The Endocannabinoid System and Atherosclerosis: Modulation of Endothelial Cell Activation and Leukocyte Recruitment

Atherosclerosis is characterized by a chronic inflammation of the arterial wall with subendothelial plaque formation.²⁶ The healthy endothelium plays a critical role in maintaining the vascular wall homeostasis and circulatory function. It represents a relatively impermeable, anti-thrombotic and anti-adhesive blood–tissue interface, which regulates vascular resistance by releasing vasoactive factors such as nitric oxide (NO). Endothelial NO is not only a vasodilator, but it also inhibits platelet aggregation, leukocyte adhesion, endothelial permeability, low-density lipoprotein (LDL) oxidation and smooth muscle cell proliferation.²⁷ Inflammatory, biochemical and physical factors (including hyperlipidaemia, turbulent flow, high blood pressure and cigarette smoking) induce endothelial dysfunction by inhibiting NO release and increasing endothelial permeability.²⁶ This is the initiating step in the development of atherosclerosis, which leads to the accumulation of LDL and leukocytes in the subendothelial space as a consequence of augmented vessel permeability and up-regulation of endothelial adhesion molecules.²⁶ Within the subendothelial space, the LDL is modified to oxidized LDL (oxLDL) which further triggers the inflammatory process. OxLDL is engulfed by infiltrating monocytes through scavenger receptors such as CD36 or SR-A1 and stored in lipid droplets in the esterified form, which leads to foam cell formation and enhanced release of pro-inflammatory cytokines.²⁸

Role of Classical Cannabinoid Receptors and Endocannabinoids

An increasing number of experimental studies highlight the relevance of endocannabinoid signalling in these early stages of atherosclerosis development (► Fig. 2). Blocking endogenous endocannabinoid signalling by pharmacological CB1 blockade with the CB1 inverse agonist rimonabant improved the endothelium-dependent vascular response in isolated aortic rings of apolipoprotein E knockout (*ApoE*^{−/−}) mice.²⁹ In primary human coronary artery endothelial cells, CB1

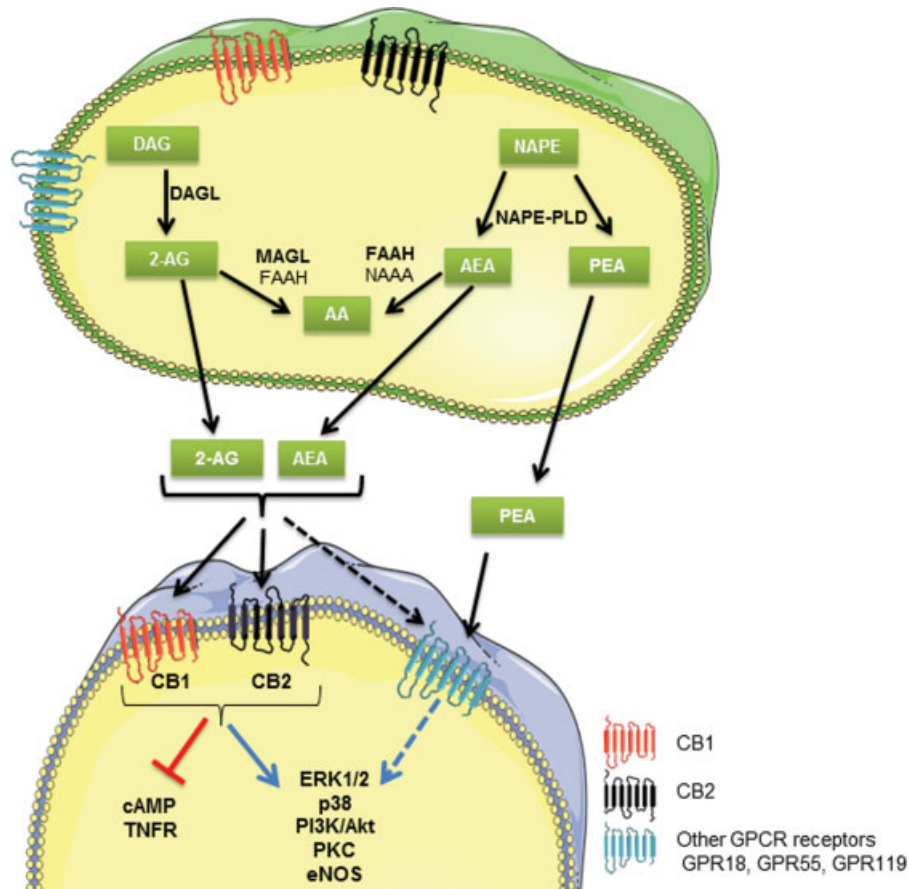


Fig. 1 Main synthesis, degradation and signalling pathways of endocannabinoids. 2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, anandamide; cAMP, cyclic adenosinemonophosphate; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; DAG, diacylglycerol; DAGL, diacylglycerol lipase; eNOS, endothelial nitric oxide synthase; ERK1/2, extracellular signal-regulated kinase 1/2; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAAA, N-acylethanolamine acid amidase; NAPE, N-acylphosphatidylethanolamines; NAPE-PLD, N-acyl phosphatidylethanolamine-specific phospholipase D; P38, p38MAPK subfamily; PEA, palmitoylethanolamide; PI3K/Akt, phosphoinositide 3-kinase/protein kinase B; PKC, protein kinaseC; TNFR, tumor necrosis factor receptor.

