ANNUAL REVIEWS

Annual Review of Physiology

Metabolic Pathways Fueling the Endothelial Cell Drive

Xuri Li,[1] Anil Kumar,[1] and Peter Carmeliet[1,2,3]

[1]State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou 510060, China; email: peter.carmeliet@kuleuven.vib.be, lixr6@mail.sysu.edu.cn

[2]Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, KU Leuven, Leuven B-3000, Belgium

[3]Laboratory of Angiogenesis and Vascular Metabolism, Center for Cancer Biology, VIB, Leuven B-3000, Belgium

Annu. Rev. Physiol. 2019. 81:483–503

The *Annual Review of Physiology* is online at physiol.annualreviews.org

https://doi.org/10.1146/annurev-physiol-020518-114731

Keywords

angiogenesis, endothelial metabolism, PFKFB3, CPT1a, GLS, glutaminase, ASNS, asparagine synthetase

Abstract

Endothelial cell (EC) metabolism is important for health and disease. Metabolic pathways, such as glycolysis, fatty acid oxidation, and amino acid metabolism, determine vasculature formation. These metabolic pathways have different roles in securing the production of energy and biomass and the maintenance of redox homeostasis in vascular migratory tip cells, proliferating stalk cells, and quiescent phalanx cells, respectively. Emerging evidence demonstrates that perturbation of EC metabolism results in EC dysfunction and vascular pathologies. Here, we summarize recent insights into EC metabolic pathways and their deregulation in vascular diseases. We further discuss the therapeutic implications of targeting EC metabolism in various pathologies.

INTRODUCTION

Blood vessels are the largest interconnecting organ; they supply nutrition and oxygen to all tissues (1). They comprise a thin layer of connected endothelial cells (ECs), which remain quiescent for long periods. Under ischemia or inflammation, ECs can quickly shift to an angiogenic mode to form new blood vessels. The process by which new blood vessels form involves EC differentiation to migratory tip cells, the proliferation of stalk cells for sprout elongation and, when the vessel is formed and perfused, lining the established perfused blood cells with quiescent phalanx cells (2). Blood vessels are involved in vasomotor regulation, leukocyte and platelet adhesion and aggregation, the secretion of perivascular cell–stimulating angiocrine factors, the formation of new blood vessels, and barrier function (3, 4).

Our understanding of the molecular mechanisms underlying the formation of blood vessels has improved substantially over the past decades (5–7). Emerging evidence suggests that metabolic pathways play a crucial role in EC adaptation to support particular functions. EC metabolic maladaptations are observed in various pathologies, including atherosclerosis, diabetes, neovascular eye disease, and cancer, and understanding the underlying metabolic perturbations is crucial to identifying EC metabolism-centric therapeutics (8, 9). Accordingly, the major goals of this review are (*a*) to describe EC-specific metabolic pathways and (*b*) to summarize recent evidence linking particular diseases to metabolic pathway disruptions, with an emphasis on the therapeutic implications of these studies. **Figure 1** provides an overview of the EC metabolic pathways discussed in this review.

GLYCOLYSIS AND ITS SIDE PATHWAYS

ECs in normal healthy adults are quiescent in nature, but they can rapidly switch gears to an intense proliferative mode during angiogenesis (5). EC proliferation relies on glycolysis, and 85% of ATP is generated by converting glucose to lactate in ECs (10). In contrast to other healthy cell types, EC glucose consumption by the glycolytic pathway is similarly high as in many cancer cells (10). Since oxidative metabolism of a glucose molecule yields more ATP molecules than anaerobic glycolytic metabolism (10), it is puzzling that quiescent ECs do not utilize oxygen, despite being in direct contact in the blood. This might be a mechanism to protect ECs from oxidative damage by keeping reactive oxygen species (ROS) levels in check (11). In addition, by utilizing anaerobic metabolism, ECs facilitate oxygen diffusion to perivascular cells (11, 12). During sprouting, tip cells migrate into a hypoxic microenvironment, where they cannot rely on oxidative metabolism, but instead must utilize anaerobic metabolism. Additionally, glycolysis produces ATP faster, allowing rapid neovascularization of hypoxic tissues (10).

During angiogenesis, the glycolytic pathway is initiated by glucose uptake into ECs via membrane-bound glucose transporters (GLUTs) (13). Glucose is then phosphorylated to glucose-6-phosphate by hexokinase 2 (HK2), a rate-controlling enzyme (14). When ECs are exposed to high-glucose levels, glycogen synthesis and the glycogen content increase, while under low-glucose conditions, the glycogen content in ECs becomes depleted (15). EC migration and viability are reduced by the inhibition of glycogen phosphorylase (GP), which catalyzes glycogen breakdown (15), suggesting that ECs use glycogen as a reserve fuel when proliferating or migrating in a low-glucose environment.

Endothelial glycolysis is activated by the key regulator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) by generating fructose-2,6-biphosphate, an allosteric activator of phosphofructokinase 1 (PFK1), another rate-controlling enzyme of glycolysis (10). Both migratory tip cell and proliferative stalk cell phenotypes rely on glycolysis. Interestingly, in quiescent

