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Dysregulated cellular metabolism in atherosclerosis: mediators and therapeutic opportunities

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Abstract

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C.S., F.S.N. and B.C. contributed equally to the drafting of the manuscript. A.C.F. provided comments on specific sections. M.A.S., K.L., O.R. and A.Y.J. reviewed and edited the manuscript. A.Y.J. oversaw the development of the Review, incorporating comments, revisions and edits. All authors contributed to the Review and approved the final version for publication.

Competing interests

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Accumulating evidence over the past decades has revealed an intricate relationship between dysregulation of cellular metabolism and the progression of atherosclerotic cardiovascular disease. However, an integrated understanding of dysregulated cellular metabolism in atherosclerotic cardiovascular disease and its potential value as a therapeutic target is missing. In this Review, we (1) summarize recent advances concerning the role of metabolic dysregulation during atherosclerosis progression in lesional cells, including endothelial cells, vascular smooth muscle cells, macrophages and T cells; (2) explore the complexity of metabolic cross-talk between these lesional cells; (3) highlight emerging technologies that promise to illuminate unknown aspects of metabolism in atherosclerosis; and (4) suggest strategies for targeting these underexplored metabolic alterations to mitigate atherosclerosis progression and stabilize rupture-prone atheromas with a potential new generation of cardiovascular therapeutics.

Atherosclerotic cardiovascular disease (ASCVD) progressively develops over decades and is characterized by chronic, non-resolving inflammation initiated by the accumulation of ApoB-containing lipoproteins[1]. Among the leading metabolic pathways driving ASCVD progression are imbalances in lipid metabolism, glucose utilization and amino acid metabolism[2–4] (Fig. 1). Dysregulations in these pathways elicit a panoply of pro-atherogenic responses arising from an intricate interplay between endothelial cells (ECs), vascular smooth muscle cells (vSMCs) and innate and adaptive immune cells (for example, monocyte-derived macrophages and lymphocytes)[5]. As the disease progresses, the lipid-rich plaque expands and eventually becomes prone to rupture or superficial erosion, resulting in thromboembolic events, such as myocardial infarction and stroke[6,7]. Despite optimal lipid-lowering treatment with statins and PCSK9-blocking antibodies[8], ASCVD remains the leading cause of morbidity and mortality worldwide[9]. Dysregulation of metabolic pathways in lesional cells that augment inflammation and hamper its resolution contribute substantially to this residual risk[10–13]. This Review explores recently identified cellular and metabolic pathways that contribute to atherosclerosis, their associated molecular mechanisms and the consequences thereof. We further highlight critical gaps in the field being addressed with emerging technologies and newly identified preclinical strategies aimed at optimizing cellular metabolism.

ECs

EC activation as an initiating factor in atherosclerosis

Before the appearance of foam cells and the formation of fatty streaks, disturbed ‘fluid shear stress’ (FSS), characterized by low magnitudes in blood flow with complex changes in direction at curvatures, branch-points and bifurcations, drives EC activation[14,15]. ECs in these regions exhibit increased inflammatory gene expression (for example, intercellular adhesion molecule 1 (*ICAM1*) and vascular cell adhesion molecule 1 (*VCAM1*)), enhanced leukocyte recruitment, and paracellular permeability[16] (Fig. 1). ApoB-containing lipoproteins accumulate in areas of paracellular pores or are transported across ECs at sites of disturbed flow via transcytosis[17–19]. These positively charged lipoproteins are retained within the negatively charged, proteoglycan-rich subendothelial matrix, where they undergo various modifications (for example, oxidation, glycation and aggregation)[20,21]. Subsequently, recruited monocyte-derived macrophages take up these modified lipoproteins

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and transform into foam cells (Fig. 1). Notably, foam cell formation compromises their beneficial immune functions, hampers their ability to clear dying cells and enhances their susceptibility to cell death[22–24].

EC metabolism in atherosclerosis

The vasculature responds to tissue metabolic demands through homeostatic processes predominantly orchestrated by the endothelium. This regulation determines vascular network formation during embryonic development and adult angiogenesis, vessel diameter and tone, transendothelial transport of macromolecules and micro-molecules, and inflammation[25]. ECs possess relatively low mitochondrial mass and generate most of their ATP via glycolysis[26], converting glucose to lactate (Fig. 2). However, most of these studies were conducted under static conditions, not considering the role of FSS that ECs experience continuously in vivo. Therefore, a comprehensive understanding of EC metabolism requires studying the effects of FSS.

Mechanical forces and EC metabolism.—Atherosclerosis preferentially forms at sites of disturbed FSS, as noted above. Recent evidence links disturbed FSS with early EC activation and metabolic dysregulation, enhanced oxidative stress, paracellular permeability and impaired nutrient trafficking. Disturbed FSS promotes aerobic glycolysis, whereby glucose is broken down in the presence of oxygen to produce lactate (Fig. 2), modulating mechanosensitive Yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) complex activation[27] (Fig. 3a), which reciprocally perpetuates glycolysis[28]. Consistently, inhibiting YAP/TAZ mitigates atherosclerosis progression[29]. Additionally, disturbed FSS promotes glycolysis through the hypoxia-inducing factor-1α (HIF1α)-mediated upregulation of glycolysis-related genes, contributing to EC activation[30] (Fig. 3a). Mechanistically, HIF1α expression is mediated by disturbed FSS-induced AMP-activated protein kinase (AMPK) activation[31] (Fig. 3a). Surprisingly, EC deletion of AMPK increases endothelial permeability and inflammatory responses and augments atherosclerosis progression[31], underscoring the complexity between mechanical forces and cellular metabolism.

Conversely, in straight regions of arteries where FSS caused by blood flow is high and unidirectional, termed ‘unilaminar FSS’, pro-inflammatory, prothrombotic and oxidative stress-inducing pathways are suppressed. Unilaminar FSS induces the transcription factors Kruppel-like factor (KLF) 2 and 4, which have a major role in preventing atherosclerosis at these sites[32]. KLF2 and KLF4 drive the expression of endothelial nitric oxide synthase (eNOS) to generate nitric oxide (NO)[32] (Fig. 2), induce thrombomodulin to limit thrombosis, and suppress nuclear factor κ-light-chain-enhanced of activated B cells (NF-κB) activation. Mechanistically, unilaminar FSS induces KLF2/KLF4 through the MEKK2/MEKK3–MEK5–ERK5 signalling cascade that mediates the binding of myocyte enhancer factor 2 (MEF2) to KLF2/KLF4 gene regulatory elements[32] (Fig. 3a). Interestingly, transcriptomic profiling revealed that unilaminar FSS reduces the expression of key glycolytic enzymes, such as 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*PFKFB3*), phosphofructokinase-1 (*PFKM*) and hexokinase 2 (*HK2*), and inhibits glycolysis in a KLF2-dependent manner[33]. Unilaminar FSS also stimulates mitochondrial oxidative

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phosphorylation (OXPHOS), leading to a moderate increase in reactive oxygen species production[34]. This rise in reactive oxygen species initiates mitophagy, which, in turn, facilitates the assembly of a protein complex that scaffolds the ERK5 signalling axis to amplify KLF2-mediated eNOS expression[34] (Fig. 3a). These insights linking the mechanosensitive and metabolic responses of ECs are crucial in developing targeted therapies for atherosclerosis.

Lipids and EC activation.—The endothelium also contributes to atherosclerosis through fatty acid uptake following hydrolysis of circulating lipoproteins by endothelial lipase[35]. Rate-limiting steps in fatty acid uptake include mitochondrial ATP production and activity of fatty acid transport proteins 3 and 4 (FATP3 and FATP4)[36,37]. Acyl-CoA synthetase long-chain 1 (ACSL1) subsequently converts long-chain fatty acids into fatty acyl-CoA esters that are either subjected to β-oxidation or stored in lipid droplets[38]. Low-density lipoprotein (LDL) particles are internalized by the LDL receptor (LDLR), cluster of differentiation 36 (CD36), activin receptor-like kinase 1 (ALK1) and scavenger receptor class B type 1 (SR-B1)[19,37,39]. Interestingly, uptake via LDLR favours utilization, whereas uptake via ALK1 or SR-B1 is coupled with transcytosis and subendothelial LDL accumulation[19,39]. Modification of subendothelial LDL promotes EC activation in the oxidative plaque microenvironment[40]. Mechanistically, bioactive lysophosphatidic acid, a cleavage product of lysophosphatidylcholine derived from oxidized LDL (oxLDL), binds the endothelial receptors LPA1 and LPA3 and drives CXCL1-mediated monocyte recruitment[41], further linking metabolic pathways and plaque inflammation.

Metabolism controls EndMT.—The transition of ECs to mesenchymal cells, termed the 'endothelial-to-mesenchymal transition' (EndMT), contributes to atherosclerosis progression and plaque instability[42,43]. Suppression of transforming growth factor-β receptor 1 and 2 (TGFBR1 and TGFBR2) signalling, essential for EndMT (Fig. 3a), not only halts the progression of atherosclerosis but also induces its regression[44]. Recent studies have demonstrated that metabolic reprogramming drives EndMT[45]. In this setting, acetate is increased via its atypical production from glucose and then converted into cytosolic acetyl-CoA by ACSS2 (ref. 45). This leads to SMAD2, SMAD4 and ALK5 acetylation, prolonging their half-life and sustaining TGFβ signalling and EndMT[45]. Importantly, EC-specific deletion of ACSS2 hampers EndMT and reduces atherosclerosis in male and female mice[45].

vSMCs

vSMCs in plaque stability

vSMCs have a crucial role in plaque stability by forming the collagen-rich fibrous cap that overlies clinically dangerous necrotic cores[46–48] (Fig. 1). Emerging evidence using fate-mapping and single-cell transcriptomics has demonstrated that neointimal vSMCs arise from either ECs undergoing EndMT or the dedifferentiation and selective expansion of medial vSMCs[49–51]. Dedifferentiation of vSMCs requires the downregulation of myocardin-mediated expression of contractile and other vSMC-related genes (for example, *MYH11* and *ACTA2*)[52], which are strongly diminished in the vast majority of vSMCs present in atherosclerotic plaques[53,54]. However, these dedifferentiated vSMCs can simultaneously

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acquire genes resembling myofibroblasts[55,56] and macrophages[53,56,57]. Additionally, vSMCs also contribute to vascular calcification due to their transition to chondrocyte-like and osteoblast-like cells[58,59]. Notably, the presence of vascular calcification has emerged as a superior predictor of cardiovascular events and cardiovascular-related mortality, surpassing all other factors or risk equations to date[60,61].

Cellular metabolism guides phenotypic modulation of vSMCs Glucose utilization controls vSMC phenotype.

