Cardiovascular cells that contribute directly to atherosclerosis and cardiac dysfunction are known to exhibit metabolic flexibility, characterized by the ability to switch from generating ATP primarily through oxidative phosphorylation to using glycolysis as the predominate energy source, and to shift from one fuel source to another. This flexibility occurs in endothelial cells (ECs), myeloid cells, and cardiomyocytes during normal development and physiology, and is thought to have evolved to protect cells with heightened energy demand from the increased oxidative stress that can be a result of oxidative phosphorylation, to shunt glucose to side branches of the glycolysis pathway, to provide energy more rapidly, or to use the most abundant fuel available. With the growing problem of systemic nutrient overload and associated insulin resistance, type 2 diabetes mellitus, and nonalcoholic fatty liver disease, metabolic flexibility and dysfunction in cells involved in cardiovascular disease have received increased attention as possible contributors to systemic inflammation and cardiovascular risk associated with these states.

Systemic insulin resistance is thought to be due primarily to nutrient overload in skeletal muscle and liver as a consequence of an inability of adipose tissue to store excess nutrients in the form of triacylglycerol-rich lipid droplets, and a subsequent increase in detrimental lipid species in liver and skeletal muscle, which are inadequately equipped to store large amounts of lipids. Accumulation of noxious lipids leads to dysfunction in liver and skeletal muscle cells characterized by insulin resistance, increased activation of the unfolded protein response, and increased production of inflammatory mediators. The lipid mediators most likely responsible are diacylglycerols and ceramides, which are associated with insulin resistance in these tissues. Insulin resistance is well known to be associated with increased cardiovascular risk. Furthermore, accumulation of hepatic lipids in subjects with nonalcoholic fatty liver disease is associated with vascular dysfunction.

Hyperlipidemia is closely linked to nutrient overload and insulin resistance and is a major contributor to cardiovascular disease. Increased intestinal nutrient handling is one process that contributes to dyslipidemia. For example, intestinal biopsies from obese insulin-resistant human subjects exhibit exaggerated triglyceride-rich lipoprotein (TRL) production, when compared with obese insulin-sensitive subjects, through a mechanism that may involve reduced insulin signaling in the intestine. Fructose was found to be particularly apt to increase TRLs in human subjects. The liver takes up TRLs and, in turn, produces very low-density lipoproteins (VLDL) through the action of the enzyme acyl-CoA:diacylglycerol acyltransferase 2. Increased VLDL and reduced high-density lipoprotein cholesterol levels are characteristics of metabolic syndrome and diabetes mellitus.

Hyperglycemia occurs in subjects with metabolic syndrome and diabetes mellitus and is often associated with dyslipidemia and other cardiovascular risk factors and endothelial dysfunction. It is still unclear to what extent hyperglycemia, directly or indirectly, contributes to cardiovascular disease in human subjects. It is, however, becoming increasingly evident that glucose metabolites play important regulatory roles in cellular activation, which is often dysfunctional in diabetes mellitus.

Is the cardiovascular disease associated with metabolic syndrome and type 2 diabetes mellitus explained by systemic factors, such as low-grade inflammation, increased adiposity, defective insulin signaling, hypertension, and dyslipidemia, or do metabolic flexibility and dysfunction in vascular and cardiac cells themselves contribute to cardiovascular pathologies? Recent advances in the research area of metabolism in cell types involved in cardiovascular disease are highlighted in this article, with special emphasis on recent research published in *ATVB*.