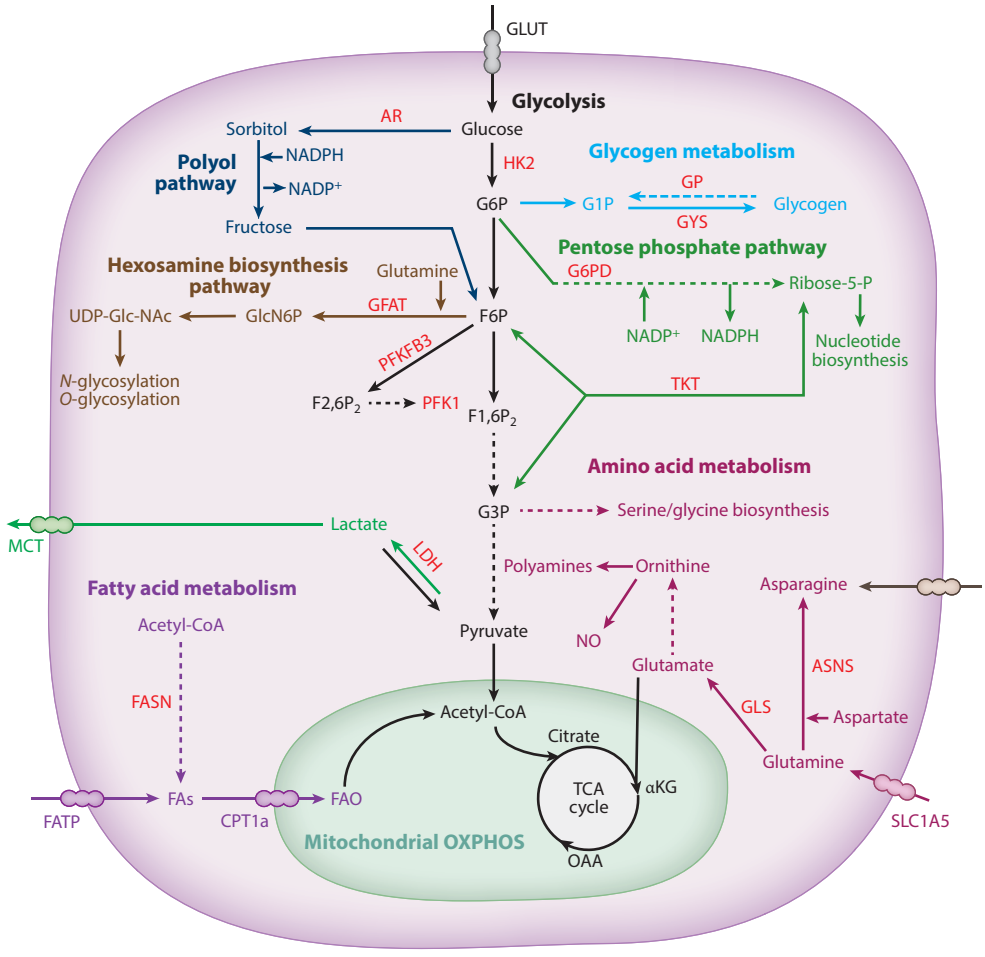

Figure 1

Schematic representation of the metabolic pathways of an endothelial cell controlling its function, as discussed in this review. Individual pathways and names are highlighted by specific colors, and enzymes are denoted by red fonts. Dashed arrows indicate the involvement of additional intermediate reactions, which are not depicted for clarity. Abbreviations: αKG, α-ketoglutarate; AR, aldose reductase; ASNS, asparagine synthetase; CPT1a, carnitine palmitoyltransferase 1a; $F1,6P_2$, fructose 1, 6-biphosphate; $F2,6P_2$, fructose 2, 6-biphosphate; F6P, fructose 6-phosphate; FA, fatty acid; FAO, fatty acid oxidation; FATP, fatty acid transport protein; FASN, fatty acid synthase; G1P, glucose 1-phosphate; G3P, glyceraldehyde 3-phosphate; G6P, glucose 6-phosphate; G6PD, glucose 6-phosphate dehydrogenase; GFAT, glutamine fructose-6-phosphate aminotransferase; GlcN6P, glucosamine-6-phosphate; GLS, glutaminase; GLUT, glucose transporter; GP, glycogen phosphorylase; GYS, glycogen synthase; HK2, hexokinase 2; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; $NADP^+$, oxidized nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OAA, oxaloacetate; OXPHOS, oxidative phosphorylation; PFK1, phosphofructokinase 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; SLC1A5, solute carrier family A1 member 5; TCA, tricarboxylic acid; TKT, transketolase; UDP-Glc-NAc, uridine diphosphate-*N*-acetylglucosamine.

ECs, glycolysis occurs in the perinuclear cytosol, but once they start to migrate, glycolysis also takes place in lamellipodia and filopodia, where it associates to the actin cytoskeleton to facilitate high local production of ATP needed for actin cytoskeleton remodeling during migration (compartmentalization of glycolysis) (10). Quiescent phalanx cells, in contrast, have reduced levels of glycolysis, in part through suppression by laminar shear stress from blood flow (see below).

Pharmacological inhibition or genetic silencing of *PFKFB3* in ECs impairs EC sprouting in vitro and postnatal murine retinal vasculature development in vivo (10, 16). Conversely, overexpression of *PFKFB3* results in increased glycolysis with a protip cell phenotype in EC spheroids, even overruling the prostalk cell cue NOTCH1 during retinal vascular development, highlighting the importance of glycolytic flux for the tip EC phenotype (15).

Pyruvate kinase (PK) is another rate-controlling enzyme of glycolysis, which converts phosphoenolpyruvate (PEP) to pyruvate. There are four distinct tissue-specific isoforms of PK. In healthy adult and malignant cells and in embryonic cells, the M2 isoform (PKM2) can exist as a dimer or a tetramer (17, 18). Tetrameric PKM2, which has a high affinity for PEP, drives glycolytic ATP production, while dimeric PKM2 has low affinity for PEP. This reduces its conversion to pyruvate, pushing glycolytic intermediates to glycolytic side pathways in part for biomass synthesis (17). Hence, in highly proliferative cells, PKM2 shifts to the dimeric form (18, 19), explaining why silencing of PKM2 in ECs reduces EC sprouting in a spheroid assay (20). Furthermore, PKM2 interacts with Jumonji C domain–containing protein 8 (Jmjd8), and knockdown of Jmjd8 also reduces EC sprouting and network formation. However, the precise mechanism by which Jmjd8 regulates PKM2 remains to be established (20). Because the PKM2 isoform is expressed in embryonic tissues (21), its role in embryonic vascular development needs to be addressed.

Glycolysis levels are lowered in quiescent phalanx ECs, in response to transcriptional regulation. Indeed, forkhead box protein O1 (FOXO1) maintains endothelial quiescence by suppressing c-MYC, a well-known regulator of anabolic metabolism and growth, resulting in decreased glycolysis (22). This finding indicates that FOXO1-MYC is an important transcriptional regulator and a gatekeeper of endothelial growth and proliferation. In addition, the expression of glycolytic regulatory genes, like *PFKFB3* and *HK2*, is suppressed by activation of Krüppel-like factor 2 (KLF2), a transcription factor induced by blood flow–induced laminar shear stress (23). Accordingly, mechanical clues influence angiogenesis in a metabolism-dependent manner (23). FOXO1 has additional context-dependent activities, as it promotes sprouting and migration of lymphatic ECs by upregulating the purigenic receptor P2RY1 when exposed to ATP (24).