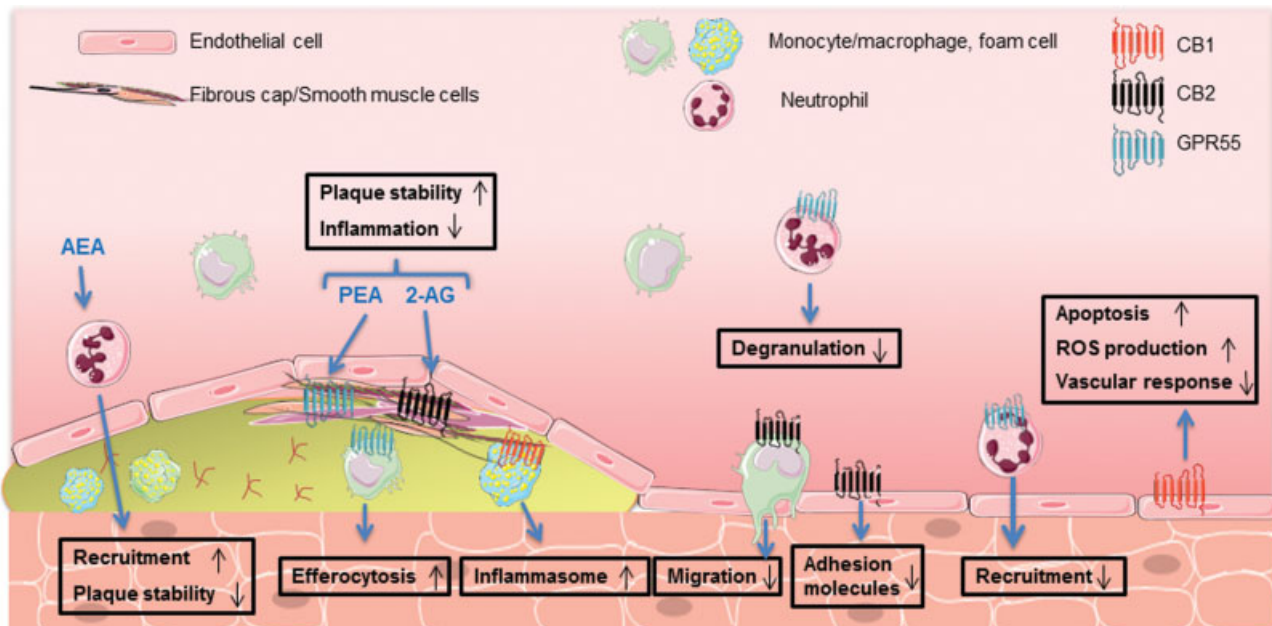


Fig. 2 Effect of endocannabinoids and cannabinoid receptor (CB1, CB2, and GPR55) activation in endothelial cells, monocytes, neutrophils and smooth muscle cells. 2-AG, 2-arachidonoylglycerol; AEA, anandamide; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; GPR55, G protein couple receptor 55; PEA, palmitoylethanolamide.

activation with AEA or a synthetic CB1-selective agonist induced endothelial cell death, reactive oxygen species production and related intracellular signalling pathways, which was attenuated by CB1 antagonists.³⁰ While rimonabant failed to limit atherosclerotic plaque growth in the *ApoE*^{-/-} model,²⁹ its chronic oral administration reduced plaque development in the LDL receptor knockout (*Ldlr*^{-/-}) mouse model of atherosclerosis, accompanied by reduced weight gain and improved plasma lipid profiles.³¹ The different findings might be related to the genetic mouse models, diet composition, rimonabant dosage and/or route of administration.

Synthetic or plant-derived cannabinoids have been shown to exhibit anti-atherogenic properties by limiting plaque macrophage accumulation, pro-inflammatory cytokine release and adhesion molecule expression.³²⁻³⁴ Additional blocking experiments with CB2 antagonists indicated that these anti-inflammatory effects are mediated by CB2 signalling.^{33,34} The atheroprotective role of CB2 was further strengthened by experimental studies employing selective CB2 agonists or mice lacking *Cnr2* (the gene encoding CB2) on *ApoE*^{-/-} as well as *Ldlr*^{-/-} background.^{35,36} One study failed to reproduce the anti-inflammatory effects of CB2 activation in the *Ldlr*^{-/-} mouse model.³⁷ In view of a potential relevance for human pathophysiology, in vitro experiments with primary human coronary endothelial cells and smooth muscle cells confirmed anti-inflammatory and anti-proliferative effects of CB2 stimulation.^{38,39}

In support of a causal role for endocannabinoid signalling in atherosclerosis, experimental data in atherosclerotic mouse models suggest that genetic ablation or pharmacological inhibition of FAAH, the endocannabinoid AEA metabolizing enzyme, may promote the development of unstable plaques^{40,41} and enhanced neointima formation after arterial injury.⁴² The pro-atherogenic effect of the FAAH inhibitor was mediated, at least in part, by an increased arterial neutrophil recruitment (► Fig. 2).⁴⁰ The enhanced neutrophil recruitment to atherosclerotic vessels might be due to the increased chemokine CXCL1 production in plaques of *Faah*^{-/-} mice or mice treated with FAAH inhibitor, respectively, but it may also involve additional mechanisms.