**Metabolic Flexibility and Dysfunction in Myeloid Cells**

The primary immune cell involved in atherosclerotic lesions is the macrophage. Lesion macrophages are well known to accumulate lipids and become lipid droplet-filled foam cells. Furthermore, a recent study published in *ATVB* demonstrates that >10% of circulating monocytes accumulate lipids from VLDL and become monocyte foam cells as early as 3 days after initiation of fat feeding in apolipoprotein E–deficient mice. This study suggests that the process of lipid accumulation in myeloid cells in response to severe dyslipidemia is initiated early and in circulating cells. The macrophage is able to handle accumulation of large amounts of lipids, mainly in the form of cholesterol esters in lipid droplets. Although lipid accumulation in macrophages was long thought to induce inflammatory activation of these cells, elegant studies have revealed that lipid loaded macrophages do in fact produce less inflammatory mediators than nonlipid loaded cells, in part,
through increased anti-inflammatory actions of liver X receptors (LXRs).21

Rather than foam cell formation per se, imbalances among lipoprotein uptake, intracellular cholesterol handling, and cholesterol efflux are likely to be responsive for metabolic and inflammatory activation of myeloid cells that promote atherosclerosis. Such mechanisms include endoplasmic reticulum stress and apoptosis induced by excess free cholesterol, inflammasome activation, and lysosomal dysfunction induced by cholesterol crystals, myelopoesis, and increased activation of toll-like receptors by accumulation of cholesterol in lipid raft domains of membranes.22,23 The ATP-binding cassette transporters, ABCA1 and ABCG1, maintain cholesterol homeostasis in macrophages by exporting free cholesterol to apolipoprotein A–I and high-density lipoprotein, and accordingly, loss of ABCA1 and ABCG1 causes cholesterol accumulation and inflammatory activation of these cells.22 Furthermore, it was recently demonstrated that human subjects with mutations in ABCA1 exhibit increased inflammatory markers systemically and in atherosclerotic lesions.24 Consistent with the anti-inflammatory actions of LXRs, activation of LXRs results in increased levels of ABCA1 and ABCG1.22 However, the ability of LXR agonists to suppress inflammation and atherosclerosis does not require myeloid cell expression of ABCA1 or ABCG1,25 nor is LXR required for the ability of macrophages to export cholesterol in vivo.26 These studies demonstrate that the mechanism of cholesterol export and LXR effects are in part distinct, and that LXR agonists induce an anti-inflammatory and antiatherosclerotic state, in part, independently of macrophage cholesterol export. One such proposed mechanism involves induction of polyunsaturated fatty acid synthesis in macrophages.27

Together, there is strong evidence that nutrient excess in the form of cholesterol directly promotes inflammatory activation and death of myeloid cells, and that this, in turn, worsens atherosclerosis and necrotic core formation in lesions. Interestingly, a recent ATVB article shows that cholesterol loading of arterial smooth muscle cells can induce a macrophage-like phenotype that might contribute to lesion progression. This conversion is mediated by the micro-RNA miR-143/145, which positively regulates the master smooth muscle differentiation transcription factor myocardin.28

Does cholesterol accumulation or systemic metabolic dysfunction induce altered metabolism in macrophages? In an interesting series of experiments, Gautier et al29 demonstrated that cholesterol accumulation in myeloid cells because of ABCA1 deficiency and ABCG1 deficiency resulted in increased glucose use and proliferation. This effect was attributed to the increased inflammatory activity of ABCA1-/ABCG1-deficient cells. Furthermore, inhibiting the glucose transporter GLUT1 prevented inflammation and proliferation of myeloid cells.29 It is, therefore, likely that cholesterol accumulation and low-grade inflammation, such as observed in metabolic syndrome and diabetes mellitus can induce metabolic changes in macrophages.