Glucose can be shunted to glycolytic side pathways for the production of macromolecules and nicotinamide adenine dinucleotide phosphate (NADPH) for redox balance (25, 26). The pentose phosphate pathway (PPP) is a side pathway of glycolysis that utilizes the glycolytic intermediate glucose 6-phosphate to generate NADPH and ribose 5 phosphate (R5P). These PPP products are utilized for nucleotide synthesis and redox homeostasis (NADPH is used for regeneration of reduced glutathione, a key cellular antioxidant) (27). Additionally, NADPH is utilized for fatty acid and nitric oxide (NO) generation, promoting angiogenic activity of the endothelium (28). PPP has two rate-limiting enzymes, i.e., glucose 6-phosphate dehydrogenase (G6PD) in the irreversible oxidation pathway and transketolase in the reversible nonoxidative pathway. Inhibition of either pathway results in decreased EC survival (15), while G6PD overexpression stimulates EC proliferation, migration, and tube formation due to increases in NO and NADPH production (29). Genetic manipulation of G6PD results in embryonic lethality (30).

The hexosamine biosynthesis pathway (HBP), another side pathway of glycolysis, accounts for a very small percentage of glucose metabolism and is associated with posttranslational protein modifications via glycosylation and the synthesis of glycolipids, proteoglycans, and glycosylphosphatidylinositol anchors (31, 32). Interestingly, VEGF receptor 2 (VEGFR2) stability, membrane expression, and signaling activity are enhanced by *N*-glycosylation (33, 34), while NOTCH signaling-related ligand interactions are affected by *O*-glycosylation. Glutamine fructose-6-phosphate amidotransferase (GFAT) is a rate-controlling enzyme of the HBP and requires fructose 6-phosphate (F6P) and glutamine to synthetize glucosamine-6-phosphate. The precise role of the HBP in EC function remains poorly understood. However, a high

concentration of glucosamine increases protein glycosylation and reduces EC tube formation and migration. Additionally, in an aortic ring angiogenesis model, impaired sprouting of ECs is associated with high levels of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc). Conversely, inhibiting protein glycosylation by overexpression of *O*-GlcNAcase (*N*-acetylglucosaminidase) increases EC tube formation and migration (35, 36). Future studies should evaluate how these HBP-dependent modifications affect EC activity.

FATTY ACID METABOLISM

The roles of fatty acid metabolism in ECs have gained recent interest. EC utilization of fatty acid metabolism differs from that of many other cell types. It requires passive diffusion from the blood or transport of fatty acid into the cell for fatty acid oxidation (FAO) (37). ECs metabolize fatty acids to acetyl-CoA to sustain the tricarboxylic acid (TCA) cycle, in conjunction with an anaplerotic substrate, in order to facilitate deoxy nucleotide triphosphate (dNTP) synthesis for EC proliferation (38). Carnitine palmitoyltransferase 1a (CPT1a) is a rate-controlling enzyme of the fatty acid metabolic pathway (39). Angiogenic stimuli induce the expression of fatty acid transport protein (FATP) and fatty acid binding protein (FABP) to regulate EC activity (40, 41). DLL4 and NOTCH signaling promoted by VEGF on ECs induces binding of the NOTCH intracellular domain to the recombination signal binding protein for immunoglobulin κJ region (RBPJκ), forming a transcription factor complex that activates *FABP4* transcription (42). Silencing of *FABP4* in ECs leads to decreased proliferation, migration, and sprouting in vitro (40, 41). Mechanistically, *FABP4* silencing results in an increased FAO rate in ECs, likely to remove unbound fatty acids, but increased FAO promotes ROS production by boosting mitochondrial respiration (43). VEGF and fibroblast growth factor 2 (FGF2) do not induce FABP4 or FABP5 expression in ECs. However, capillary ECs express FABP4 and FABP5, which facilitate the transport of fatty acids across the endothelium and toward fatty acid-consuming tissues, such as the muscle and heart (44).

Interestingly, VEGF-B induces transendothelial fatty acid transport by FATP3 and FATP4, and *Vegf-b* deletion in mice leads to accumulation of fatty acids in the heart and muscle (45). However, the relationship between VEGF-B and insulin resistance remains debated (46, 47). Lipoprotein lipase (LPL), secreted by muscle and fat cells, hydrolyzes triglyceride-rich lipoprotein in the capillary lumen. ECs support transendothelial fatty acid transport by facilitating the transport of LPL from muscle and fat cells to the capillary lumen (48).

The CPT1a isoform of the CPT family is abundant in ECs. Pharmacological or genetic deletion of *CPT1a* leads to reduced EC proliferation and sprouting defects both in vitro and in vivo, without affecting EC migration (38). Supplementing *CPT1a*-depleted cells with acetate, a precursor of acetyl-CoA, to fuel the TCA cycle restores dNTP levels and rescues the vascular sprouting defect (38). Interestingly, both glucose and glutamine metabolism fail to compensate for this FAO metabolic defect. Thus, rather than using fatty acid metabolism for energy production, proliferating ECs incorporate fatty acid-derived carbons in deoxyribonucleotides for DNA synthesis to maintain EC proliferation during vessel sprouting (38). Furthermore, vessel leakage caused by *Cpt1a* inhibition is associated with the disruption of Ca^{2+} homeostasis (49). Additionally, *Cpt1a*-driven FAO modulates lymphangiogenesis via epigenetic regulation. The transcription factor Prox1, critical for venous-to-lymphatic EC differentiation, binds to the CPT1a promoter, thereby enhancing CPT1a gene transcription, FAO, and production of acetyl-CoA, which is then used by the histone acetylase p300 to acetylate histones at lymphangiogenic genes, thereby promoting lymphatic EC differentiation (50). Because p300 forms a complex with Prox1, this epigenic regulation occurs more specifically at lymphangiogenic than angiogenic genes (50). Hence, Prox1 highjacks FAO to enhance its own transcriptional activity to induce lymphatic EC differentiation.

These findings suggest that different metabolic changes or differential activity levels of specific pathways can drive the distinct EC subtype phenotypes. For example, boosting glycolysis supports EC tip cell behavior, whereas reducing glycolysis by targeting *Pfkfb3* impairs both tip and stalk cell behavior (10). The blockage of FAO targets proliferating stalk cells by blocking de novo synthesized nucleotides needed for cell division (38). Therefore, different EC subtypes are driven by different metabolic changes.