—Recent studies interrogating the complex metabolic interactions within atherosclerotic plaques have uncovered a critical role for glucose uptake and glycolysis in controlling vSMC dedifferentiation[62]. The soluble mediators platelet-derived growth factor increase GLUT1-mediated glucose uptake and glycolysis in vSMCs[63], leading to their dedifferentiation, proliferation and migration (Fig. 3b)[63]. Enhancing glycolysis via GLUT1 overexpression in vSMCs accelerates atherosclerosis in male and female mice that exhibit features resembling metabolic syndrome in humans[64]. Furthermore, deletion of pyruvate kinase muscle isozyme M2 (PKM2), which catalyses the final step in glycolysis, inhibited platelet-derived growth factor-induced vSMC proliferation and migration and hampered neointimal hyperplasia[65,66]. Single-cell RNA sequencing (scRNA-seq) revealed that vSMC dedifferentiation during atherosclerosis requires metabolic reprogramming towards glycolysis[67]. This was supported by unbiased network preservation analysis using vSMCs isolated from a multi-ethnic cohort of 151 heart transplant donors[68]. Also, mass spectrometry imaging (MSI) revealed elevated lactate in unstable human atheromas compared to stable plaques[69]. This is particularly interesting considering that vSMCs residing in a microenvironment rich in lactate show a more synthetic phenotype[70]. Although these studies point to a critical role for glycolysis in vSMC phenotypic modulation, a shift in glucose utilization towards the hexosamine biosynthesis pathway and the pentose phosphate pathway (PPP) is also observed (Fig. 2)[71]. The PPP has a crucial role in maintaining cellular redox balance via the production of nicotinamide adenine dinucleotide phosphate (NADPH)[72,73]. Activation of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the PPP (Fig. 2), mitigates vSMC apoptosis by maintaining redox homeostasis[72,73] (Fig. 3b). Given that vSMC apoptosis accelerates features of plaque instability[74], enhancing glucose-6-phosphate dehydrogenase-mediated flux through the PPP may offer a novel strategy for stabilizing rupture-prone atheromas.

The mammalian target of rapamycin complex 1 (mTORC1) is a pivotal regulator of nutrient sensing and metabolism and has a central role in vSMC phenotypic modulation[75]. This complex comprises the core catalytic subunit, mTOR, and the regulatory associated subunit, Raptor[75]. Conditional deletion of Raptor in vSMCs, essential for mTORC1 activation, impairs relaxation and contractility in the aorta accompanied by alterations in autophagic signalling[76]. mTORC1 activation is suppressed by the tuberous sclerosis complex (TSC), and deletion of TSC1 leads to chronic mTORC1 hyperactivation. Interestingly, chronic mTORC1 activation in vSMCs achieved by vSMC-specific TSC1 deletion diminished vSMC contractile genes and led to a degradative vSMC phenotype that caused extracellular matrix proteolysis and progressive aortic disease[77]. These studies highlight the critical role of mTORC1 in vascular health and disease.

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vSMC amino acid metabolism in plaque stability.—Arginine metabolism has a central role in vSMC phenotypic modulation and plaque stability. Through the NOS family of enzymes, including nNOS, iNOS and eNOS, arginine metabolism leads to the generation of citrulline and NO[78] (Fig. 2). EC-generated NO stimulates vasodilation via cyclic GMP-mediated activation of soluble guanylate cyclase in vSMCs, and vSMC-generated NO operates as a free radical that inhibits proliferation and migration and promotes apoptosis[79,80] (Fig. 3b). NOS enzymes and arginases compete for available arginine. Thus, flux through arginase 1 (ARG1) limits NO production and promotes survival, proliferation and collagen synthesis while inhibiting lipopolysaccharide (LPS)-induced inflammation[79–81] (Figs. 2 and 3b). Furthermore, ARG1 overexpression in vivo, which substantially increases lesional polyamines (small, positively charged molecules integral to crucial cellular processes), enhances vSMC proliferation, drives the production of pro-resolving cytokines and promotes features associated with plaque stability[82]. Treatment with the polyamine spermidine mitigates necrotic core expansion by stimulating vSMC autophagy and preventing lipid accumulation, supporting polyamine synthesis as an important mediator[83].

In addition to altered arginine metabolism, dysregulated tryptophan metabolism in vSMCs affects vascular calcification and atherosclerosis (Fig. 3b). Importantly, individuals with coronary artery calcification show evidence of altered tryptophan metabolism[84]. Because lesional vSMCs adopt osteoblast-like properties during atherosclerosis, dysregulation in tryptophan metabolism probably contributes to vSMC-mediated vascular calcification. Indeed, vSMC-specific deletion of the tryptophan-catabolizing enzyme, indoleamine 2,3-dioxygenase 1 (IDO1), increases runt-related transcription factor 2 (RUNX2) expression and exacerbates vascular calcification, which can be reversed by administering the IDO1 product kynurenine[84] (Fig. 3b). By activating the aryl hydrocarbon receptor, kynurenine limits the transition of vSMCs to chondrocytes and preserves the integrity of the fibrous cap[85]. However, observational studies in humans have demonstrated inconsistent correlations between the tryptophan–kynurenine pathway to ASCVD and stroke[86–88]. Additionally, the tryptophan metabolite 3-hydroxyanthranilic acid activates NF-κB and increases MMP2 expression in vSMCs[89]. Furthermore, 3-hydroxyanthranilic acid treatment in mice augments the formation of abdominal aortic aneurysms[89]. Although these insights into the metabolic reprogramming of vSMCs highlight their role in atherosclerosis, the exact implications of tryptophan metabolism remain to be fully resolved.

Macrophage immunometabolism

Macrophages show remarkable plasticity in their function and occupy a range of phenotypes that extend from pro-inflammatory to pro-resolving states[90,91]. This plasticity is essential as certain phenotypes are adapted for combating acute infections, and others aid in tissue repair. A plethora of studies have established a link between the metabolic activities of macrophages and their phenotypic states. For instance, pro-inflammatory macrophages display increased aerobic glycolysis, flux through the PPP, and iNOS-mediated NO production[92–95] (Fig. 2). By contrast, pro-resolving macrophages are characterized by sustained tricarboxylic acid (TCA) cycle flux, elevated fatty acid β-oxidation (FAO), increased oxygen consumption, enhanced glutaminolysis and polyamine biosynthesis[92,93,96]

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(Fig. 2). Importantly, the balance between pro-inflammatory and pro-resolving macrophages in atherosclerotic plaques has important consequences in their clinical manifestations, as they can either drive plaque instability or stabilize rupture-prone atheromas. As discussed below, metabolic reprogramming of lesional macrophages is highly dynamic, and emerging studies suggest that correcting dysregulated macrophage metabolism is a promising therapeutic approach.

Cholesterol metabolism in foam cell formation

Dysregulated cholesterol metabolism in macrophages is intricately linked to the pathogenesis of atherosclerosis. The cell-surface lipid-sensing triggering receptor expressed on myeloid cells 2 (TREM2), identified in foamy plaque macrophages[97], upregulates the expression of the scavenger receptor CD36 (ref. 98). Uptake of modified LDLs through scavenger receptors, such as CD36 or SR-B1, bypasses the regulatory feedback that normally limits cholesterol accumulation. Internalized cholesterol is esterified by acyl-CoA:cholesterol acyltransferase (ACAT) to store cholesterol as cholesterol esters in lipid droplets, transforming macrophages into foam cells[99]. However, dysregulations in sterol metabolism prevent the safe disposition of cholesterol into lipid droplets and promote the formation of membrane-damaging cholesterol crystals[100,101]. This is further exacerbated as normal cholesterol efflux through the transporters ABCA1 and ABCG1 becomes compromised during atherosclerosis. Although these foam cells are not inflammatory, their diminished migratory capability and the constant barrage of atherogenic stimuli eventually lead to their death through apoptosis[102–106]. If these apoptotic foam cells are not rapidly cleared, they further proceed into post-apoptotic necrosis, spilling their contents into the plaque microenvironment[102,103,107]. The uptake of cholesterol crystals activates the NLRP3 inflammasome[108,109]. Activation of this multiprotein complex leads to caspase-1-mediated secretion of the pro-inflammatory and pyrogenic cytokines, interleukin (IL)-1β and IL-18 (refs. 110,111). Notably, suppression of the inflammasome via enhancing cholesterol efflux or by inhibiting NLRP3 assembly blunts atherosclerosis progression and enhances features of plaque stability[101,112–114].

Glycolysis and OXPHOS in inflammation and its resolution

Inflammatory stimuli have been shown to enhance the expression of the key glycolytic enzymes GLUT1 (encoded by *SLC2A1*)[115], hexokinase 3 (*HK3*)[116], 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*PFKFB3*)[116,117] and *PKM2* (refs. 118–120). This glycolytic shift is further driven by atherogenic factors, such as cytokines, oxLDL and cholesterol crystals, which increases their polarization towards an inflammatory phenotype[121]. The concept that pro-inflammatory lesional macrophages readily take up glucose has been leveraged in positron emission tomography using the glucose analogue [^{18}F]-fluoro-2-deoxy-D glucose (^{18}F-FDG) to detect advancing atheromas in humans[122,123].

Enhanced glycolysis in pro-inflammatory macrophages breaks the TCA cycle, leading to the accumulation of the TCA cycle intermediates, succinate and citrate[124,125]. These intermediates drive reactive oxygen species-mediated IL-1β production and tumour necrosis factor (TNF) translation[124,125] (Fig. 3c). Moreover, pro-inflammatory macrophages exposed to bioactive components of modified LDL increase citrate production. This is subsequently

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converted into oxaloacetate by ATP citrate lyase (ACLY), promoting HIF1α-mediated IL-1β production[126]. Although likely due to modulation of fatty acid and cholesterol metabolism rather than glycolysis, deleting ACLY in myeloid cells enhances fibrous cap formation and decreases necrotic core size[127]. Despite these clear connections between glycolysis and pro-inflammatory responses, the role of glycolysis in macrophages during atherosclerosis is complex. For instance, mice heterozygous for the *Slc2a1* gene in their haematopoietic cells showed reductions in monocyte recruitment to plaques and attenuations in atherosclerosis[128]. By contrast, myeloid-specific GLUT1 deletion augmented features of plaque instability[129]. Also, transgenic overexpression of GLUT1 in $CD68^+$ cells enhanced glucose uptake and increased glycolysis, but atherosclerosis progression or features of plaque stability were unaffected[116]. These data suggest a difference between limiting versus abolishing glucose flux in macrophages. As discussed later, this may be partly due to the recently discovered effects of lactate stimulating inflammation resolution.