An opposite effect on atherosclerotic plaque development has been reported when targeting the endocannabinoid 2-AG metabolizing enzyme MAGL. Mice with genetic deficiency of *Magl* on *ApoE*^{-/-} background and 9 weeks of Western-type diet feeding developed larger but more stable plaques with increased smooth muscle cell and collagen content as well as thicker fibrous caps, whereas plaque lipid and macrophage content were reduced (► Fig. 2).⁴³ Treatment with a CB2 inverse agonist prevented the observed plaque phenotype in *ApoE*^{-/-} *Magl*^{-/-} mice, suggesting that inhibiting the MAGL pathway exhibits anti-inflammatory effects via enhanced 2-AG/CB2 signalling.⁴³ CB2 is predominantly expressed by immune cells and is considered to play an anti-inflammatory and atheroprotective role.^{32,35,44} The effect of genetic MAGL deficiency on early atherosclerotic plaque formation was not addressed in the initial study.⁴³ Using a pharmacological inhibitor of MAGL, Jehle et al recently reported that inhibiting this enzyme during early atherogenesis promotes plaque

formation.⁴⁵ *ApoE*^{-/-} mice were treated for 4 weeks with the MAGL inhibitor JZL184 in parallel to 4 weeks high-fat diet feeding. The analysis of atherosclerotic plaques in aortic root cross-sections revealed significantly larger plaques and more aortic macrophages in mice treated with MAGL inhibitor compared with vehicle-treated mice.⁴⁵ This seems somewhat conflicting with the reported anti-atherosclerotic effects in *ApoE*^{-/-} *Magl*^{-/-} mice at advanced stage.⁴³ As opposed to Jehle et al, our own recent data support an atheroprotective effect of genetic *Magl* deficiency or pharmacological blockade of this enzyme. In our experimental study, blocking the MAGL pathway during atherosclerosis onset led to CB1 desensitization, which translated into an atheroprotective B1a-IgM phenotype.⁴⁶ The atheroprotective effect was dependent on CB2 signalling, as confirmed in *Cnr2*^{-/-} mice. It is possible that the efficiency of MAGL inhibition (depending on the selected dose, way and frequency of administration) in the study from Jehle et al is less potent than global genetic deficiency of the enzyme or the higher dose of the MAGL inhibitor used in our study. A varying efficiency of MAGL inhibition may differentially affect cannabinoid receptor signalling or cannabinoid receptor-independent actions related to altered eicosanoid levels.⁴⁷ As to a possible modulation of cannabinoid receptor signalling, it has been shown that genetic deficiency or chronic MAGL inhibition results in CB1 desensitization, due to chronically elevated 2-AG levels,^{48,49} and this was also observed in our own experimental atherosclerosis study targeting MAGL.⁴⁶ In support of dose-dependent effects of MAGL inhibition, it has been previously reported that CB1 receptor activity and expression are attenuated following high-dose JZL184 administration (16 or 40 mg/kg), but are maintained at low-dose JZL184 treatment (1.6, 4 or 8 mg/kg).⁵⁰ The putative role of 2-AG in atherosclerosis becomes even more complex when considering the effect of genetic *Dagl* deficiency, the gene encoding a major 2-AG biosynthesizing enzyme. Myeloid cell-specific deletion of the *Dagl* isoform α in the *ApoE*^{-/-} background inhibited atherosclerotic plaque formation.⁵¹ Given that DAGL α is more relevant for brain 2-AG production than DAGL β , while the latter is more important for liver 2-AG release,⁵² it would be interesting to further address the relevance of DAGL β in atherosclerosis, which might be the more relevant isoform in cardiovascular disease. In support of this hypothesis, online accessible murine microarray data from the Immunological Genome Project (www.immgen.org) show much higher expression levels of the DAGL β isoform than DAGL α in myeloid cells.

Possible Role of Cannabinoid-Sensitive Receptor GPR55 in Atherosclerosis

So far, little is known about the contribution of other cannabinoid-sensitive receptors in atherosclerosis. The orphan receptor GPR55 has been proposed as a novel cannabinoid receptor, based on high-affinity binding to synthetic cannabinoid CP55940 in radioactive ligand binding assays with the cloned human receptor.⁵³ GPR55 was also found to exhibit high binding affinities for endogenous ligands, including endocannabinoid-related palmitoylethanolamide (PEA) as well as endocannabinoids AEA and 2-AG.⁵³ However, further

investigation revealed that lysophosphatidylinositols were more potent endogenous ligands than endocannabinoids.⁵⁴ According to quantitative polymerase chain reaction data, GPR55 is highly expressed in the brain, adrenals, small intestine and, to a lower extent, in the spleen.⁵³ The flow cytometric analysis of GPR55 surface levels on human blood leukocytes revealed high receptor expression by monocytes and natural killer cells.⁵⁵ In vitro data with THP1-derived macrophages further suggest a role for GPR55 in macrophage oxLDL accumulation.⁵⁶ Montecucco et al tested the effect of chronic treatment with the GPR55 antagonist CID16020046 on atherosclerosis in *ApoE*^{-/-} mice fed with either a normal chow diet for 16 weeks or a high cholesterol diet for 11 weeks.⁵⁷ Independent of normal or atherogenic diet, the plaque size was not affected by CID16020046 although in both experimental setups intra-plaque MMP-9 was increased, while the collagen content was reduced compared with the vehicle-treated group. Only in mice fed with normal chow diet, which develop less advanced atherosclerotic lesions, treatment with CID16020046 resulted in plaques with higher neutrophil content (►Fig. 2). Mechanistically, they found that the GPR55 antagonist systemically increased the circulating levels of chemokines mediating neutrophil recruitment under normal diet conditions and induced neutrophil degranulation in vitro. These findings may suggest that GPR55 negatively regulates neutrophil chemotaxis and activation through still unknown mechanisms. However, the effects of GPR55 on atherosclerosis might be masked under high-fat diet conditions, at least in this specific experimental study.⁵⁷