AMINO ACID METABOLISM

In addition to glucose-derived carbons, the metabolism of glutamine, a nonessential amino acid, can supply anaplerotic substrates for the TCA cycle. Out of all amino acids, ECs consume the most glutamine and sustain growth by proliferation and vascular expansion (51, 52). In fact, glutamine is found in the circulating human plasma (accounting for 0.65 mM of the ~2.5 mM of total free amino acids in the plasma) (53), and ECs from both venous and arterial vessels express glutaminase (GLS), the first enzyme that catabolizes glutamine to glutamate and ammonia. Glucose-deprived ECs can oxidize glutamine and glutamate (up to 68 nM/h/mg protein) as well as alanine (35 nM/h/mg protein) at a high rate (54). In ECs, 30% of the TCA carbons are derived from glutamine, comparable to glycolysis and FAO-derived carbon (38). Glutathione produced by ECs via glutamine is used for redox homeostasis, and depletion of glutamine makes ECs vulnerable to ROS-induced damage (51, 55). Glutamate generated by catabolism of glutamine can be converted to ornithine to generate proangiogenic factors, such as NO and polyamine (56, 57). Genetic deletion of *Gls1* in ECs and inhibition by CB-839 decrease EC proliferation and migration and vessel sprouting (51). In chimeric EC spheroids, containing a mixture of GFP^+ *Gls1*-silenced and $mCherry^+$ wild-type ECs, *Gls1*-silenced ECs display reduced competitive behavior to reach the tip position. These findings illustrate that glutamine metabolism plays a prominent role in tip and stalk cell positioning during vessel sprouting (51).

Asparagine in combination with the anaplerotic substrate α-ketoglutarate rescues the defects of glutamine-deprived ECs, while asparagine alone partially rescues proliferation of these ECs, suggesting a key role of asparagine in the EC response to glutamine deprivation (51). Further illustrating the importance of asparagine for vessel sprouting, EC proliferation is impaired by silencing asparagine synthetase (ASNS), the enzyme responsible for producing asparagine from glutamine-derived nitrogen and aspartate (51). Mechanistically, decreased mTOR activity and endoplasmic reticulum (ER) stress contribute to the decreased EC proliferation upon glutamine depletion (51).

Interestingly, the amino acid arginine is a source for endothelial nitric oxide synthase (eNOS) to generate vasoprotective NO (58, 59). In agreement, arginine depletion from EC cultures leads to eNOS dysfunction (60). Increased arginase levels during vascular inflammation lead to the depletion of L-arginine, resulting in decreased NO production with impaired EC-dependent vasodilation. Inhibition of arginase or supplementation of L-arginine restores EC function (61, 62).

ECs can take up serine directly or produce this amino acid from the glycolytic intermediate 3-phosphoglycerate in a multistep enzymatic reaction controlled by phosphoglycerate dehydrogenase (PHGDH) (63). Serine can be interconverted to glycine, which contributes to one-carbon folate metabolism for nucleotide biosynthesis and redox homeostasis (64). N5-methyl-tetrahydrofolate is used to generate methionine from homocysteine. EC-specific loss of PHGDH leads to severe vascular defects due to reduced EC proliferation and survival, partly from defective heme synthesis. These exciting results related to amino acid metabolism in EC merit further investigation.

Table 1 List of vascular diseases associated with endothelial cell (EC) metabolic deregulation

Vascular disease	Metabolic perturbation	Consequences
Aneurysm aortic dissection	↑NOX activity, ↑ROS production	Inflammation, increased protease levels, vascular remodeling
Atherosclerosis	↑ADMA, ↓NO, ↑arginase, eNOS uncoupling, ↑NOX, ↑ROS	EC dysfunction, deregulation of vascular tone, plaque formation
Cancer	↑GLUT1, ↑PFKFB3, ↑LDH-B, ↑glycolysis, ↑nucleotide synthesis	EC hyperproliferation, broken EC lining, leakage, increased cancer cell intravasation
Diabetes	↑Glucose levels, ↑NOX activity, ↑ROS, ↑AGE levels, ↑protein glycosylation, ↓glycolysis, ↑glycolysis side pathway activity	EC dysfunction–oxidative damage, decreased ROS scavenging, vessel leakage, pathological angiogenesis
Neovascular eye disease	↑PFKFB3-driven glycolysis, ↑CPT1a-driven FAO	Acellular capillaries, inflammation, leaky endothelial barrier, pathological angiogenesis
Pulmonary arterial hypertension	Mitophagy, ↑glycolysis, ↑PPP, ↑GLS1, ↓FAO, ↓NO	Vascular remodeling, loss of vasodilative response, excess angiogenesis

Abbreviations: ADMA, asymmetric dimethylarginine; AGE, advanced glycation end product; CPT1a, carnitine palmitoyltransferase 1a; eNOS, endothelial nitric oxide synthase; FAO, fatty acid oxidation; GLS1, glutaminase 1; GLUT1, glucose transporter 1; LDH-B, lactate dehydrogenase B; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOX, NADPH oxidase; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PPP, pentose phosphate pathway; ROS, reactive oxygen species.

ENDOTHELIAL METABOLISM DURING VASCULAR DYSFUNCTION

Quiescent ECs maintain vascular barrier integrity, tone, and redox homeostasis. However, these otherwise quiescent vascular ECs can become dysfunctional in multiple vascular diseases, including atherosclerosis, diabetes, pulmonary hypertension, cancer, ocular diseases, and aging. EC metabolism is perturbed or maladapted in these vascular pathogeneses. **Table 1** summarizes the key metabolic perturbations in ECs during these diseases.

Atherosclerosis

Atherosclerosis is a chronic, inflammatory, fibroproliferative disease of arteries, fueled by lipid accumulation; it is the leading cause of morbidity in Western society (65). EC dysfunction plays a major role in the progression of atherosclerosis (66, 67). The generation of high levels of toxic ROS with subsequent impairment of NO and hydrogen sulfide (H_2S) production, which are needed for vascular homeostasis, increases oxidative stress; this further promotes EC dysfunction with leukocyte adhesion, platelet aggregation, and smooth muscle cell accumulation (68). Decreased NO and citrulline levels result from eNOS uncoupling, which leads to ROS production in conditions of limited availability of arginine and tetrahydrobiopterin (BH_4) (69).