Although pro-resolving macrophages show enhanced FAO-mediated oxygen consumption, FAO is dispensable for their polarization[130,131]. Interestingly, inhibiting macrophage FAO by deleting CPT1α and CPT2 enhanced CD36 expression and increased foam cell formation (Fig. 3c), ultimately driving atherosclerosis progression[132]. This process is influenced by oxLDL/CD36 signalling, which stimulates the NLRP3 inflammasome and cytokine production, shifting the metabolic balance in macrophages from OXPHOS to glycolysis[133]. The potent anti-inflammatory cytokine IL-10 suppresses LPS-induced glycolysis and drives OXPHOS, which blunts inflammasome-mediated IL-1β secretion[134]. The establishment of FAO driving IL-10 and, reciprocally, IL-10 promoting FAO, highlights the existence of a feed-forward loop that augments resolution[135]. Peroxisome proliferator-activated receptor gamma (PPARγ) critically regulates this pathway as its deletion reduces oxygen consumption and exacerbates atherosclerosis[136,137]. PPARγ deletion lowers the expression of isocitrate dehydrogenase 1 (IDH1) and increases the expression of immune-responsive gene 1 (IRG1, encoded by *ACOD1*), resulting in itaconate accumulation[136,138]. This metabolite inhibits succinate dehydrogenase and subsequently leads to increased succinate levels, which block macrophage polarization towards a pro-resolving phenotype[119,138,139] (Fig. 3c). Although manipulating these pathways is an attractive therapeutic strategy, the risk of unintended consequences, such as heightened susceptibility to infections and tumorigenesis[140], cannot be ignored. This delicate interplay between metabolism, inflammation and resolution in atherosclerosis highlights the need for precise and carefully considered therapeutic interventions.

Amino acid metabolism in macrophages

Accumulating evidence over the past two decades has increasingly highlighted a critical role for amino acid metabolism in macrophages. The amino acids glutamine, arginine, tryptophan, serine and glycine have been shown to have important roles in macrophage function, particularly during atherosclerosis. As an example, glutamine can be transaminated by glutaminase 1 (GLS1) into glutamate[141] (Fig. 2). This is then channelled by the malate–aspartate shuttle via aspartate aminotransferase (GOT; Fig. 3c). Macrophages deficient in GLS1 show deficiencies in OXPHOS and are unable to meet the high-energy demand for cytoskeletal rearrangement required for efferocytosis. Mice with myeloid-specific deletion

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of GLS1 manifest large necrotic cores during atherosclerosis[141]. As another example, macrophages can metabolize arginine through the NO pathway, in which iNOS converts arginine to NO, or the polyamine pathway, in which arginine is converted to ornithine and then into polyamines (Fig. 2). The temporal features of arginine metabolism through these routes have been demonstrated in models of inflammation and its resolution. iNOS-mediated NO production is high during acute inflammation[142], which is useful when eliminating pathogens. At later phases, arginase mediated ornithine formation and polyamine biosynthesis dominates[142]. As mentioned earlier, macrophages exist among a spectrum of phenotypes and often express iNOS and ARG1 simultaneously in vivo. However, the metabolism of arginine by one pathway often suppresses the other. For instance, NO-mediated nitrosylation on ornithine decarboxylase (ODC1) suppresses its activity and prevents polyamine synthesis[143]. Conversely, NO production is restricted by ARG1-mediated polyamine synthesis, as polyamines suppress iNOS translation[144–146]. Interestingly, low expression of ARG1 in macrophages is associated with susceptibility to atherosclerosis[147], and deletion of ARG1 in myeloid cells blunts atherosclerosis regression in male mice, where $ARG1^{+}$ macrophages are abundant[148].

The degradation of tryptophan into kynurenine by IDO1 (Fig. 2) contributes to the non-inflammatory property of efferocytosis[149]. Furthermore, kynurenine possesses diagnostic and prognostic value for atherosclerosis, and a deviation in this pathway, particularly a downregulation of kynurenic acid branch enzymes, is associated with unstable atherosclerosis[150,151]. Tryptophan degradation through this pathway exerts anti-inflammatory effects due to the subsequent accumulation of uncharged tRNAs that activate the serine/threonine kinase general control nonderepressible 2 (GCN2). Consequently, GCN2 activation modulates ribosome assembly and induces a repertoire of transcription factors that promote *Il10* and *Tgfb* expression while simultaneously suppressing *Il12* production[149]. Treating macrophages with the tryptophan metabolite 3-hydroxyanthralinic acid blocks inflammasome activation, lowers oxLDL uptake and decreases atherosclerosis progression[152,153]. These pathways probably account for the finding that deleting or inhibiting IDO1 in apolipoprotein-E-deficient ($Apoe^{-/-}$) male mice enhances atherosclerosis, increases necrotic core area and augments the expression of pro-inflammatory cytokines[154,155]. Additionally, treating atheroprone male mice with the IDO1 inhibitor 1-methyl-DL-tryptophan (1-MT) blocked atherosclerosis regression elicited by the oral administration of eicosapentaenoic acid[156]. Furthermore, preclinical and clinical studies demonstrate that the synthetic tryptophan metabolite 3,4-dimethoxycinnamoyl anthranilic acid (3,4-DAA, also known as ‘Tranilast’) reduced atherosclerosis progression in mice, lowered the restenosis rate in patients with ASCVD after transluminal angioplasty and lowered myocardial infarction rates in humans in the Prevention of REStenosis with Tranilast and its Outcomes (PRESTO) trial[154,157–159]. However, other studies suggest a more complicated role of IDO1 in atherosclerosis. For instance, inhibiting IDO1 using 1-MT or deleting IDO1 in haematopoietic cells enhanced high-density lipoprotein (HDL) and increased IL-10 production[160,161]. Moreover, MSI studies revealed that the tryptophan metabolite 5-hydroxyindoleacetic acid, associated with metabolic syndrome and inflammation[162], is enriched in the fibrous cap of unstable human plaques[69]. Additionally,

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tryptophan metabolism, as assessed by the ratio of kynurenine to tryptophan, was positively correlated with stroke severity[163].

Serine supports a complex network of metabolic pathways that contribute to cell proliferation and synthesis of nucleotides, NADPH and *S*-adenosyl-methionine (SAM) through one-carbon metabolism[164,165] (Fig. 2). Additionally, serine has a crucial role in maintaining redox homeostasis by its conversion to glycine via serine hydroxymethyltransferases 1 and 2 and subsequently into the potent anti-oxidant glutathione (GSH; Figs. 2 and 3c). Despite the role of GSH in suppressing oxidant stress and inflammation, reducing GSH synthesis by serine deprivation decreases LPS-induced IL-1β production[166]. Another study corroborated this finding yet suggested that serine has a role in IL-1β production through SAM-mediated epigenetic reprogramming by histone methylation rather than by reducing GSH[167]. Nonetheless, GSH is among the metabolites most decreased in pro-inflammatory macrophages, and in vivo strategies that enhance GSH production decrease atherosclerosis[168–170]. Furthermore, glycine, a GSH precursor, is negatively associated with ASCVD in humans[171,172], and glycine-based treatments reduce atherosclerosis through de novo GSH biosynthesis in macrophages[173].

Mechanistic insights into efferotabolism

Macrophage-mediated clearance of apoptotic cells (ACs), termed ‘efferocytosis’, is vital to tissue homeostasis[107]. Monocyte-derived macrophages in early atherosclerotic lesions display intact AC clearance[107] (Fig. 1). However, as atherosclerosis advances, efferocytosis becomes defective, which drives the formation of large necrotic cores that are intimately linked to clinically dangerous unstable atheromas[23,174–177]. Preclinical studies aimed at restoring efferocytosis in mouse models of atherosclerosis demonstrated that enhancing the clearance of ACs diminishes necrotic core area and increases the size of the protective fibrous cap[178–181]. Emerging evidence indicates that the efficient clearance of ACs in vivo requires successive rounds of phagocytosis[148,182,183], which is metabolically distinct from single phagocytic events. After the internalization of an AC, phagocytes degrade and process the AC-derived macromolecules through a process requiring microtubule-associated protein 1A/1B light chain 3 (refs. 184–186). This metabolically demanding process, which we have termed ‘efferotabolism’[187], describes the process by which phagocytes break down, metabolize and respond to AC-derived products (Fig. 4). Efferotabolism is necessary to trigger a resolution response and has been associated with features of plaque stability. Conversely, impaired processing of AC-derived macromolecules sustains inflammation and is now considered a hallmark of advanced atherosclerosis[188].

After AC degradation in phagolysosomes, intracellular cholesterol levels rise, especially when digesting cholesterol-rich apoptotic foam cells[189]. To manage this, macrophages direct AC-derived cholesterol to ACAT in the endoplasmic reticulum (ER)[190] (Fig. 4). Concurrently, AC-derived sterols stimulate LXRs and PPARs, enhancing the expression of cholesterol transporters ABCA1 and ABCG1 (refs. 191–193; Fig. 4). Cholesterol efflux through these transporters has a vital role in macrophage survival after efferocytosis[189,194]. Furthermore, TREM2 deficiency leads to a failure of LXR-mediated cholesterol efflux and efferocytosis as well as increasing ER stress responses. Despite these pro-atherogenic

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responses, TREM2 deletion in monocytes reduced atherosclerosis[195]. However, this initial benefit diminishes as the disease progresses[196]. In addition to cholesterol, intracellular arginine levels also increase after efferocytosis[148]. Pro-resolving macrophages expressing high levels of ARG1 hydrolyse arginine into ornithine, which is subsequently decarboxylated by ODC1 to generate putrescine. Putrescine biosynthesis leads to Rac1-mediated cytoskeletal remodelling that permits the internalization of multiple ACs (Figs. 3c and 4). Deletion of ARG1 from myeloid cells or silencing ODC1 in lesional macrophages using macrophage-targeting nanoparticles leads to a selective defect in continual efferocytosis, lowers IL-10 production and blunts atherosclerosis regression in male mice[148,197].

The binding of an AC with a macrophage triggers CD36-dependent ERK1/ERK2 activation, leading to prostaglandin E2 biosynthesis and TGFβ1 expression[198]. However, CD36-mediated ERK1/ERK2 activation is insufficient to surmount the repression imposed by dual specificity phosphatase 4 (DUSP4). This regulatory feedback is overcome through a methionine salvage pathway originating from the phagolysosomal degradation of an AC[198]. AC-derived methionine is enzymatically converted to SAM by methionine adenosyltransferase 2A[198]. SAM is then utilized by DNA methyltransferase 3A (DNMT3A) to repress *DUSP4* expression via DNA methylation, permitting ERK1/ERK2–PGE2-mediated TGFβ1 upregulation[198]. Haematopoietic deletion of DNMT3A impairs this ERK1/ERK2–PGE2–TGFβ1 signalling axis in mice and drives features of plaque instability[198] (Figs. 3c and 4). In parallel, nucleotides salvaged from AC degradation potentiate macrophage proliferation via a process termed 'efferocytosis-induced macrophage proliferation' (EIMP)[199] (Figs. 3c and 4). Similarly to CD36, AC binding with a macrophage activates MerTK to stimulate ERK1/ERK2. Simultaneously, mTORC2 is stimulated by DNA-PK-mediated Rictor activation. These pathways converge on the transcriptional repressor BHLEH40, which represses c-Maf-mediated suppression of cell cycle progression, a process contingent on Myc. Importantly, mTORC2 deletion in macrophages lowered IL-1β production during atherosclerosis progression and impaired proliferation of efferocytosis-competent macrophages during regression[199,200].