More recent findings propose that GPR55 might also play a role at advanced stages of atherosclerosis, by modulating macrophage-resolving properties.⁵⁸ GPR55 is a possible receptor for mediating anti-inflammatory effects of the endocannabinoid-related lipid mediator PEA. In the *ApoE*^{-/-} mouse model, PEA treatment inhibited plaque formation at early stage and promoted signs of plaque stability in pre-established atherosclerosis as evidenced by reduced macrophage accumulation and necrotic core size, increased collagen deposition and down-regulation of pro-inflammatory macrophage markers.⁵⁸ In vitro experiments with bone marrow-derived murine macrophages revealed that PEA increased the expression of the phagocytosis receptor MerTK and enhanced macrophage efferocytosis, which was blunted in *Gpr55*-deficient macrophages obtained from knockout mice.⁵⁸

Endocannabinoid System and Lipid Metabolism

Hepatic and Bile Acid Lipid Metabolism

Mice with genetic deficiency of *Magl* on *ApoE*^{-/-} background not only develop less atherosclerosis, but also have an altered hepatic cholesterol metabolism and lipid-dependent gut transit.⁵⁹ These mice develop less pronounced liver steatosis upon Western-type diet feeding. Under fasting conditions, a reduced level of plasma-free glycerol concentrations and free fatty acids was found in *Magl*^{-/-} *ApoE*^{-/-} mice compared with *ApoE*^{-/-} controls, as well as reduced hepatic triglyceride

levels and reduced very low density lipoprotein (VLDL) secretion. However, there were no changes in fasting plasma levels of triglycerides and total cholesterol, and no effect on body weight after atherogenic diet feeding. These findings suggest that *Magl*-deficient mice exhibit a moderate lipolytic defect. The reduced hepatic lipid levels were not due to altered liver cholesterol synthesis or uptake, but rather a consequence of increased intestinal cholesterol secretion via bile acid and reduced bile acid re-uptake.⁵⁹ The authors suggest an involvement of effects which are independent of CB1, although they did not specifically clarify which receptors are involved in the hepatic effects of *Magl* deficiency. However, it is conceivable that CB1 desensitisation contributes at least partially to the metabolic changes, given that hepatic *Cnr1* mRNA expression was not detectable in *Magl*^{-/-} *ApoE*^{-/-} mice.⁵⁹ This may also explain the absence of *Cnr1* expression in *ApoE*^{-/-} mice fed with atherogenic diet, as a consequence of increased 2-AG endocannabinoid levels.^{40,60} There is additional experimental evidence reporting that hepatic CB1 activation increases bile acid synthesis.⁶¹ Moreover, hepatic CB1 stimulates fatty acid synthesis and thereby mediates hepatic steatosis and related metabolic dysfunction⁶² (►Fig. 3).

Obesity is associated with increased circulating endocannabinoid levels and decreased HDL.⁶ To clarify whether endocannabinoids reduce HDL by inhibiting the expression of its primary structural lipoprotein apolipoprotein A1 (ApoA1), in vitro experiments with human hepatocyte and intestinal epithelial cell lines have been performed. Indeed, treatment of HepG2 and Caco-2 cells with AEA or 2-AG reduced ApoA1 secretion in these cells.⁶³ The endocannabinoid-mediated reduction of ApoA1 provides a mechanistic explanation for the decreased HDL levels in obese individuals. To further address the link between elevated endocannabinoid levels and lipid metabolism, other investigators used a pharmacological in vivo approach to inhibit endocannabinoid metabolism. They found that increased endocannabinoid levels through inhibition of their enzymatic hydrolysis by isopropyl dodecyl-fluorophosphonate resulted in elevated plasma triglyceride levels, which were associated with reduced plasma triglyceride clearance.⁶⁴ The effect was mediated via CB1 signalling, which was confirmed with *Cnr1*^{-/-} mice and additional experiments with CB1 antagonists.

Macrophage Cholesterol Metabolism

OxLDL which accumulates in atherosclerotic plaques is taken up by macrophages via scavenger receptors.²⁸ The cholesterol may be stored as intracellular lipid droplets or transported out of the cell via ABCA1 and ABCG1 to ApoA1 or HDL. This process is named reverse cholesterol transport. In vitro data indicate that oxLDL increases endocannabinoid signalling, which in turn decreases reverse cholesterol transport.⁶⁵ In particular, oxLDL was shown to increase 2-AG and AEA levels as well as CB1 and CB2 expression in RAW264.7 and rat peritoneal macrophages. The synthetic cannabinoid WIN55,212-2 reduced the expression of ABCA1 in RAW264.7 cells, while increasing scavenger receptor CD36. This resulted in increased cellular cholesterol levels in RAW264.7 macrophages. The effect was sensitive to pretreatment with CB1 antagonist