High plasma levels of asymmetric dimethylarginine (ADMA), an analogue of arginine and a competitive inhibitor of NOS, are correlated with EC dysfunction in patients with atherosclerosis (70). The ratio of arginine to ADMA is considered a sensitive risk factor for atherosclerosis (71, 72). Arginine methylation leads to the generation of ADMA and symmetric dimethylarginine. Inactivation of the enzyme dimethylarginine dimethyl-aminohydrolase (DDAH) by oxidative stress enhances ADMA accumulation (73, 74). Endothelial DDAH1 is, however, not a critical determinant of plasma ADMA, vascular reactivity, or hemodynamic homeostasis, though it stimulates angiogenesis (75). Dietary delivery of L-arginine restores NO levels and attenuates high-fat diet–induced atherogenesis in an animal model (76). However, other studies failed to show such

a benefit, emphasizing the need for additional exploration (77). Endothelial FOXO1 increases eNOS by inhibiting ADMA via downregulation of DDAH, responsible for ADMA degradation (78). Additionally, NADPH oxidase (NOX) levels are downregulated in vessels of *Ldlr*$^{-/-}$ mice lacking FOXO1 in ECs, highlighting the role of FOXO1 (79).

Elevated plasma levels of homocysteine and *S*-adenosyl-homocysteine (SAH) are associated with DNA hypomethylation in vascular diseases, such as atherosclerosis (80). Increased SAH levels cause ER stress, which promotes EC dysfunction by oxidative stress and inflammation (81). Plasma levels of SAH and homocysteine might be sensitive biomarkers for EC dysfunction in atherosclerosis (82). Oxidized low-density lipoprotein (OXLDL), a major contributor to atherosclerotic plaque formation, increases the expression of arginase and competes with eNOS for the substrate arginine, thereby reducing NO availability, which explains why genetic deletion of arginase restores EC function in an atherosclerotic model (83). Small-molecule inhibitors have been identified for the pharmacological inhibition of arginase (84). Interestingly, in a recent clinical trial, pharmacological arginase blockade improves endothelial function in patients with familial hypercholesterolemia (61).

The transcription factor KLF2 promotes EC quiescence, barrier function, and alignment in the vascular flow axis (85). In atherosclerosis, LDL impairs endothelial KLF2 expression, leading to EC activation and dysfunction (86). KLF2 expression is suppressed by miR-92a and upregulated by OXLDL, while inhibition of miR-92a reduces EC inflammation and atherosclerotic burden in mice (87). Interestingly, EC glucose metabolism is also regulated by KLF2. Indeed, overexpression of KLF2 lowers glucose uptake by partially repressing *PFKFB3* promoter activity (23) and promotes EC quiescence, elucidating the impact of KLF2 on EC metabolism.

Aneurysms are associated with life-threatening complications, characterized by abnormally weak protruding blood vessels due to EC injury, extracellular matrix remodeling, and a loss of smooth muscle cells (88). Aneurysms are associated with oxidative stress and inflammation, resulting from NOX-derived ROS produced by infiltrating macrophages, fibroblasts, and ECs (89). Endothelial overexpression of NOX2 in mice leads to increased ROS, which enhances the susceptibility to aortic dissection in response to Ang II stimulation. Mechanistically, ECs enhance the secretion of cyclophylin A, which triggers inflammation, protease expression, and vascular remodeling. Thus, targeting EC ROS generation is a potential therapeutic approach to prevent aortic dissection (90).

Diabetes

Diabetes is a metabolic disorder with a rapidly increasing global prevalence (91); it is characterized by high blood glucose levels, which contribute to EC dysfunction and subsequently to vascular inflammation (92). EC dysfunction during diabetes is complicated by excessive or impaired angiogenesis. Unwarranted angiogenesis contributes to diabetic retinopathy, nephropathy, and atherosclerotic plaque disruption. Impaired angiogenesis prompts coronary artery disease, ischemic stroke, impaired wound healing, and skin ulcers (93). Early intervention studies have shown that the development and progression of micro- and macrovascular complications can be decreased by reducing hyperglycemia (94). Endothelial ROS levels during hyperglycemia are induced by NOXs, the protein kinase C (PKC) pathway, endothelial xanthine oxidase, and eNOS uncoupling (95). In agreement, inhibition of PKC reduces the levels of NOX protein subunits (p22phox, p47phox, p67phox) in vessels from diabetic coronary artery patients (96). A metabolomics study using streptozotocin-diabetic nephropathy rats established that hyperglycemia induces high levels of xanthine oxidase and perturbs purine metabolism (97). In a peripheral neuropathy model (98) and a diabetic mouse model (99), eNOS uncoupling induces EC dysfunction. The interrupted metabolism of arginine and cofactors BH_4 and NADPH promotes endothelial insulin resistance

(reviewed in 95), aberrant *O*-glycosylation (reviewed in 95), increased levels of advanced glycation end products (AGEs) (100), and the restriction of PPP flux (78). In diabetic ECs, perturbed mitochondria contribute to EC dysfunction through increased ROS formation and apoptosis (101).

During hyperglycemia, metabolic regulators, such as peroxisome proliferator-activated receptor gamma (PPARγ), coactivator 1 alpha (PGC1α), AMP-activated protein kinase (AMPK), and nuclear factor erythroid 2–related factor 2 (Nrf2), counteract the effects of ROS. PGC1α, upregulated by NO, protects ECs from ROS and regulates mitochondrial antioxidant defenses in ECs. Activation of AMPK by rosiglitazone (a PPARγ agonist that activates AMPK) in hyperglycemic ECs reduces oxidative stress (102). Manganese superoxide dismutase (MnSOD), induced by AMPK activation via metformin or 5-aminoimidazole-4-carboxamide-riboside (AICAR) alleviates hyperglycemic oxidative stress (103) and induces mitochondrial biogenesis via PGC1α (104). In vivo studies established the beneficial effects of AMPK activation for the prevention of EC dysfunction in diabetes (105). Nrf2, a regulator of cellular redox homeostasis (106), regulates the activity of the antioxidant enzyme glyoxalase 1 (GLO1) in ECs exposed to hyperglycemia (107). Indeed, overexpression of Glo1 reduces vascular AGE formation and normalizes impaired angiogenesis in vitro and in hyperglycemia animal models (108). Additionally, evidence suggests that the cross talk between Nrf2 and AMPK prevents cardiomyopathy (109). Nonetheless, antioxidant therapies in clinical trials have not been effective, despite these promising findings.