Interestingly, macrophage ingestion of an AC stimulates both glycolysis and OXPHOS[135,182]. Unbiased metabolomics revealed that macrophages use fatty acids from ACs to fuel mitochondrial β-oxidation, increasing NAD^+ levels[135]. Inhibiting complex III of the electron transport chain limited IL-10 production, which can be restored by supplying NAD^+ precursors. Additionally, RNA-seq of efferocytes revealed upregulation of GLUT1, enhancing glucose uptake and stimulating a transient increase in glycolysis dependent on PFKFB2 activation[182,201]. Efferocytosis-mediated glycolysis promotes cytoskeleton remodelling and enhances the expression of AC receptors to drive continual efferocytosis[182,201] (Fig. 4). Furthermore, lactate generated in macrophages after efferocytosis is exported via MCT1 into the surrounding microenvironment[182,202]. Lactate also promotes inflammation resolution through epigenetic modifications whereby lactate moieties are covalently attached to lysine residues on histone proteins[203]. Histone lactylation in macrophages enhances ARG1 expression, increases their phagocytic capacity and redirects macrophage polarization towards a pro-resolving phenotype[204,205]. Interestingly,

histone lactylation is tightly coupled to mitochondrial dynamics as mitochondrial fission, an early event in efferocytosis[206], drives histone lactylation[205] (Fig. 4).

A recent integrative study combining transcriptomic and proteomic approaches identified two distinct states in macrophages under chronic physiological hypoxia (~1% oxygen) —the ‘primed’ and ‘poised’ states[207]—that are associated with enhanced conversion of glucose into a noncanonical PPP loop. This increases the production of NADPH that drives phagolysosomal acidification and maintains cellular redox homeostasis, which are necessary for continual efferocytosis. These findings are particularly interesting because advanced human atheromas that are stable show signs of inflammation resolution and may involve metabolic reprogramming of lesional macrophages[208]. Gaining a comprehensive understanding of the interplay between various metabolic pathways in macrophages during efferocytosis will provide valuable insights into the progression of ASCVD and offers potential avenues for therapeutic interventions.

T cell metabolism in atherosclerosis

T cell function in atherosclerosis

T cells are critical modulators of atherogenesis. Regulatory T (T_{reg}) cells, which express the transcription factor FOXP3 and the high-affinity IL-2 receptor CD25, exert anti-inflammatory and pro-resolving effects[209]. By enhancing efferocytosis and stimulating the production of pro-resolving mediators that promote atherosclerosis regression, T_{reg} cells have been repeatedly shown to be atheroprotective[210–212]. Alternatively, specific $CD4^+$ helper T cells are associated with pro-atherogenic responses. This is most clear for the T_H1 subset of helper T cells[210]. Type 2 helper T (T_H2) cells, although probably having a minor impact on atherosclerosis[210], warrant further investigation. Moreover, the intricate interplay between T cell metabolism and features of autoimmunity adds another layer of complexity that needs to be elucidated for a more comprehensive understanding of atherosclerosis pathogenesis.

T cell-mediated autoimmunity in atherosclerosis

Clonal expansion of T cells and B cells in atherosclerosis provides increasing evidence for the essential role of autoimmunity in atherosclerosis[213]. Individuals with autoimmune disorders, such as systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease and systemic sclerosis, exhibit an increased risk for ASCVD[214]. Cancer immunotherapies increase the risk of ASCVD, which is assumed to be mediated by disinhibition of autoreactive T cells[215]. Accordingly, growing evidence has revealed the critical role of $CD4^+$ T cell-mediated autoimmunity in atherosclerosis[216–221]. Recent single-cell T cell antigen receptor sequencing has revealed a break in tolerance in atherosclerosis, whereby adaptive immune cells no longer distinguish self-antigens from non-self-antigens[218,219]. T_{reg} cells and effector T (T_{eff}) cells recognize epitopes in plaque-associated autoantigens[210]. The most studied atherosclerosis-related autoantigen is ApoB, the core protein of LDL, chylomicrons and other lipoproteins. In the 1990s, Hansson and colleagues isolated $CD4^+$ T cells responding to oxLDL from human atherosclerotic plaques[222]. Whereas this T cell response was suggested to rely on the formation of LDL-

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neoepitopes through oxidation[222], subsequent work identified $CD4^+$ T cells that recognize epitopes in the native ApoB protein, restricted by binding to major histocompatibility complex (MHC). $CD4^+$ T cells recognize peptides bound to MHC class II, and $CD8^+$ T cells recognize peptides bound to MHC class I. Many MHC class II-restricted epitopes have been discovered in the native ApoB protein in mice[216,220,223] and humans[217,220], and vaccine-based strategies using ApoB peptides substantially decrease atherosclerosis in animal models[224]. Phenotyping by flow cytometry and RNA-seq has revealed that ApoB-reactive ($ApoB^+$) $CD4^+$ T cells predominantly exhibit T_{reg} cell-like signatures in healthy mice and humans[216,221].

T_{reg} cells can become unstable during atherosclerosis and convert into exT_{reg} cells[216], described as T_H1-like, T_{FH}-like, T_H17-like or cytotoxic[216,220,225–227]. $ApoB^+$ T cells are particularly susceptible to adopting T_H1/T_H17 effector cell characteristics with minimal T_{reg} cell signatures and to switching into exT_{reg} cells[216,221]. Conversely, exT_{reg} cells upregulate T_{eff} cell markers[216]. Notably, exT_{reg} cells are highly pro-inflammatory and pro-atherogenic[221], and recent lineage tracing studies have shown that some exT_{reg} cells are a cytotoxic type of $CD4^+$ T cells[228]. The switch from antigen-specific T_{reg} cells to exT_{reg} cells may participate in the break in tolerance to self during atherogenesis. To date, the mechanisms responsible for this switch are unknown[210,229]. Cellular metabolic changes might have a critical role in governing this process as the conversion from T_{reg} cells to exT_{reg} cells is accelerated in mice fed a Western diet[216].

Metabolic reprogramming determines the function and fate of T cells

Accumulating evidence suggests that T cell responses are dynamically regulated by metabolic signals[230]. Following activation, quiescent and naive $CD4^+$ T cells reprogramme their cellular metabolism to meet the increased energy demand required for proliferation and differentiation[230]. Specific metabolic programmes are required for differentiation into pro-inflammatory effector $CD4^+$ T cells (for example, T_H1, T_H2 and T_H17 cells) or anti-inflammatory T_{reg} cells. Whereas T_{eff} cells are highly glycolytic, T_{reg} cells predominantly utilize mitochondrial OXPHOS of fatty acids or pyruvate for energy production[231,232]. The PI3K–Akt–mTORC1 signalling pathway is one of the central regulators of pro-glycolytic metabolism in $CD4^+$ T cells, which is characterized by GLUT1 upregulation[233]. GLUT1 expression is essential for the activation and proliferation of T_{eff} cells but not required for the functionality of T_{reg} cells [233]. Additionally, pyruvate dehydrogenase kinase 1 (PDK1)[232], which inhibits the conversion of pyruvate into acetyl-CoA and thereby prevents subsequent OXPHOS, or the glycolysis-promoting transcription factor HIF1α[234], are both highly expressed in T_{eff} cells but low in T_{reg} cells. $CD4^+$ T cell-specific deletion and/or pharmacological depletion of GLUT1, GLUT3, PDK1 or HIF1α all protect mice from experimental autoimmune diseases by attenuating T_{eff} cell differentiation and activation[232–235]. In *Apoe*$^{-/-}$ mice, PDK1 inhibition by dichloroacetate reduced plaque infiltration by $CD4^+$ T cells, induced a shift from pro-inflammatory to anti-inflammatory T cell responses, and conferred atheroprotection[236]. By contrast, T_{reg} cell-specific deletion of the metabolic sensor liver kinase B1, critical for maintaining OXPHOS, reduces T_{reg} cell number and impairs their function, thereby inducing a fatal autoimmune disorder in mice[237].

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Besides affecting T cell differentiation and functions, metabolic alterations can shift pre-existing lineage decisions. Specifically, uncontrolled glycolysis impairs T_{reg} cell stability (Fig. 3d) and induces spontaneous autoimmunity in mice[238–240]. T_{reg} cells lacking the phosphatase PTEN, which reduces the pro-glycolytic activity of mTORC2 by inhibiting PI3K–Akt signalling, lose their suppressive capacity as well as their expression of FOXP3 and give rise to autoimmunity in mice[238,239]. Accordingly, T_{reg} cells expressing constitutively active GLUT1 have diminished suppressive capacity and lose FOXP3 expression[240]. Although the effects of energy metabolism and intermediate metabolites on T cells in the context of atherosclerosis remain largely unexplored, it is likely that metabolic reprogramming contributes to these responses.

A recent study demonstrated that $CD4^+$ T cells are susceptible to metabolic exhaustion in advanced atherosclerosis. Prolonged Western diet feeding to *Apoe*$^{-/-}$ male mice reduced glycolysis in naive $CD4^+$ T cells, which led to increased apoptosis and a diminished proliferative capacity[241] (Fig. 3d). Moreover, compelling evidence suggests that hypercholesterolaemia also contributes to the instability of T_{reg} cells during atherogenesis (Fig. 3d). A shift from high to normal cholesterol intake reduces the loss of lesional T_{reg} cells and plaque progression in male and female *Ldlr*$^{-/-}$ mice[242]. In a related study, treating *Apoe*$^{-/-}$ mice with ApoAI, the core protein of HDL, diminished Western diet-related intracellular cholesterol accumulation in T_{reg} cells and prevented their conversion into exT_{reg} cells [227]. By contrast, exaggerated cholesterol accumulation in T cells induced apoptosis and impaired T cell functionality (Fig. 3d), which was associated with reduced atherosclerosis in aged *Ldlr*$^{-/-}$ mice[243]. Because unrestrained glycolysis renders T_{reg} cells unstable in autoimmune disease models[238–240], hypercholesterolaemia-driven T_{reg} cell instability might involve a similar mechanism. However, the specific impact of hypercholesterolaemia on metabolic reprogramming of T cells in the context of atherosclerosis must be determined by future studies.