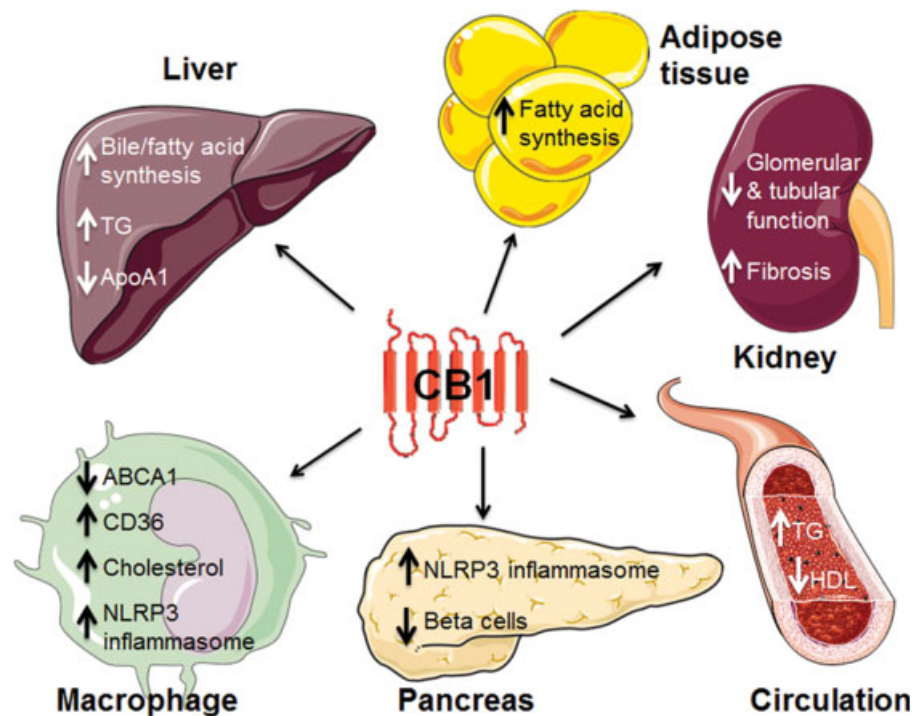


Fig. 3 Peripheral and metabolic effects of CB1 signalling. CB1 signalling affects several peripheral organs. In liver, CB1 activation leads to stimulation of bile/fatty acid synthesis, the accumulation of TG and the reduction of ApoA1 secretion, which result in decreased HDL and increased TG levels in circulation. Besides, CB1 activation stimulates fatty acid synthesis also in adipose tissue. In macrophages, CB1 stimulation causes intracellular cholesterol accumulation as a consequence of increased uptake (CD36) and decreased efflux (ABCA1). In pancreas, CB1 activation in infiltrating macrophages directly activates the NLRP3 inflammasome leading to β cell loss. In the kidney, CB1 signalling in podocytes has been linked to glomerular and tubular dysfunction and fibrosis. ABC, ATP-binding cassette transporter; ApoA1, apolipoprotein A1; CB1, cannabinoid receptor 1; HDL, high-density lipoprotein; NLRP3, NLR family pyrin domain-containing 3; TG, triglycerides.

and inverse agonist AM251, which indicates a possible pathophysiological mechanism by which CB1 signalling may contribute to atherosclerotic plaque inflammation. Macrophage stimulation with the endocannabinoid-related endogenous mediator PEA resulted in an up-regulation of SR-B1 levels, a receptor mediating bidirectional lipid transport in macrophages. This effect was independent of GPR55 and might be mediated by PPAR- α .⁵⁸

Excessive cholesterol accumulation impairs the capacity of macrophages to clear apoptotic cells and may lead to the formation of cholesterol crystals that are able to induce NLRP3 inflammasome activation.⁶⁶ In an experimental model of type 2 diabetes, CB1 was shown to directly activate the NLRP3 inflammasome complex in pancreas-infiltrating macrophages, thereby contributing to β cell loss.⁶⁷ Moreover, impaired clearance of apoptotic cells, leading to secondary necrosis, and extracellular lipid accumulation contribute to the formation of a necrotic core. Secondary necrosis as well as alternative non-apoptotic cell death pathways occurring within advanced lesions further entrain the local inflammatory milieu, thereby contributing to plaque destabilization.⁶⁸ Freeman-Anderson and colleagues investigated the effect of genetic CB2 deficiency on oxLDL-induced apoptosis and found that the apoptosis rate was significantly reduced in peritoneal macrophages lacking CB2.⁶⁹ Mechanistically, the deactivation of the Akt pro-survival pathway was impaired in the absence of CB2 after 7-ketocholesterol exposure,

suggesting that CB2 expression increases the susceptibility of macrophages to oxLDL-induced apoptosis. However, a potential relevance of this mechanism in atherosclerotic plaque macrophages has not been confirmed thus far.

Evidence for a Peripheral Endocannabinoid Regulation of Lipid and Glucose Metabolism

Peripherally active CB1 antagonists have been shown to improve metabolic disorders in mouse models without blocking central CB1 receptor signalling.^{67,70–73} These drugs do not pass the blood–brain barrier and therefore are devoid of centrally mediated psychiatric side effects, such as anxiety and depression.^{74,75} In addition, experimental evidence based on cell-specific genetic deficiency of CB1 receptors is emerging that strengthens the relevance of peripheral endocannabinoid regulation in metabolic functions (**Fig. 3**). Liver-specific *Cnr1* deficiency blunted glucocorticoid-induced dyslipidemia, but not the obesity phenotype.⁷¹ More recently, inducible adipocyte-specific *Cnr1* deficiency was shown to be sufficient for protecting mice from diet-induced obesity and associated metabolic alterations. Moreover, induction of adipocyte-specific *Cnr1* deficiency in mice with already established obesity reversed the phenotype in these mice.⁷⁶ Podocyte-specific deletion of *Cnr1* prevented glomerular and tubular dysfunction in a mouse model of diabetic nephropathy.⁷⁷ As mentioned above, CB1 signalling in pancreatic macrophages induces β cell

loss in type 2 diabetes by activating the NLRP3 inflammasome complex.⁶⁷ A limitation is that the experimental in vivo evidence in this study was solely based on combined treatment with peripherally active CB1 antagonists and clodronate liposomes for macrophage depletion or siRNA-mediated *Cnr1* knockdown, respectively.