Aerobic glycolysis is essential to the activation of many types of immune cells, including macrophages. Resting macrophages primarily derive their energy from oxidative phosphorylation30; however, during activation, macrophages undergo metabolic reprogramming. Classically activated inflammatory (M1) macrophages stimulated with lipopolysaccharide and interferon-γ in vitro markedly increase their aerobic glycolysis.1 Conversely, alternative activated (M2) macrophages induced by interleukin-4 stimulation in vitro use fatty acid oxidation (FAO) to fuel their longer-term tissue repair and healing functions, at least in the mouse.1 Accordingly, lipopolysaccharide and other inflammatory molecules induce expression of GLUT1 and enzymes promoting glycolysis, such as hexokinase and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3).31,32 Furthermore, GLUT1 and glycolysis are required for inflammatory activation of macrophages.1,32 However, although increased glucose uptake and glycolysis clearly is an integral part of inflammatory activation of macrophages, forcing macrophages to increase glycolysis by overexpressing GLUT1 can increase cytokine production in cell lines in vitro, but does not result in inflammatory activation or increased atherosclerosis in LDL receptor–deficient (Ldlr−/−) mice.31,32 A recent article published in ATVB has expanded our knowledge of how glycolysis is regulated during inflammatory activation. Tawakol et al33 demonstrated that glycolysis and proinflammatory activation in macrophages depend on the transcription factor hypoxia-inducible factor-1α, which in turn is required for PFKFB3 induction and cytokine production. Furthermore, inhibition of glycolysis in activated, but not resting, macrophages induced cell death. Inhibition of PFKFB3 in vivo produced a similar effect (reduced cytokine expression and increased caspase 3 activity as a marker of apoptosis) in lesions of atherosclerosis of apolipoprotein E–deficient mice.

Together, these studies suggest that excess cholesterol accumulation in macrophages results in inflammatory activation associated with increased glucose use. Increased glucose uptake and glycolysis are required for cytokine production, proliferation, and survival of activated inflammatory macrophages, but glycolysis is not sufficient to drive inflammatory activation and atherosclerosis in nonactivated myeloid cells. The relevance of these findings to metabolic disease in humans needs to be evaluated.

**Metabolic Flexibility and Dysfunction in ECs**

In healthy adults, ECs are quiescent and maintain barrier function and tissue homeostasis. Quiescent ECs derive most of their energy from glycolysis.2 They maintain the capacity to quickly form new vasculature in response to angiogenic factors induced by injury or in pathological conditions, such as hypoxia, nutrient deprivation, or tissue damage. When stimulated to revascularize tissues, ECs undergo a rapid increase of glycolytic flux.2 Recent studies have highlighted the role of the glycolytic enzyme PFKFB3 in both angiogenesis and pathological neovascularization.34 De Bock et al35 showed that PFKFB3 is a key regulatory enzyme in glycolysis in ECs, as in many other cells. Silencing PFKFB3 reduces EC proliferation, migration, and vessel sprouting both in vitro and in vivo.35,36

Conversely, recent research has revealed a role of PFKFB3 suppression in maintaining ECs at a resting state under laminar shear stress. Laminar shear stress promotes anti-inflammatory, antithrombotic, and antioxidative properties in ECs.
and helps maintain quiescence largely via the transcription factor Krüppel-like factor 2 (KLF2). Doddaballapur et al.\textsuperscript{46,47} reported that laminar shear stress and KLF2 reduce EC glucose uptake and glycolysis. Gene expression of multiple enzymes involved in glycolysis, including PFKFB3, hexokinase-2, and phosphofructokinase platelet isoform 1, were significantly lowered. This effect of shear stress was mediated by KLF2 in part by inhibition of PFKFB3 promoter activity. Thus, KLF2 overexpression reduced glucose uptake, glycolysis, mitochondrial content, and basal mitochondrial respiration. Overexpression of PFKFB3 partially restored glycolysis and sprouting in KLF2-overexpressing ECs. This study sheds new light on the importance of PFKFB3 suppression to maintain EC quiescence. Because high-glucose exposure can suppress EC KLF2 expression, it is possible that systemic metabolic dysfunction counteracts the ability of KLF2 to prevent glycolysis and activation of ECs, which could potentially contribute to cardiovascular dysfunction. Elevated glucose has been shown to increase several pathways and processes likely to be pathological in ECs, including reactive oxygen species levels, glycosylation and advanced glycation endproducts, the polyl pathway, and to alter gene expression.\textsuperscript{41,42} These studies are primarily based on in vitro studies. However, activation of the polyl pathway by adole reductase overexpression in ECs results in increased atherosclerosis in diabetic apolipoprotein E–deficient mice,\textsuperscript{43} suggesting that increased EC glucose flux through this pathway promotes atherosclerosis. It is still uncertain to what extent hyperglycemia directly alters EC metabolism in vivo.\textsuperscript{44}