Nutrients modulate T cell responses in autoimmunity

The typical Western diet of the 21st century, characterized by an excess intake of refined sugars, saturated fats, animal protein, salt and cholesterol, is known to increase the risk of ASCVD[244,245]. Although the effects of diet on T cell-mediated immunity in atherosclerosis remain largely unexplored, emerging evidence strongly suggests that specific nutrients, mainly glucose and amino acids, directly influence T cell fate and function. For example, glucose restriction leads to AMPK-mediated inhibition of mTORC1 signalling, suppressing T_{eff} cell responses and promoting T_{reg} cell differentiation[246] (Fig. 3d). Consistently, high glucose intake exacerbates experimental autoimmunity by induction of T_H17 cells[247]. Notably, increased mTORC1 signalling has also been implicated in T_{reg} cell lineage instability. Leucine-induced mTORC1 activation drives $CD4^+$ T cell differentiation into T_H1 and T_H17 cells, but T_{reg} cell differentiation remains unaffected[248]. α-Ketoglutarate, a cell-permeable metabolite of glutamine, impairs T_{reg} cell differentiation and induces the generation of pro-inflammatory T cells[249]. In line with this, glutamine deprivation shifts the differentiation of naive $CD4^+$ T cells under T_H1 polarizing conditions towards a T_{reg} cell phenotype by attenuating mTORC1 activation[250]. Interestingly, transient inhibition of glutaminase, which catalyses the first step of glutaminolysis, induces differentiation towards

a T_H1 phenotype by increasing histone methylation and thereby enhancing mTORC1 signalling[251].

In addition to leucine, other amino acids also regulate T cell responses. Serine fuels proliferating T cells with glycine and one-carbon units for de novo purine nucleotide synthesis (Figs. 2 and 3d), independent of glucose[252]. Methionine, found in meat, fish and dairy products, is a critical substrate for the methyl donor SAM (Fig. 2), which is needed for histone methylation in activated T cells, and thus regulates the expression of genes involved in T_{eff} cell proliferation and cytokine production[253]. Furthermore, dietary methionine restriction reduces the expansion of pathogenic T_H17 cells[253]. Tryptophan metabolism also has a notable role in T cell behaviour. IDO1-mediated tryptophan depletion and biosynthesis of kynurenine and its downstream metabolites drives T_{reg} cell differentiation and blunts inflammatory responses[254,255]. Consistently, IDO1 deletion in dendritic cells stimulates IL-6 production and differentiation of T cells towards T_H17 cells[256]. IDO1 expression by vSMCs has been shown to inhibit T cell infiltration into the arterial media, a phenomenon that is called the 'medial immunoprivilege'[257]. T_{reg}cells induce IDO1 expression in vSMCs, ECs and macrophages through their co-inhibitory surface receptor CTLA4. Notably, the induction of the 'T_{reg}–IDO axis' in the vessel wall is atheroprotective[258].

Future research investigating the following aspects will further elucidate the role of dysregulated T cell metabolism in atherosclerosis: (1) whether and how T cells, especially those responding to disease-mediating autoantigens such as ApoB, are metabolically reprogrammed during atherogenesis; (2) the impact of metabolic adaptations on lineage stability of T_{reg} cells; and (3) whether atheroprotective immunity can be enhanced by experimental targeting of distinct metabolic pathways. Ultimately, understanding the causes and consequences of metabolic adaptations in T cells during atherosclerosis could be pivotal in development of novel immunotherapies.

Intercellular metabolic cross-talk in atherosclerosis

Studies exploring the molecular mechanisms of dysregulated metabolism in atherosclerosis often either use purified cell types in vitro or involve the deletion of critical metabolic enzymes in specific cells in vivo. However, these approaches may overlook vital intercellular communications that involve various cell types and multiple enzymes, potentially missing important connections that occur during atherosclerosis in humans. Additionally, metabolites can act as communicative factors between lesional cells. The soluble factors they produce can also influence the metabolism in distant cells, governing their fate and function. This interplay between ECs, vSMCs and immune cells can either accelerate atherosclerosis and enhance plaque instability or attenuate atherosclerosis progression and drive regression or stabilization. Additionally, alterations in metabolic pathways may be pro-atherogenic in one cell type or anti-atherogenic in others. Furthermore, due to the epidemic of obesity, dyslipidaemia and insulin resistance, the common use of medications treating these diseases is creating a new population of individuals experiencing long-term metabolic alterations. This warrants careful consideration in the design of preclinical models of atherosclerosis and the development of novel therapies thereof. The following sections highlight the

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mechanisms driving pathological metabolic cross-talk during atherosclerosis and cover the pleiotropic effects of commonly used metabolism-based drugs.

Intercellular metabolic communication between lesional cells

Glycolysis promotes atherosclerosis by driving EC activation and vSMC dedifferentiation while limiting the differentiation of $CD4^+$ T cells towards T_{reg} cells[30,67,70] (Fig. 3). ECs in regions of disturbed FSS probably experience a persistent state of glycolysis[30], suggesting that low levels of lactate may be released into the subendothelial space. This scenario merits exploration owing to its potential implications in vSMC dedifferentiation, proliferation, migration and calcification[70,259]. However, glycolysis simultaneously exerts anti-atherogenic properties by promoting efferocytosis and stimulating the production of pro-resolving mediators by macrophages[182,201].

The processing of AC-derived cargo in the context of efferotabolism is metabolically demanding as evidenced by the simultaneous stimulation of glycolysis, FAO glutaminolysis and the PPP[135,141,182,201,260]. These changes in cellular metabolism following efferocytosis have a key role in their cross-talk with vSMCs[261]. Additionally, the ACs themselves release polyamines and nucleotides that directly serve as tissue messengers[262]. Secreted metabolites from ACs or macrophages following efferocytosis act in an autocrine or paracrine manner to further increase the successive clearance of dying cells and promote the expression of IL-10 and TGFβ[261,262]. However, disparate responses by other cell types may antagonize these atheroma-stabilizing properties. For example, TGFβ secreted from efferocytes may also promote EndMT, which has a central role in plaque instability[43]. In addition, efferocytes secrete the potent angiogenic factor vascular endothelial growth factor A, which may inadvertently enhance intraplaque angiogenesis[263], which has been shown to permit haemorrhage and promote plaque instability[264–266].

In addition, the mechanisms necessary to process the metabolic burden following efferocytosis are only partially understood but may involve novel and unexpected pathways. Mitochondria from an ingested AC may escape phagolysosomal degradation and fuse with the phagocyte mitochondria to increase mitochondrial mass and enhance respiration, as was seen for endosymbiosis. The exchange of respiring mitochondria between adipocytes and macrophages has also been observed[267–269]. Transfer of endocytic organelles via nanotubes may also occur, an emerging and exciting field of research that raises questions about how cells cope with metabolic stress[270]. Organelle transfer becomes particularly intriguing when considering a hypothetical scenario where non-phagocytic cells residing in atheromas could share the metabolic burden of processing ingested ACs.

The modulation of T cell metabolic reprogramming and regulation of immune responses hinge on the actions of EC-secreted metabolites[271]. NO and sphingosine-1-phosphate primarily shape T cell metabolism and its subsets (Fig. 3). NO, a potent messenger molecule derived from ECs, influences various physiological and pathological conditions by stimulating glycolysis and concurrently impairing mitochondrial reserve capacity[272]. Furthermore, NO-mediated nitrosylation of very long-chain acyl-CoA dehydrogenase[273], which catalyses the first step in FAO, potentially alters T cell subset differentiation. This implies its critical role in dictating lipid metabolism and mitochondrial respiration and,

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thus, indirectly forming T cell subsets. By contrast, sphingosine-1-phosphate, an important EC metabolite, promotes the survival of naive T cells by maintaining mitochondrial content[274,275], adding a new facet to the dynamic regulation of T cell differentiation and survival. In addition, IDO1 manages tryptophan catabolism and curbs excessive immune responses, with elevations in IDO1 activity by ECs indirectly determining immune cell apoptosis, thereby steering T cell polarization and overall inflammation[276,277]. These intersecting metabolic pathways form a complex network of EC–T cell cross-talk extending beyond basic cell function to dictate broader immune and inflammatory responses that could be relevant to ASCVD.

In the complex microenvironment of atherosclerotic plaques, a variety of metabolite-sensing G-protein-coupled receptors (GPCRs) have a critical role in orchestrating intercellular communication[278]. These GPCRs enable cross-talk between different cell types, with metabolites produced by one cell acting as extracellular signals for receptors on others. As an example, succinate, often released by hypoxic cells within atherosclerotic plaques, activates SUCNR1/GPR91 on ECs and immune cells[279,280]. This interaction triggers $G_{q/11}$, leading to phospholipase C-mediated signalling, calcium mobilization, NF-κB activation and inflammasome-mediated IL-1β secretion[236,279,280]. GPR35, responsive to kynurenic acid from the kynurenine pathway, acts conversely by dampening inflammatory responses[281]. However, consistent with the currently unresolved role of tryptophan metabolism in ASCVD, deleting the GPR35 receptor in haematopoietic cells does not affect atherosclerosis[282]. Binding of medium-chain fatty acids to GPR84 on macrophages promotes pro-inflammatory cytokine production[283]. Additionally, short-chain fatty acids, such as acetate and propionate, activate OLFR78 on vSMCs, altering vascular tone and blood pressure[284,285]. These GPCRs highlight the dynamic and multifaceted role of metabolites in governing intercellular communication within atherosclerotic lesions.

Effects of metabolism-altering therapeutics on lesional cells Statins.—Statins are best known for lowering cholesterol but exhibit atheroprotective pleiotropic effects even in normocholesterolaemic settings[286]. One such benefit is the inhibition of leukocyte–endothelial interactions due to decreased adhesion molecule presentation on ECs[287]. Additionally, inhibiting HMG-CoA reductase by statins prevents isoprenylation, lowers membrane levels of small GTPases, increases NO production by ECs and enhances vascular reactivity[288,289]. These effects probably involve statin-induced increases in KLF2 driving the expression of anti-inflammatory genes, including eNOS, which decreases pro-inflammatory signalling by inhibiting NF-κB and YAP/TAZ[29,290–292]. Despite these benefits, nearly 50% of individuals treated with statins still experience recurrent cardiovascular events[293]. Notably, the rate of statin use among the global population is increasing, coinciding with a shift in the prevalence of acute clinical events towards superficial plaque erosion rather than plaque rupture[294,295]. Plaque erosion has been mechanistically linked to the formation of neutrophil extracellular traps (NETs)[296]. EC-targeting nanoparticles carrying inhibitors to peptidyl arginine deiminase-4, which mediates histone citrullination necessary for NETosis[297], mitigate the formation of NETs and preserves endothelial integrity[298].