Accumulating evidence indicates that a loss of insulin signaling promotes EC dysfunction (110). In many tissues, insulin signaling promotes glucose uptake via intracellular trafficking of GLUT4, but ECs take up glucose via GLUT1, which is not altered by insulin. Instead, its expression is regulated by the high glucose levels in circulation, thus explaining the susceptibility of ECs to hyperglycemia-induced oxidative stress (111). How the excess uptake of glucose leads to aberrant glucose metabolism in ECs is further discussed below.

The enzyme fatty acid synthase (FAS) induced by systemic insulin generates palmitate. It is involved in the preferential *S*-acylation of eNOS to localize NO production to the plasma membrane and to retain endothelial function (112). In diabetic animal models, reduced levels of endothelial FAS decrease eNOS palmitoylation, impairing angiogenesis and promoting inflammation via increased oxidative stress. However, EC insulin also regulates palmitoylation of proteins responsible for insulin-induced EC migration and angiogenesis (113). A proteomic analysis could be useful to identify novel insulin-dependent palmitoylation targets that are dysregulated by insulin resistance in diabetes. Notably, branched amino acid metabolism has been linked to transendothelial fatty acid transport and lipid accumulation in muscle, contributing to diabetes (114). Indeed, 3-hydroxyisobutyrate (3-HIB), a catabolic intermediate of the branched amino acid valine that is secreted from muscle cells, activates transendothelial flux of fatty acids, stimulates muscle fatty acid uptake, and promotes lipid accumulation in muscle, leading to insulin resistance in mice.

Dietary fructose, which can be metabolized to glycolytic intermediates, increases the cardiovascular risk by promoting EC dysfunction, atherosclerosis, and cardiovascular complications in diabetes (115, 116). In addition to glucose, elevated levels of fructose can promote hazardous reactive protein modifications (peptide glycation by fructose).

Cancer

Aberrant angiogenesis in cancer is initiated by several angiogenic factors, including VEGF, FGF, angiopoietins, and other molecules (6, 117). Low oxygen tension in tumors steers HIF1α activation, which triggers the expression of several growth factors that promote tumor angiogenesis (118). Compared to normal ECs (NECs), tumor ECs (TECs) have high proliferation rates and are resistant to serum starvation–induced apoptosis (117); they also display certain genomic aberrations with higher rates of aneuploidy, which are intensified during tumor progression (95, 119).

TEC metabolism has not been fully characterized. The metabolic profile of tumor tissues is often characterized by high lactate levels (40 mM) and low glucose levels (0.12 mM). TECs exhibit hyperglycolytic activity (95, 120) and upregulate glycolytic enzymes, such as lactate dehydrogenase B (LDH-B) (121). LDH-B catalyzes the generation of pyruvate from lactate, which is taken up by monocarboxylate transporter 1 (MCT1) (122), fueling the TCA cycle. Moreover, lactate uptake by ECs induces ROS-mediated NF-κB activation and interleukin-8 (IL-8) expression (122). Additionally, lactate also regulates levels of the putative tumor suppressor protein N-Myc downstream-regulated gene 3 (*NDRG3*), which can stimulate angiogenesis during hypoxia. Depletion of *NDRG3* in ECs reduces tube formation (123), suggesting that lactate can act as a proangiogenic stimulus.

Compared to NECs, TECs from tumor-infiltrated livers display a distinct metabolic profile (120); TECs exhibit a hyperglycolytic metabolism, which they use for biomass production. Indeed, in vitro tracing studies using ^{14}C-glucose and ^{13}C-glucose showed that TECs use glucose carbons for nucleotide synthesis (120). EC-specific deletion of a single allele of the glycolytic regulator *Pfkfb3* (*Pfkfb3*$^{+/\Delta EC}$) in tumor-bearing mice lowers hyperglycolysis by 15–20%, which reduces intravasation of cancer cells and metastasis without affecting tumor size. Interestingly, the tumor vasculature in *Pfkfb3*$^{+/\Delta EC}$ mice displays vessel enlargement with reduced vessel tortuosity and improves perfusion dynamics with continuous stable VE-cadherin adherens junctions and pericyte coverage—structural hallmarks of a normalized vasculature (120). Pharmacological blockade or cell autonomous endothelial deletion of *Pfkfb3* promotes pericyte quiescence and adhesion by enhancing the expression of N-cadherin, thus supporting tumor vessel normalization (95, 120). Because glycolytic ATP production is required for endocytosis of junctional molecules in ECs, *Pfkfb3* inhibition promotes higher levels of the junctional molecule VE-cadherin on the EC surface, thereby tightening the vascular barrier and impairing cancer cell intravasation (120). Thus, targeting TEC metabolism might provide an alternative therapeutic strategy to reduce metastasis, normalize the tumor vasculature, and improve chemo- and immunotherapy.

Notably, tumor-associated macrophages, genetically engineered to be highly glycolytic, outcompete TECs for glucose usage, which thwarts vascular hyperactivation and promotes the formation of a more quiescent, normalized tumor vasculature (124). Thus, targeting perivascular cells in the tumor stroma may also offer novel therapeutic opportunities.

Neovascular Eye Diseases

Several ocular diseases are impacted by aberrant neovascularization, as in retinopathy of prematurity (ROP), diabetic retinopathy (DR), neovascular age–related macular degeneration (NV-AMD), and corneal neovascularization. These morphologically abnormal, leaky vasculatures due to a loss of junctional integrity eventually end with fibrotic scar, a major cause of vision loss.

ROP accounts for 6–18% of childhood blindness and is associated with premature birth and oxygen supplementation (125). In an ROP model, postnatal pups (postnatal day 7, or P7) are exposed to hyperoxic environment for 5 days (P12), and the developing retinal endothelium is pruned due to high oxygen tension. Upon exposure to a normoxic environment for 5 days (P12–P17), the retina becomes hypoxic, promoting neovascularization in the retina (125). Treatment of ROP mice with the small-molecule compound 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), an inhibitor of PFKFB3, mitigates pathological vascular tuft formation (16). Similarly, ROP pups treated with etomoxir (an inhibitor of the mitochondrial enzyme carnitine palmitoyltransferase 1 (CPT1), regulating FAO) exhibit reduced vascular tuft formation (38). These results indicate the impact of EC metabolism on vascular tuft development.