Conclusion

Emerging evidence suggests that enhanced endocannabinoid signalling affects atherosclerosis via multiple effects, including a modulation of vascular inflammation, leukocyte recruitment and macrophage cholesterol metabolism, which influences atherosclerotic plaque stability. A few experimental studies revealed somewhat conflicting findings, which might be partly related to the pharmacology of available cannabinoid compounds, which can act as agonists, partial agonists, inverse agonists or antagonists for several cannabinoid receptors.¹⁵ The biological response to the stimulation with a synthetic cannabinoid receptor agonist also depends on the presence of endogenously produced ligands in response to cellular activation. Despite some controversy in pro- or anti-inflammatory effects of inhibiting selective enzyme pathways of endocannabinoid metabolism, there is overwhelming evidence for a pathophysiological role of excessive CB1 activation by endocannabinoids in atherosclerosis and related metabolic complications. Thus, blocking CB1 signalling in the vasculature and peripheral organs might represent a promising therapeutic target in atherosclerosis. As opposed to other emerging anti-inflammatory drugs for the treatment of atherosclerosis, blocking CB1 signalling might offer the advantage to exhibit multiple anti-atherogenic actions by inhibiting vascular inflammation and improving metabolic risk factors.

Funding

This work was supported by grants from the Deutsche Forschungsgemeinschaft STE1053/6–1 to S. Steffens, SFB1123 TP B09 to S. Steffens and S. Herzig, and SFB1118 TP A01 to S. Herzig.

Conflict of Interest

None declared.

References

- Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011–2012. *JAMA* 2014; 311(08):806–814
- Inoue Y, Qin B, Poti J, Sokol R, Gordon-Larsen P. Epidemiology of obesity in adults: latest trends. *Curr Obes Rep* 2018;7(04):276–288
- Finucane MM, Stevens GA, Cowan MJ, et al; Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group (Body Mass Index). National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9.1 million participants. *Lancet* 2011;377(9765):557–567
- Collaboration NRF; NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* 2016;387(10026):1377–1396
- World Health Organization. Obesity and overweight. Available at: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>
- Tchernof A, Després JP. Pathophysiology of human visceral obesity: an update. *Physiol Rev* 2013;93(01):359–404
- Skinner AC, Perrin EM, Moss LA, Skelton JA. Cardiometabolic risks and severity of obesity in children and young adults. *N Engl J Med* 2015;373(14):1307–1317
- Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. *Nat Neurosci* 2005;8(05):585–589
- Szmitko PE, Verma S. The endocannabinoid system and cardiometabolic risk. *Atherosclerosis* 2008;199(02):248–256
- Pacher P, Steffens S. The emerging role of the endocannabinoid system in cardiovascular disease. *Semin Immunopathol* 2009;31(01):63–77
- Valenta I, Varga ZV, Valentine H, et al. Feasibility evaluation of myocardial cannabinoid type 1 receptor imaging in obesity: a translational approach. *JACC Cardiovasc Imaging* 2018;11(2, Pt 2):320–332
- van Eenige R, van der Stelt M, Rensen PCN, Kooijman S. Regulation of adipose tissue metabolism by the endocannabinoid system. *Trends Endocrinol Metab* 2018;29(05):326–337
- Di Marzo V, Després JP. CB1 antagonists for obesity—what lessons have we learned from rimonabant? *Nat Rev Endocrinol* 2009;5(11):633–638
- Pacher P, Steffens S, Haskó G, Schindler TH, Kunos G. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. *Nat Rev Cardiol* 2018;15(03):151–166
- Console-Bram L, Marcu J, Abood ME. Cannabinoid receptors: nomenclature and pharmacological principles. *Prog Neuropsychopharmacol Biol Psychiatry* 2012;38(01):4–15
- Irving A, Abdulrazzaq G, Chan SLF, Penman J, Harvey J, Alexander SPH. Cannabinoid receptor-related Orphan G protein-coupled receptors. *Adv Pharmacol* 2017;80:223–247
- Sugamura K, Sugiyama S, Nozaki T, et al. Activated endocannabinoid system in coronary artery disease and antiinflammatory effects of cannabinoid 1 receptor blockade on macrophages. *Circulation* 2009;119(01):28–36
- Engeli S, Böhnke J, Feldpausch M, et al. Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 2005;54(10):2838–2843
- Blüher M, Engeli S, Klötting N, et al. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. *Diabetes* 2006;55(11):3053–3060
- Côté M, Matias I, Lemieux I, et al. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. *Int J Obes* 2007;31(04):692–699
- Quercioli A, Pataky Z, Montecucco F, et al. Coronary vasomotor control in obesity and morbid obesity: contrasting flow responses with endocannabinoids, leptin, and inflammation. *JACC Cardiovasc Imaging* 2012;5(08):805–815
- Quercioli A, Pataky Z, Vincenti G, et al. Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. *Eur Heart J* 2011;32(11):1369–1378
- Cappellano G, Uberti F, Caimmi PP, et al. Different expression and function of the endocannabinoid system in human epicardial adipose tissue in relation to heart disease. *Can J Cardiol* 2013;29(04):499–509
- Pacher P. Cannabinoid CB1 receptor antagonists for atherosclerosis and cardiometabolic disorders: new hopes, old concerns? *Arterioscler Thromb Vasc Biol* 2009;29(01):7–9
- Van Gaal LF, Scheen AJ, Rissanen AM, Rössner S, Hanotin C, Ziegler O; RIO-Europe Study Group. Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. *Eur Heart J* 2008;29(14):1761–1771
- Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011;473(7347):317–325
- Li H, Förstermann U. Nitric oxide in the pathogenesis of vascular disease. *J Pathol* 2000;190(03):244–254