ACLY inhibitors.—ACLY has emerged as a crucial enzyme linking carbohydrate and lipid metabolism. By converting citrate to acetyl-CoA, ACLY has a vital role in fatty acid and cholesterol biosynthesis[299], processes central to the pathophysiology of ASCVD. Bempedoic acid (ETC-1002) is a first-in-class inhibitor of ACLY, offering a unique approach to modulating lipid metabolism[300]. By inhibiting ACLY, bempedoic acid increases hepatic LDLR expression and lowers circulating cholesterol[300]. In statin-intolerant patients, bempedoic acid reduced the risk of major adverse cardiovascular events[301]. Interestingly, the use of bempedoic acid extends beyond its primary application as a lipid-lowering agent, potentially exerting anti-atherogenic effects by modulating macrophage-mediated inflammation within atherosclerotic lesions. As an example, ACLY is activated in inflammatory macrophages and its deletion from macrophages enhances collagen cap thickness and decreases necrotic core area[127].

Thiazolidinediones.—Thiazolidinediones have been lauded for their ability to lower blood glucose in type 2 diabetes, primarily by enhancing insulin sensitivity in peripheral tissues, leading to improved glucose utilization and reduced insulin resistance[302]. Additionally, pioglitazone has demonstrated beneficial effects on lipid profiles, increasing HDL cholesterol and reducing triglyceride levels[303], with evidence of reduced atherosclerosis[304]. However, pioglitazone also results in weight gain, a bona fide risk factor for atherosclerosis[305]. Its tendency to cause fluid retention leading to oedema could also exacerbate heart failure in susceptible patients. Moreover, while enhancing macrophage apoptosis in a PPARγ-independent manner, pioglitazone and rosiglitazone increase AC clearance by macrophages, albeit with a net effect of increasing ACs and plaque necrosis[306]. Thus, the above risks highlight the importance of individualizing therapy, weighing the beneficial glucose-lowering and possible cardiovascular benefits against potential complications.

Metformin.—Metformin, primarily used for type 2 diabetes management, benefits ASCVD beyond its glucose-lowering capability[307,308]. For instance, metformin increases eNOS-mediated NO production in ECs[309–311]. Through a similar mechanism, metformin also suppresses vascular calcification and vSMC senescence[312,313]. As another example, metformin drives macrophage polarization towards a pro-resolving phenotype via regulating an AMPK–mTOR–NLRP3 signalling axis that accelerates wound healing[314]. As discussed below, metformin, in combination with drug-eluting stents (DESs), delays re-endothelialization and, potentially, heightens the risk for in-stent thrombosis[315].

Insulin.—Whereas metformin exerts multiple beneficial effects beyond its glucose-lowering capability, insulin therapy has a more intricate relationship with respect to ASCVD. Insulin is an irreplaceable component in managing type 1 diabetes and frequently becomes necessary in the later stages of type 2 diabetes[316]. Its potent glucose-lowering abilities improve glycaemic control, which limits microvascular complications such as nephropathy, retinopathy and neuropathy[317]. Nonetheless, insulin therapy carries challenges. Using exogenous insulin can lead to weight gain, potentially exacerbating insulin resistance and the risk of atherosclerosis[318]. High-dose insulin therapy has also been implicated in cardiovascular concerns, promoting sodium retention, vasoconstriction and vSMC

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proliferation, migration and inflammation, factors that contribute to atherosclerosis[319–321]. The relationship between insulin therapy and atherosclerosis is complex and influenced by factors such as the patient's underlying metabolic status and concurrent therapies[322].

mTOR inhibitors.—The advent of first-generation DESs coated with the potent mTOR inhibitor rapamycin (for example, sirolimus) marked an important advance in interventional cardiology. This was primarily due to its superior ability to maintain vessel patency compared to its bare-metal counterparts[323]. Acting as a central regulator of nutrient sensing and metabolism, mTOR has a pivotal role in the proliferation and migration of vSMCs. Inhibiting the mTOR pathway using rapamycin induces *CNN1*, *ACTA2* and *TAGLN* gene expression, all associated with a quiescent vSMC phenotype[324–326]. This inhibition also blocks nutrient sensing, impeding cell cycle progression and subsequent proliferation. This, in turn, reduces restenosis, offering a compelling advantage over bare-metal stents[323]. However, these first-generation DESs also delay re-endothelialization, thereby heightening the risk of stent thrombosis[327–329]. As a result, patients receiving first-generation DESs require an extended period of anticoagulation therapy. Interestingly, newer-generation DESs, which still contain rapamycin or its derivatives, are overcoming this hurdle due to improved drug release mechanisms, polymer coatings and stent design[330–332]. Thus, the success of mTOR inhibitors in DESs underscores the potential of exploiting metabolic pathways for therapeutic purposes.

Conclusions and future directions

Our understanding of ASCVD has evolved beyond the simplistic notion of lipid accumulation, embracing the intricate interplay of dysregulated cellular metabolism and complex interactions between cell types that drive disease progression. To optimize cellular metabolism and address the persistent cardiovascular risk despite existing therapies, we must prioritize critical areas for exploration. Understanding the complex links between metabolic pathways, cellular activities and cell differentiation within the plaque microenvironment is essential. This knowledge will drive the development of targeted therapeutics that address specific metabolic pathways. In this regard, the explosion of new metabolomic technologies, such as single-cell metabolomics, MSI, spatial transcriptomics and cytometry by time of flight (CyTOF), has begun to reveal the complexity of cell metabolism in atherosclerosis (Box 1).

Although these technologies have provided invaluable insights into atherosclerosis, they also open new avenues for exploration and highlight gaps in our understanding. The complexity of cellular interactions within plaques, particularly in metabolic processes, remains a challenge. Advances in single-cell metabolomics, such as SCENITH and SpaceM[333,334], promises to unravel this complexity. As an example, a recent study combining RNA-seq and MSI in human atheromas uncovered previously unrecognized metabolic pathways involved in plaque instability[69]. Future explorations integrating MSI with mass-tagged antibodies could offer profound insights by merging single-cell, spatial metabolomics with spatial transcriptomics. Nonetheless, integration of data from genomics, transcriptomics, proteomics and metabolomics in a broader approach will require continued advances in computational methods, machine learning and imaging techniques.

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Additionally, methods to target metabolic pathways for treating ASCVD, including the exploration of cell-type-specific, nanoparticle-based strategies for delivering inhibitors, mRNA and RNAi, hold great promise. Although isolated metabolic pathways are relatively well understood, further exploration is needed to comprehensively characterize the contribution of noncanonical pathways, dysregulation within these pathways and the mechanisms underlying intercellular cross-talk. Innovation in these areas is set to provide a more comprehensive understanding of atherosclerosis at both the molecular and cellular levels, ultimately revealing potential biomarkers and novel therapeutic strategies to treat ASCVD.

One area deserving further investigation is the protective role of efferotabolism in atherosclerosis, which remains understudied. Strategies that increase lesional efferocytosis have been repeatedly shown to mitigate atherosclerosis progression and even enhance its regression. However, macrophages must undergo metabolic reprogramming to effectively take up and process multiple ACs. Thus, gaining a deeper understanding of the interplay among various metabolic processes in efferotabolism will not only shed light on the progression of ASCVD but also reveal strategies for therapeutic intervention. Moreover, because most research interrogating EC metabolism has been predominantly conducted under static conditions, the metabolism of ECs under pathophysiological conditions in the setting of FSS warrants attention. A comprehensive understanding of EC metabolism is critical, given their role on leukocyte adhesion and phenotypic transition to mesenchymal cells. Similarly, insights from T cell studies in autoimmune diseases could deepen our understanding of their metabolism in atherosclerosis. This is particularly important as recent studies have clearly demonstrated that alterations in T cell specification rely on metabolic reprogramming. Bridging knowledge gaps between interconnected metabolic pathways within lesional cells could open new avenues for therapeutic manipulation. By focusing on these underexplored aspects of dysregulated cellular metabolism and their interdependencies, we can unlock a new era of effective interventions to mitigate ASCVD (Boxes 1 and 2).

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Box 1

Single-cell technologies reveal novel insights into atherosclerosis

Unbiased single-cell technologies, such as scRNA-seq, single-cell assay for transposase-accessible chromatin with high-throughput sequencing, CITE sequencing and single-cell mass cytometry, are powerful platforms that have ushered in a new age for understanding cellular dynamics while also revealing molecular signatures of newly identified cell types in atherosclerotic plaques[335–339]. Specifically, these technologies have permitted cellular indexing of transcriptomes (scRNA-seq), allowed for the analysis of genetically open regions of chromatin that are available for active transcription (single-cell assay for transposase-accessible chromatin with high-throughput sequencing), elucidated cell-surface markers and epitopes (CITE sequencing) and profiled single-cell proteomics in heterogeneous samples using metal isotopes (CyTOF). Furthermore, advancements in computational modelling have allowed for the deconstruction of plaques to reveal cell–cell communications through specific ligand–receptor interactions. These approaches have revealed inflammatory *Il1b*$^{+}$*Nlrp3*$^{+}$ macrophages in plaques[97]. The clinical relevance of these findings was highlighted by the CANTOS trial where blocking IL-1β signalling reduced cardiovascular events[12]. CyTOF uncovered the expansion of monocytes, plasmacytoid dendritic cells and CD11c^{+} immune cells with a simultaneous diminishment of CD206^{+}CD169^{+} macrophages and type 2 conventional cells during atherosclerosis[340]. In the non-immune cell compartment, single-cell omics platforms confirmed the plasticity of vSMCs and revealed a new vSMC-derived population, termed the 'SEM', which can either maintain vSMC properties or differentiate into macrophage-like or fibrochondrocyte-like cells[56]. Additionally, spatial transcriptomics using MERFISH identified a new population of dedifferentiated vSMCs in males dominated by the abundance of *CARTPT*[341]. Profiling the transcriptional landscape of human atheromas revealed genes in vSMCs commonly associated with bone mineralization, vascular calcification and matrix remodelling in the necrotic cores of plaques. Lineage tracing coupled to scRNA-seq further identified *Tcf21* as a critical component of vSMC dedifferentiation and their trajectory resembling other cell types[55]. Interestingly, stem cell antigen 1^{+} vSMCs, which show a loss of expression in differentiated vSMC markers[50], were found in the core of atherosclerotic plaques but were absent in the fibrous cap[53].