Choroidal neovascularization (CNV) in NV-AMD triggers irreversible blindness and is the leading cause of visual impairment in the aged population. NV-AMD is projected to impact

288 million individuals globally by 2040 (126). In a preclinical model of NV-AMD, CNV can be induced by laser injury of Bruch's membrane, resulting in hypoxia, tissue traction, and inflammation. A recent study established that glycolytic flux is a determinant of CNV. Indeed, targeting *Pfkfb3* by 3PO reduces CNV lesions and improves the efficiency of antiangiogenic therapy by VEGFR2 inhibition (16).

In DR, early vascular cell death and acellular capillaries are instigated by abnormal glucose metabolism (28). Elevated oxidative stress in hyperglycemic ECs leads to the accumulation of glycolytic intermediates through inactivation of the glycolytic enzyme GAPDH. Excess glucose is then shunted to the polyol pathway, which consumes NADPH and increases oxidative stress (127). Glycolytic intermediates, such as F6P, activate GFAT of HBP, a pathway involved in the glycosylation of Akt (35). The accumulation of intermediates and triosephosphates generates methylglyoxal, glyoxal, and 3-deoxyglucosone, contributing to AGE formation, which results in ROS (28). Dihydroxyacetone phosphate (DHAP) is converted to diacylglycerol (an activator of PKC), which stimulates ROS production by NOX (95). Collectively, these findings demonstrate the significance of EC metabolism in various aspects of neovascular eye diseases.

Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a progressive vasculopathy with significant morbidity and mortality. PAH is characterized by increased resistance of pulmonary arteries and arterioles (128). The pathophysiology of PAH includes aberrant angiogenesis due to excessive growth of apoptosis-resistant ECs, extensive vascular remodeling, and pulmonary vascular lesion formation (128, 129). These extensive changes lead to increased pulmonary arterial pressure (>25 mm Hg) at rest and eventually to right ventricular cardiac failure and premature death (130). A key feature of PAH is that mitochondrial remodeling and mitophagy result in suppressed glucose oxidation and the secondary upregulation of glycolysis in ECs (95, 131).

As a consequence of EC apoptosis, residual ECs become hyperproliferative and apoptosis resistant (95). The hyperproliferative EC phenotype can be partly attributed to the upregulation of the PPP and polyamine biosynthesis pathway (95). Furthermore, pulmonary vascular remodeling upregulates glutaminase 1 (GLS1), lactate dehydrogenase A (LDH-A), and pyruvate carboxylase to promote glutamine metabolism in addition to glycolysis and glucose carbon anaplerosis to meet the demands of hyperproliferative ECs (132). Importantly, PAH has recently been characterized by intracellular lipid accumulation and decreased fatty acid consumption due to reduced FAO, which results in right ventricular lipotoxicity and cardiac failure (133).

Oxidative stress in ECs plays an important role in vascular remodeling in PAH (95). Levels of eNOS, a key regulator of vessel tone (134), and the bioavailability of NO are decreased in PAH, with increasing levels of endothelin-1, a potent vasoconstrictor (134). Increased threonine (T495) phosphorylation of eNOS via PKC is responsible for a reduction in NO levels (135). Interestingly, NO bioavailability is restored in PAH-associated ECs by β-blocker–mediated inhibition of PKC activity (135). Additionally, in a rat model of PAH, the application of eNOS-transduced endothelial progenitor cells resulted in the regeneration of the pulmonary endothelium and significantly improved survival (95).

METABOLIC CROSS TALK BETWEEN THE ENDOTHELIUM AND OTHER CELL TYPES

Metabolite-based communication between ECs and other cell types is mediated by the uptake and transport of nutrients, such as sugars, fatty acids, and amino acids. EC metabolism can be altered by neighboring stromal or immune cells via various stimuli (VEGF, FGF, DLL4). Cancer cells

can promote EC proliferation by secreting the TCA cycle intermediate succinate or by stimulating glucose uptake (25, 136). Lactate secreted in part by cancer cells or cancer-associated fibroblasts can also promote EC proliferation (137) and angiogenesis by enhancing VEGF signaling via HIF1α (6) and the PI3K/AKT pathway (2). Tumor-generated lactate enters ECs via MCT1 and promotes tumor angiogenesis by stimulating the NF-κB/IL-8 pathway in a ROS- and IκBα-dependent manner (122). The acidic microenvironment created by the accumulation of lactate causes ER stress and induces an inflammatory response (138). Tumor-associated macrophages (TAMs) secrete high amounts of VEGF, which promotes EC proliferation, but they also express arginase 1, which reduces local arginine levels and impairs T cell function (76, 83, 117, 139). The proangiogenic nature of TAMs is dependent on the enzyme glutamine synthetase. Targeting glutamine synthesis in TAMs decreases glutamine levels, leading to tumor vessel normalization and reduced tumor metastasis (140).

Upon anticancer treatment, metabolic cross talk in the tumor becomes more complex, leading to compensatory mechanisms. For example, glycolytic cancer cells in the hypoxic tumor microenvironment consume glucose and generate lactate, which is then utilized as a substrate of oxidative metabolism by neighboring cancer cells located in the proximity of blood vessels (2, 95). This symbiotic metabolism can cause resistance to antiangiogenic therapy, as cancer cells in oxygenated areas are fed by cancer cells in hypoxic regions (141). Likewise, the antitumor effect of mTOR inhibitors is partially hindered by positive effects on TAMs, which can promote tumor angiogenesis. Further investigations are needed to determine the overall efficacy of combination strategies targeting metabolism.

EC-secreted lactate can induce a vasoactive signal in pericytes. This metabolite-based communication seems to be regulated in the context of cellular energy homeostasis. In low energy supply conditions, lactate initiates vasodilation, whereas in abundant energy supply situations, lactate acts as a vasoconstrictor (95). Additionally, lactate can regulate gene transcription in skeletal muscle and neurons (2, 142) and polarization of immune cells (95). By lowering the glycolytic activity of T cells through downregulating Hk1 and inhibiting Pfk, lactate inhibits chronic inflammation, boosted by IL-17A secretion by cytotoxic T cells (143). Lactate also activates HIF1α and thereby stimulates tumor angiogenesis, and this mechanism is reduced by targeting MCT1 in ECs (122). Additional investigations are needed to verify the efficacy of targeting the EC lactate transporter MCT1.