- 28 Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: a dynamic balance. *Nat Rev Immunol* 2013;13(10):709–721
- 29 Tiyerili V, Zimmer S, Jung S, et al. CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. *Basic Res Cardiol* 2010;105(04):465–477
- 30 Mukhopadhyay P, Pan H, Rajesh M, et al. CB1 cannabinoid receptors promote oxidative/nitrosative stress, inflammation and cell death in a murine nephropathy model. *Br J Pharmacol* 2010;160(03):657–668
- 31 Dol-Gleizes F, Paumelle R, Visentin V, et al. Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2009;29(01):12–18
- 32 Steffens S, Veillard NR, Arnaud C, et al. Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature* 2005;434(7034):782–786
- 33 Zhao Y, Liu Y, Zhang W, et al. WIN55212-2 ameliorates atherosclerosis associated with suppression of pro-inflammatory responses in ApoE-knockout mice. *Eur J Pharmacol* 2010;649(1–3):285–292
- 34 Zhao Y, Yuan Z, Liu Y, et al. Activation of cannabinoid CB2 receptor ameliorates atherosclerosis associated with suppression of adhesion molecules. *J Cardiovasc Pharmacol* 2010;55(03):292–298
- 35 Hoyer FF, Steinmetz M, Zimmer S, et al. Atheroprotection via cannabinoid receptor-2 is mediated by circulating and vascular cells in vivo. *J Mol Cell Cardiol* 2011;51(06):1007–1014
- 36 Netherland CD, Pickle TG, Bales A, Thewke DP. Cannabinoid receptor type 2 (CB2) deficiency alters atherosclerotic lesion formation in hyperlipidemic Ldlr-null mice. *Atherosclerosis* 2010;213(01):102–108
- 37 Willecke F, Zeschky K, Ortiz Rodriguez A, et al. Cannabinoid receptor 2 signaling does not modulate atherogenesis in mice. *PLoS One* 2011;6(04):e19405
- 38 Rajesh M, Mukhopadhyay P, Bátkai S, et al. CB2-receptor stimulation attenuates TNF-alpha-induced human endothelial cell activation, transendothelial migration of monocytes, and monocyte-endothelial adhesion. *Am J Physiol Heart Circ Physiol* 2007;293(04):H2210–H2218
- 39 Rajesh M, Mukhopadhyay P, Haskó G, Huffman JW, Mackie K, Pacher P. CB2 cannabinoid receptor agonists attenuate TNF-alpha-induced human vascular smooth muscle cell proliferation and migration. *Br J Pharmacol* 2008;153(02):347–357
- 40 Lenglet S, Thomas A, Soehnlein O, et al. Fatty acid amide hydrolase deficiency enhances intraplaque neutrophil recruitment in atherosclerotic mice. *Arterioscler Thromb Vasc Biol* 2013;33(02):215–223
- 41 Hoyer FF, Khoury M, Slomka H, et al. Inhibition of endocannabinoid-degrading enzyme fatty acid amide hydrolase increases atherosclerotic plaque vulnerability in mice. *J Mol Cell Cardiol* 2014;66:126–132
- 42 Molica F, Burger F, Thomas A, et al. Endogenous cannabinoid receptor CB1 activation promotes vascular smooth-muscle cell proliferation and neointima formation. *J Lipid Res* 2013;54(05):1360–1368
- 43 Vujic N, Schlager S, Eichmann TO, et al. Monoglyceride lipase deficiency modulates endocannabinoid signaling and improves plaque stability in ApoE-knockout mice. *Atherosclerosis* 2016;244:9–21
- 44 Montecucco F, Di Marzo V, da Silva RF, et al. The activation of the cannabinoid receptor type 2 reduces neutrophilic protease-mediated vulnerability in atherosclerotic plaques. *Eur Heart J* 2012;33(07):846–856
- 45 Jehle J, Schöne B, Bagheri S, et al. Elevated levels of 2-arachidonoylglycerol promote atherogenesis in ApoE-/- mice. *PLoS One* 2018;13(05):e0197751
- 46 Guillamat Prats R, Rami M, Ring L, et al. Deficiency of monoacylglycerol lipase enhances IgM plasma levels and limits atherogenesis in a CB2-dependent manner. *Thromb Haemost* 2019;119(02):348–351
- 47 Grabner GF, Zimmermann R, Schicho R, Taschler U. Monoglyceride lipase as a drug target: at the crossroads of arachidonic acid metabolism and endocannabinoid signaling. *Pharmacol Ther* 2017;175:35–46
- 48 Schlosburg JE, Blankman JL, Long JZ, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* 2010;13(09):1113–1119
- 49 Taschler U, Eichmann TO, Radner FPW, et al. Monoglyceride lipase deficiency causes desensitization of intestinal cannabinoid receptor type 1 and increased colonic μ -opioid receptor sensitivity. *Br J Pharmacol* 2015;172(17):4419–4429
- 50 Kinsey SG, Wise LE, Ramesh D, et al. Repeated low-dose administration of the monoacylglycerol lipase inhibitor JZL184 retains cannabinoid receptor type 1-mediated antinociceptive and gastroprotective effects. *J Pharmacol Exp Ther* 2013;345(03):492–501
- 51 Jehle J, Hoyer FF, Schöne B, et al. Myeloid-specific deletion of diacylglycerol lipase α inhibits atherogenesis in ApoE-deficient mice. *PLoS One* 2016;11(01):e0146267
- 52 Gao Y, Vasilyev DV, Goncalves MB, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J Neurosci* 2010;30(06):2017–2024
- 53 Ryberg E, Larsson N, Sjögren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152(07):1092–1101
- 54 Ross RA. L- α -lysophosphatidylinositol meets GPR55: a deadly relationship. *Trends Pharmacol Sci* 2011;32(05):265–269
- 55 Chiurchiù V, Lanuti M, De Bardi M, Battistini L, Maccarrone M. The differential characterization of GPR55 receptor in human peripheral blood reveals a distinctive expression in monocytes and NK cells and a proinflammatory role in these innate cells. *Int Immunol* 2015;27(03):153–160
- 56 Lanuti M, Talamonti E, Maccarrone M, Chiurchiù V. Activation of GPR55 receptors exacerbates oxLDL-induced lipid accumulation and inflammatory responses, while reducing cholesterol efflux from human macrophages. *PLoS One* 2015;10(05):e0126839
- 57 Montecucco F, Bondarenko AI, Lenglet S, et al. Treatment with the GPR55 antagonist CID16020046 increases neutrophil activation in mouse atherogenesis. *Thromb Haemost* 2016;116(05):987–997
- 58 Rinne P, Guillamat-Prats R, Rami M, et al. Palmitoylethanolamide promotes a proresolving macrophage phenotype and attenuates atherosclerotic plaque formation. *Arterioscler Thromb Vasc Biol* 2018;38(11):2562–2575
- 59 Vujic N, Korbelius M, Leopold C, et al. Monoglyceride lipase deficiency affects hepatic cholesterol metabolism and lipid-dependent gut transit in ApoE-/- mice. *Oncotarget* 2017;8(20):33122–33136
- 60 Montecucco F, Matias I, Lenglet S, et al. Regulation and possible role of endocannabinoids and related mediators in hypercholesterolemic mice with atherosclerosis. *Atherosclerosis* 2009;205(02):433–441
- 61 Chanda D, Kim YH, Li T, et al. Hepatic cannabinoid receptor type 1 mediates alcohol-induced regulation of bile acid enzyme genes expression via CREBH. *PLoS One* 2013;8(07):e68845
- 62 Osei-Hyiaman D, DePetrillo M, Pacher P, et al. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J Clin Invest* 2005;115(05):1298–1305
- 63 Haas MJ, Mazza AD, Wong NC, Mooradian AD. Inhibition of apolipoprotein A-I gene expression by obesity-associated endocannabinoids. *Obesity (Silver Spring)* 2012;20(04):721–729
- 64 Ruby MA, Nomura DK, Hudak CS, et al. Overactive endocannabinoid signaling impairs apolipoprotein E-mediated clearance of triglyceride-rich lipoproteins. *Proc Natl Acad Sci U S A* 2008;105(38):14561–14566
- 65 Jiang LS, Pu J, Han ZH, Hu LH, He B. Role of activated endocannabinoid system in regulation of cellular cholesterol metabolism in macrophages. *Cardiovasc Res* 2009;81(04):805–813