An increased dependence on glycolysis is also a characteristic of pulmonary arterial ECs in pulmonary hypertension, a fatal disease characterized by EC proliferation, oblitative vascular remodeling in the lungs, and a progressive increase in pulmonary artery pressure.\textsuperscript{45} It has recently been proposed that the altered EC phenotype may be attributed to suppressed glucose oxidation and upregulated glycolysis through mitochondrial remodeling.\textsuperscript{46} A study published in \textit{ATVB} used intermittent hypoxia exposure of ECs as a model of pulmonary hypertension to study the role of mitochondrial uncoupling protein 2 in ECs in mice.\textsuperscript{47} This study demonstrated that EC-targeted uncoupling protein 2-deficiency resulted in higher right ventricular systolic pressure accompanied by increased mitophagy, decreased mitochondrial biogenesis, and increased apoptosis. Importantly, pulmonary artery ECs from patients with pulmonary hypertension showed a phenotype similar to the uncoupling protein 2-deficient ECs, suggesting a role for mitophagy in ECs in pulmonary hypertension.

Accumulation of the TCA cycle intermediate succinate in ischemic tissues has been reported to play an important role in angiogenesis and pathological retinal neovascularization through interacting with G-coupled protein receptor (GPR) 91.\textsuperscript{46,47} GPR91 is expressed in highly vascularized tissues, however, it is not expressed on ECs.\textsuperscript{46,47} Succinate binds to GPR91, often on neuronal cells, which mediates the release of several angiogenic factors, including vascular endothelial growth factor (VEGF). Recent studies have elucidated the role of succinate in murine models of cerebral hypoxia/ischemia revascularization and diabetic retinopathy.\textsuperscript{48,49} Using GPR91-deficient mice, Hamel et al.\textsuperscript{48} demonstrated increased succinate levels in close proximity to brain infarcts, and GPR91 enhanced microvascular density and reduced infarct size. The effect of succinate on VEGF release was mediated by GPR91 and prostaglandin E\textsubscript{2}. In the case of diabetic retinopathy,\textsuperscript{49} excessive succinate levels in the eye are thought to contribute to pathological neovascularization associated with diabetes mellitus. Succinate levels were increased in retinas of diabetic rats. GPR91 was primarily localized to the retinal ganglion cells, and knockdown of GPR91 lowered prostaglandin E\textsubscript{2} release and VEGF protein levels in the retina and protected diabetic rats from developing dysfunctional retinal vasculature. Additional studies will be needed to dissect the metabolic cross-talk between different cell types.

FAO also plays important roles in ECs. For example, FAO maintains integrity of the EC layer, and loss of activity of carnitine palmitoyltransferase-1A, which shuttles long-chain fatty acids into mitochondria for oxidation, induces hyperpermeability.\textsuperscript{50} Furthermore, Schoors et al.\textsuperscript{51} have recently reported a new role for FAO in ECs. Loss of carnitine palmitoyltransferase-1A decreased vessel sprouting in vitro and in vivo because of a reduction in EC proliferation. Using a series of elegant experiments, the authors demonstrated that a reduction of FAO in ECs did not cause energy depletion or disturb redox homeostasis, but instead that fatty acid carbons were required for replenishment of TCA cycle intermediates used for de novo nucleotide synthesis, and that FAO is required for efficient DNA replication and EC proliferation. Complete inhibition of FAO with etomoxir (an irreversible carnitine palmitoyltransferase-1 inhibitor) reduced vessel sprouting in vivo, strongly suggesting an exciting new role for FAO in angiogenesis.