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Box 2

From lipids to proteins and amino acids: a paradigm shift in nutrient metabolism and atherosclerosis

The critical role of lipid/lipoprotein metabolism in ASCVD has been extensively studied, leading to the development of effective therapeutics that reduce LDL cholesterol and cardiovascular risk[342,343]. Despite this, numerous individuals with normal LDL cholesterol levels still suffer from major cardiovascular events[344,345], emphasizing the importance of other metabolic pathways. Over the past decades, a growing body of evidence uncovered essential roles for protein and amino acids in ASCVD pathogenesis. In *Apoe*$^{-/-}$ mice, high-protein diets enhance the size and complexity of atherosclerotic plaques[346,347]. In humans, however, high-protein intake can either increase or decrease the cardiovascular risk[348–350], highlighting the importance of understanding the roles of specific amino acids, rather than total protein intake.

Among 79,838 participants, a higher intake of essential amino acids (for example, branched-chained amino acids (BCAAs)) was associated with increased cardiovascular mortality, whereas a higher intake of non-essential amino acids (for example, glycine) was associated with a lower risk[351]. Individuals with ASCVD show a distinct pattern of circulating amino acids, where BCAAs are increased, and glycine is decreased in association with disease severity[171–173,352,353]. Although it was shown that increased BCAAs drive glycine depletion through activation of the pyruvate–alanine cycle[354], recent isotope tracing studies revealed that increased synthesis of serine through reversed serine hydroxymethyltransferase activity drives glycine depletion independent of BCAAs[355]. Consistent with its lower levels in coronary artery disease, glycine depletion enhances, whereas glycine-based treatment reduces, atherosclerosis in *Apoe*$^{-/-}$ mice through lipid-lowering effects and inducing de novo GSH biosynthesis in macrophages[173,356]. The role of BCAAs, however, is controversial[357,358]. Supplementation of BCAAs or leucine alone lowers atherosclerosis in *Apoe*$^{-/-}$ mice by reducing LDL cholesterol and pro-atherogenic/inflammatory chemokines[359,360]. In lipid-laden macrophages, leucine reduces intracellular cholesterol and triglycerides by increasing cholesterol efflux and inhibiting very-low-density lipoprotein uptake[361,362]. By contrast, leucine activates mTORC1 in macrophages and synergizes with atherogenic lipids to enhance mitochondrial dysfunction and apoptosis[347]. Thus, although higher circulating BCAAs are linked with ASCVD, dysregulated BCAA metabolism and the effects of specific BCAAs on different cell types of the atherosclerotic plaque warrant further research.

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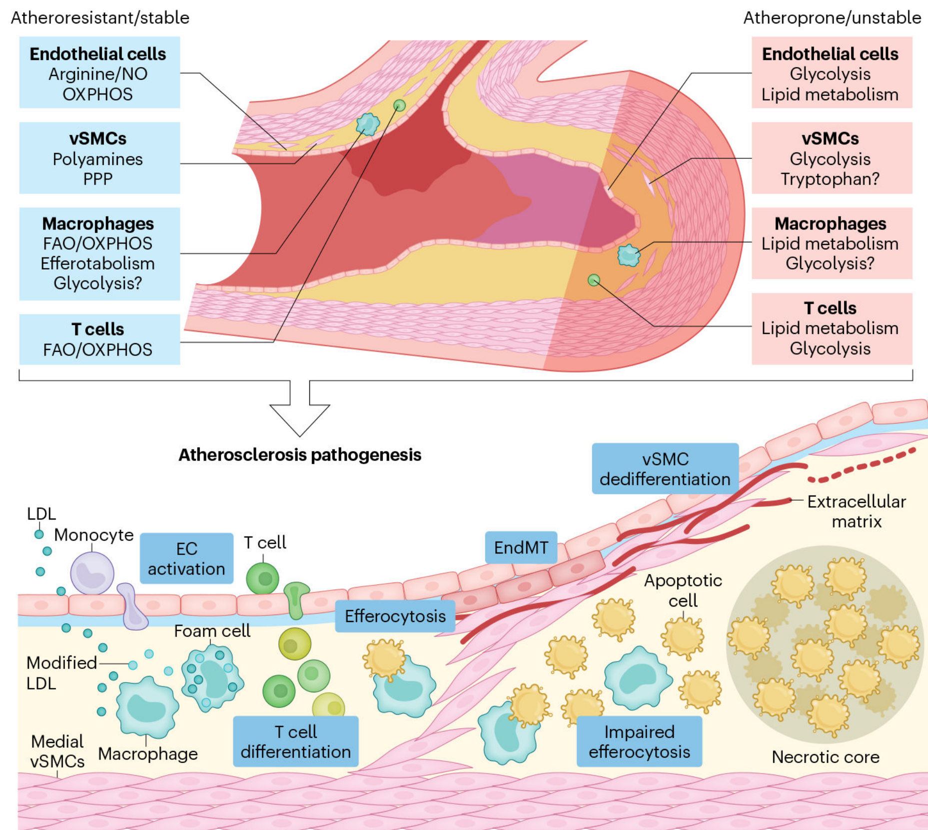

Fig. 1 |. Cellular events leading to the inception, progression and manifestation of cardiovascular events.

Top, Metabolic pathways upregulated in atheroresistant/stable disease areas are in blue, and pathways enriched in atheroprone/unstable disease areas are in red. Bottom, Circulating monocytes bind to adhesion molecules presented on the surface of activated ECs and transmigrate into the vessel wall. Monocytes then mature into macrophages and become foam cells, which can later become apoptotic due to uncontrolled uptake of modified LDL. In early atherosclerosis, these dead cells are efficiently cleared by macrophages. However, as atherosclerosis advances, the capacity for AC removal becomes impaired and promotes necrotic core formation. T cells similarly extravasate into the vessel wall and differentiate into subsets that influence the function of lesional cells. Upon a variety of atherogenic insults, medial vSMCs dedifferentiate and migrate towards the intima, where they initially assemble extracellular matrix in the fibrous cap. In addition, vSMCs can also derive from ECs that have undergone a mesenchymal transition. At later phases of atherosclerosis, vSMCs can adopt multiple cellular phenotypes that can destabilize the fibrous cap and drive the formation of rupture-prone atheromas.

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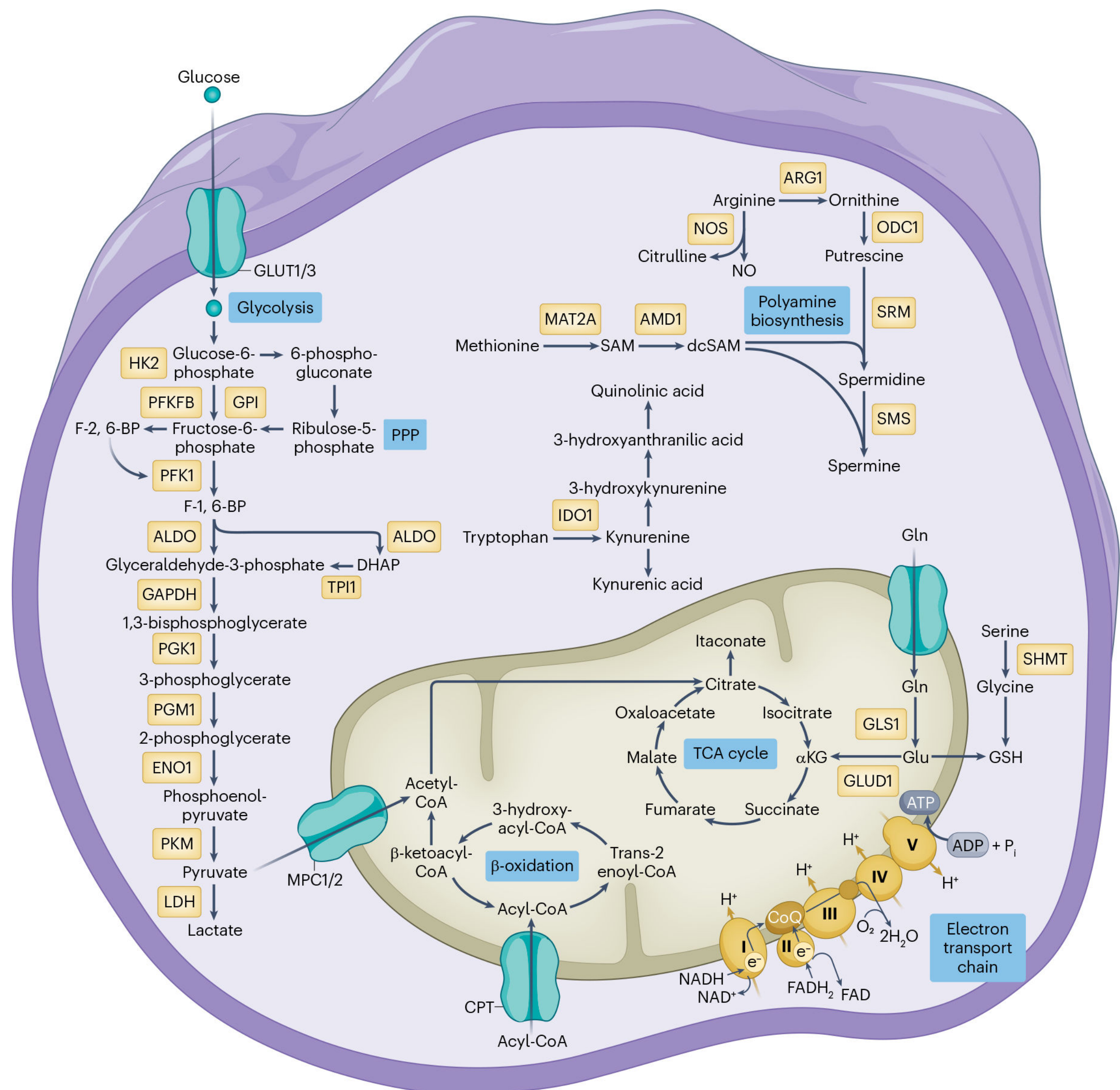

Fig. 2 |. Metabolic pathways in lesional cells relevant to atherosclerosis.