- 66 Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010;464(7293):1357–1361
- 67 Jourdan T, Godlewski G, Cinar R, et al. Activation of the Nlrp3 inflammasome in infiltrating macrophages by endocannabinoids mediates beta cell loss in type 2 diabetes. *Nat Med* 2013;19(09):1132–1140
- 68 Rayner KJ. Cell death in the vessel wall: the good, the bad, the ugly. *Arterioscler Thromb Vasc Biol* 2017;37(07):e75–e81
- 69 Freeman-Anderson NE, Pickle TG, Netherland CD, Bales A, Buckley NE, Thewke DP. Cannabinoid (CB2) receptor deficiency reduces the susceptibility of macrophages to oxidized LDL/oxysterol-induced apoptosis. *J Lipid Res* 2008;49(11):2338–2346
- 70 Tam J, Vemuri VK, Liu J, et al. Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity. *J Clin Invest* 2010;120(08):2953–2966
- 71 Bowles NP, Karatsoreos IN, Li X, et al. A peripheral endocannabinoid mechanism contributes to glucocorticoid-mediated metabolic syndrome. *Proc Natl Acad Sci U S A* 2015;112(01):285–290
- 72 Jourdan T, Szanda G, Rosenberg AZ, et al. Overactive cannabinoid 1 receptor in podocytes drives type 2 diabetic nephropathy. *Proc Natl Acad Sci U S A* 2014;111(50):E5420–E5428
- 73 Gruden G, Barutta F, Kunos G, Pacher P. Role of the endocannabinoid system in diabetes and diabetic complications. *Br J Pharmacol* 2016;173(07):1116–1127
- 74 Silvestri C, Di Marzo V. Second generation CB1 receptor blockers and other inhibitors of peripheral endocannabinoid overactivity and the rationale of their use against metabolic disorders. *Expert Opin Investig Drugs* 2012;21(09):1309–1322
- 75 Kunos G, Osei-Hyiaman D, Bátkai S, Sharkey KA, Makriyannis A. Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends Pharmacol Sci* 2009;30(01):1–7
- 76 Ruiz de Azua I, Mancini G, Srivastava RK, et al. Adipocyte cannabinoid receptor CB1 regulates energy homeostasis and alternatively activated macrophages. *J Clin Invest* 2017;127(11):4148–4162
- 77 Jourdan T, Park JK, Varga ZV, et al. Cannabinoid-1 receptor deletion in podocytes mitigates both glomerular and tubular dysfunction in a mouse model of diabetic nephropathy. *Diabetes Obes Metab* 2018;20(03):698–708