In addition to providing energy through oxidation, fatty acids exert many other effects in ECs. For example, exposure of ECs to excess saturated fatty acids leads to expression of adhesion molecules and chemokines involved in atherosclerosis,\textsuperscript{52} and omega-3 polyunsaturated fatty acids exert effects on angiogenesis.\textsuperscript{53} In addition, intracellular fatty acid handling in ECs plays important roles in EC proliferation and angiogenesis, and perhaps in transendothelial transport of fatty acids to underlying tissues. The fatty acid-binding protein (FABP)-4 is required for EC proliferation and VEGF-induced neovascularization.\textsuperscript{54,55} Loss of FABP4 most likely acts by increasing free unbound fatty acids intracellularly or by preventing chaperoning to correct intracellular locale. Recently, Iso et al.\textsuperscript{56} demonstrated that capillary ECs in fatty acid consuming tissues, such as the heart and skeletal muscle, express FABP4 and FABP5 and that FABP4 and FABP5 facilitate transport of fatty acids into these tissues.\textsuperscript{56} Loss of both FABP4 and FABP5 reduced fatty acid uptake and increased glycolysis in the heart and skeletal muscle. Importantly, fatty acid uptake was not altered in isolated cardiomyocytes ex vivo, suggesting that ECs can transport fatty acids via FABP4 and FABP5 to cardiomyocytes.

Together, these studies highlight the role of EC glycolysis, its branch pathways, and FAO, in angiogenesis, pathological neovascularization, and atherosclerosis, and the response of ECs to dysfunctional metabolism.
Metabolic Flexibility and Dysfunction in Cardiac Cells

The human heart, our most energy-requiring organ, relies to a large extent on FAO to meet its energy needs, but can use glucose, lactate, amino acids, and ketones when needed. The metabolic flexibility of the adult heart allows it to switch between different energy sources, for example, to use more lactate during increased workload when lactate is produced by skeletal muscle. However, changes in cardiomyocyte metabolism might lead to heart failure and cardiomyopathy. Thus, in response to pathological cardiac hypertrophy, the heart uses relatively more glucose through glycolysis, and diabetes mellitus is associated with excessive fatty acid uptake, utilization, and lipid accumulation, which are thought to contribute to diabetic cardiomyopathy. The mechanism whereby fatty acid uptake into the heart is increased by diabetes mellitus is likely multifactorial. One probable contributor is an increased availability of circulating free fatty acids or fatty acids stored as triacylglycerols in TRLs and VLDL. The triacylglycerols in TRLs and VLDL are hydrolyzed by lipoprotein lipase for uptake of fatty acids into the heart. Another contributor might be the release of factors from ECs that mediate release of lipoprotein lipase from cardiomyocytes in diabetes mellitus.

Several studies have demonstrated that triacylglycerol accumulation, or accumulation of the triacylglycerol precursor diacylglycerol or other lipids, such as ceramides, is toxic to the heart and promotes cardiomyopathy. The exact mechanism whereby cardiac toxicity is induced by lipids is still debated. One theory is that excess mitochondrial activity and reactive oxygen species production is responsible, in part, for cardiomyopathy and sudden cardiac death due to arrhythmia. This concept is supported by a recent study by Wang et al demonstrating that mice deficient in neuropilin-1 in cardiomyocytes and smooth muscle cells exhibited cardiomyopathy and sudden cardiac death due to arrhythmia.

In the context of metabolism and the heart, it is noteworthy that alteration of mesenchymal stem cell metabolism has generated interest as a possible strategy to increase survival of these cells in ischemic cardiac tissue. Zhu et al recently demonstrated that increased glycogen storage in mouse mesenchymal stem cells by hypoxic preconditioning improved their subsequent survival in ischemic muscle, presumably because of increased glycogen stores, which can be used as an energy source through glycogenolysis and subsequent glycolysis.

Further studies are needed to elucidate the role of cardiac metabolism in cardiovascular disease associated with metabolic dysfunction in humans.

Summary

Recent articles published in ATVB and elsewhere have highlighted the existence and role of metabolic flexibility and dysfunction in cell types directly involved in atherosclerosis and heart disease. These studies underscore the importance of cardiovascular cellular metabolism to cardiovascular disease, and will no doubt lead to new research discoveries in the area of cardiovascular metabolism in metabolic and inflammatory diseases.

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None.

References


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