Glucose is transported through the GLUT1 transporter and then proceeds down the glycolysis pathway. This pathway can give rise to lactate or feed into the TCA cycle. Pyruvate, a product of glycolysis, can enter the mitochondria through MPC1 or MPC2 and be converted to acetyl-CoA, which then enters the TCA cycle. Simultaneously, acyl-CoA can be transported into the mitochondria through the carnitine palmitoyltransferase (CPT) 1A and 2 enzymes and enter β-oxidation, generating acetyl-CoA that also feed into the TCA cycle. By-products of these pathways also fuel electron transport chain activity. Glutaminolysis occurs through the enzymes GLS1 and GLUD1, which generate α-ketoglutarate. Additionally, arginine can be converted into NO through the NOS enzymes (eNOS, nNOS or iNOS) or into the polyamine biosynthetic pathway, which also relies on methionine metabolism. Tryptophan is degraded by IDO1 into kynurenine and generates kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic

acid or quinolinic acid. Serine is converted to glycine by SHMT1 or SHMT2 and gives rise the formation of the potent anti-oxidant GSH. GLUT1, glucose transporter 1; HK2, hexokinase 2; GPI, glucose-6-phosphate isomerase; PFKFB, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatases; PFK1, phosphofructokinase-1; TPI1, triosephosphate isomerase 1; ALDO, aldolase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PGK1, phosphoglycerate kinase 1; PGM1, phosphoglycerate mutase 1; ENO1, enolase 1; PKM, pyruvate kinase isozymes M1/M2; LDH, lactate dehydrogenase; MPC1/MPC2, mitochondrial pyruvate carriers 1 and 2; SHMT, serine hydroxymethyltransferase; IDO1, indoleamine 2,3-dioxygenase 1; ODC1, ornithine decarboxylase; SRM, spermidine synthase; SRM, spermine synthase; MAT2A, methionine adenosyltransferase 2A; AMD1, adenosylmethionine decarboxylase 1; GLUD1, glutamate dehydrogenase 1.

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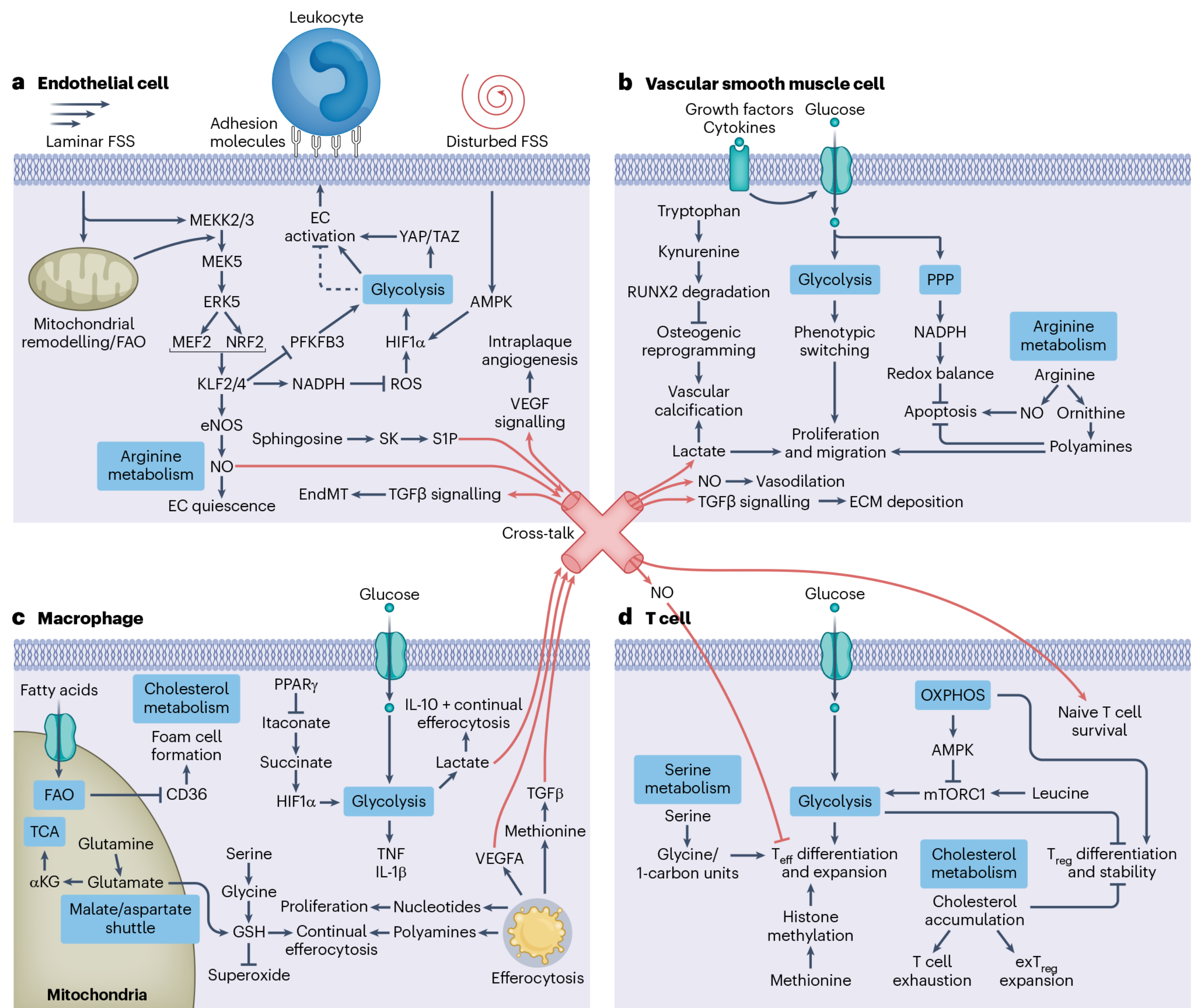

Fig. 3 |. Metabolic pathways in lesional cells and their consequences.

a, Unilaminar FSS induces the expression of transcription factors KLF2 and KLF4, which promote eNOS-mediated NO production, suppressing NF-κB activation and endothelial permeability. Mechanistically, FSS induces KLF2/KLF4 through the MEKK2/MEKK3–MEK5–ERK5 signalling cascade. FSS also decreases glycolysis in a KLF2-dependent manner, supporting mitochondrial metabolism, NADPH production and redox homeostasis. By contrast, disturbed FSS enhances glycolysis through HIF1α, contributing to EC activation. However, some reports indicate a complicated role for glycolysis in EC activation (dashed line). **b**, Growth factors and cytokines stimulate glucose uptake and promote glycolysis, resulting in dedifferentiation and phenotypic switching. Additionally, the PPP helps to maintain redox balance and inhibits apoptosis, which is counteracted by NOS-mediated NO. Alternatively, arginine metabolism into the polyamine biosynthetic pathway drives proliferation, migration and collagen deposition. IDO1-mediated kynurenine synthesis suppresses osteogenic reprogramming of vSMCs by stimulating the degradation of RUNX2, thereby restraining vascular calcification. **c**, Glycolysis stimulates pro-

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inflammatory cytokine secretion. However, lactate simultaneously drives a robust pro-resolving response—stimulating both IL-10 and continual efferocytosis. FAO prevents foam cell formation by suppressing CD36 expression and reducing foam cell formation. GSH synthesis drives continual efferocytosis and lowers superoxide levels. Efferotabolism of AC-derived cargo further stimulates continual efferocytosis, drives TGFβ production, and expands pro-resolving macrophages through the process known as 'EIMP'. **d**, mTORC1-mediated glycolysis, serine metabolism into glycine and one-carbon units, and methionine-mediated histone methylation support the differentiation and expansion of T_{eff} cells. Additionally, cholesterol accumulation destabilizes T_{reg} cell differentiation and promotes T cell exhaustion and exT_{reg} cell expansion. OXPHOS stimulates AMPK-mediated repression of glycolysis and drives T_{reg} cell differentiation and stability. Cross-talk pathways driven by metabolites and soluble factors are shown in red.

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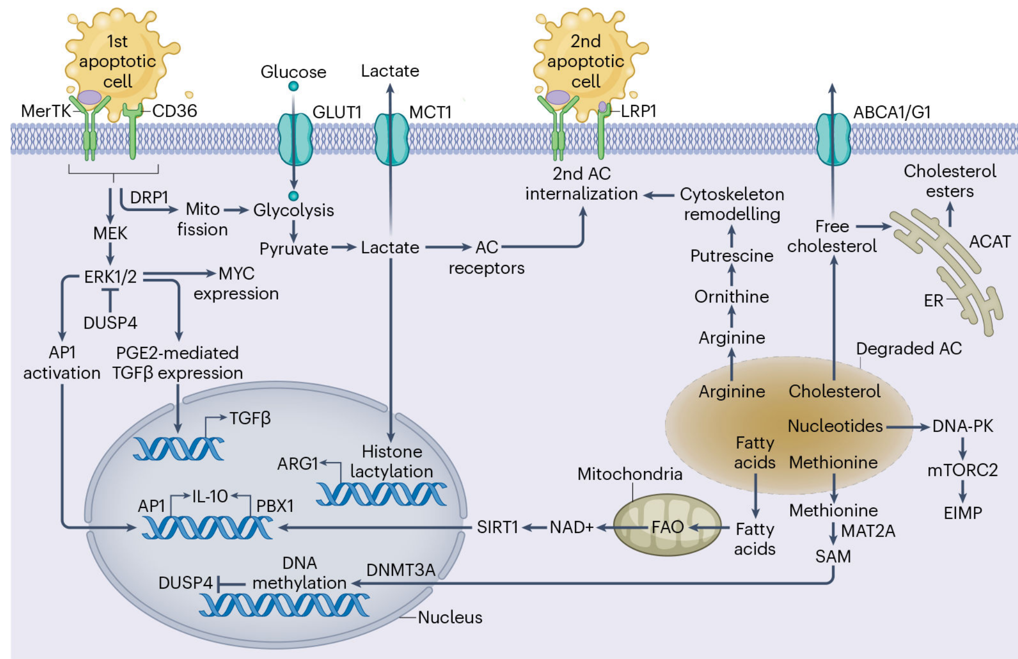

Fig. 4 |. Current understanding of efferocytosis, the metabolism of AC-derived cargo and consequences thereof.

Binding of an AC to a macrophage activates cell-surface receptors that drive ERK1/ERK2 activation and DRP1-mediated mitochondrial fission. Free cholesterol from a degraded AC is either esterified by ACAT in the ER or exported by the cholesterol transporters ABCA1 and ABCG1. Methionine from a degraded AC is converted to SAM, which is used for DNA methylation that subsequently suppresses *DUSP4* expression. This lifts repression of ERK1/ERK2 activation. ERK1/ERK2 signalling drives MYC expression, PGE2-mediated TGFβ production and AP1-mediated IL-10 secretion. AC-derived arginine is converted into putrescine and triggers cytoskeleton remodelling and continual efferocytosis. Recycling of AC-derived nucleotides mediates EIMP through a DNA-PK–mTORC2 signalling cascade. Fatty acids derived from ACs are used for FAO that promotes NAD^{+}–SIRT1–PBX1 signalling that drives IL-10 expression. Concurrently, GLUT1-mediated glucose uptake promotes glycolysis and lactate production. Lactate is secreted from the macrophage via MCT1 to prime a pro-resolving microenvironment. Guided by mitochondrial fission, lactate is utilized for histone lactylation, which drives an epigenetic programme that promotes ARG1 expression.