An increasing number of studies have focused on extracellular vesicles (EVs). A broad variety of cells communicates through these extracellular signals. EVs contain several factors, including metabolites, proteins, and nucleic acids (mRNA, DNA, and miRNA), with the potential to stimulate targeted cells (144). Especially under glycemic changes, cardiomyocytes and cardiac ECs can influence each other via EVs. Glucose-starved cardiomyocytes remodel the EV content and are able to stimulate recipient EC proliferation and angiogenesis (145). These remodeled EVs from cardiomyocytes increase the expression of glucose transporters (GLUT1 and 4) of ECs (145). Reciprocally, ECs secrete pyruvate in their microenvironment, likely to maintain cardiomyocyte function (145). The EC-derived EV content might reflect the status of the cell and EVs can act as diagnostic markers in vascular disorders.

THERAPEUTIC IMPLICATIONS

EC metabolism has emerged as a key regulator of angiogenesis and is becoming an attractive therapeutic target. Anti-VEGF therapies in the clinical setting are limited by insufficient efficacy, intrinsic refractoriness, and the development of resistance in the majority of patients (146). As discussed above, recent studies deciphered how ECs rewire their metabolic network to promote pathological vascular growth. PFKFB3 is a key activator of glycolysis, which provides the necessary

ATP and promotes biomass synthesis to maintain the angiogenic switch. Thus, antiglycolytic therapy using the PFKFB3 blocker 3PO reduces glycolysis and pathological tumor angiogenesis. PFKFB3-targeted therapy is safer than anticipated, as it only transiently and partially lowers glycolysis, without causing deleterious systemic effects on glycolysis-dependent healthy tissues (16). Additionally, low-dose 3PO treatment is sufficient to reduce metastasis in tumor models by normalizing the tumor vasculature, while normalized vessels improve the efficacy of chemotherapy by ameliorating drug delivery (16). TECs are sensitive to the partial inhibition of glycolysis because they rely on glycolysis more than do other cell types. However, the complete inhibition of glycolysis by 2-deoxy-D-glucose (2DG) affects healthy quiescent vessels and other cell types that rely on it. Patients with cancer undergoing 2DG treatment present adverse effects with disease progression. The 3PO derivative PFK158 is being tested in a phase 1 clinical trial for patients with solid malignancies (study NCT02044861).

Recent studies also demonstrated the beneficial therapeutic effect of CPT1 inhibition by etomoxir in pathological ocular angiogenesis (38). Etomoxir reduced tumor growth in an FAO-dependent tumor model, though it remains to be determined if this is also due to tumor angiogenesis inhibition (147). Perhexiline is another inhibitor of CPT1 with limited efficacy (148). The reliance of solid tumor cells and leukemia cells on glutamine provided a basis for the development of GLS1 inhibitors. BPTES is an irreversible allosteric inhibitor of glutaminase but has a moderate efficacy as a GLS1 inhibitor (51). Another noncompetitive allosteric inhibitor, compound 968, with an antiproliferative effect on glutamine-addicted cancer cells, has limited therapeutic use owing to its hydrophobic properties (149). CB-839 is a potent selective and reversible inhibitor of human GLS1; it affects the progression of various preclinical tumor models (149, 150). CB-839 is in phase 2 and phase 1/2 clinical studies with standard chemo-immunotherapeutic agents and patients with renal carcinoma, melanoma, non-small-cell lung cancer, triple negative breast cancer, and colorectal cancer (studies NCT03163667, NCT02771626, NCT02071862, and NCT02861300). The effects of GLS1 inhibitors on tumor angiogenesis remain to be determined.

The EC metabolic link between glutamine and asparagine and the importance of endothelial ASNS for angiogenesis raises the question whether inhibitors of the asparagine metabolism enzyme ASNS (such as *N*-acylsulfonamide compound 6) could be useful to inhibit pathological angiogenesis. Adenylated sulfoximine also blocks human ASNS with an improved affinity (151). Efforts to synthesize new sulfoximine derivatives with improved metabolic stability and bioavailability may be useful in the future. **Table 2** summarizes the current status of metabolic drug development.

Table 2 Endothelial cell metabolic pathways targeted by small molecules and current status of drug development

Metabolic pathway	Enzyme targeted	Inhibitors	Current status
Glycolysis	PFKFB3	3PO	Preclinical
		PFK-158	Clinical phase 1
		Phenoxyindole	Preclinical
Fatty acid oxidation	CPT1	Etomoxir	Preclinical
		Perhexiline	Preclinical
Amino acid metabolism	GLS1	BPTES	Preclinical
	ASNS	Compound 968	Preclinical
		CB-839	Clinical phase 1/2
		N-acylsufonamide 6	Preclinical
		Adenylated sulfoximine	Preclinical

Abbreviations: 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one; ASNS, asparagine synthetase; CPT1, carnitine palmitoyltransferase 1; GLS1, glutaminase 1; PFK-158, phosphofructokinase-158; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3.

CONCLUSION AND PERSPECTIVES

Emerging evidence highlights the role of metabolic pathways in regulating both physiological and pathological angiogenesis, and targeting EC metabolism can provide a new therapeutic strategy for the treatment of cancer or ocular diseases, characterized by excessive angiogenesis (see above). Recent studies have also demonstrated the impact of glycolysis, FAO, and amino acid metabolism on the proliferation, migration, and survival of ECs. Few metabolic enzymes in ECs have been characterized; studies of these enzymes have shed light on the critical role of metabolism in vascular biology, establishing a basis for the development of new antimetabolic drugs. Many questions remain as to how ECs integrate the regulation of the multiple metabolic pathways, though few regulators have been identified (such as FOXO1 and KLF2 that lower glycolysis, whereas hypoxia-inducible factors upregulate glycolysis). The impact of the metabolic cross talk on the functions of ECs and their neighboring cells is an outstanding question for future research, especially in EC dysfunction-related pathologies, e.g., cancer, atherosclerosis, ocular pathologies, and diabetes. Deregulated EC metabolism can be clarified via global untargeted metabolomics to determine the metabolic signature of ECs from patients with disease and mice with abnormal vessel growth. Metabolic profiling by utilizing multi-omics technologies and employing proteomics and transcriptomics to analyze metabolic genes would represent a powerful approach, as it offers optimal discovery potential to identify unsuspected and novel metabolic pathways involved in EC function.

DISCLOSURE STATEMENT

P. Carmeliet is named as inventor on patent applications on subject matter related to results discussed in this review. The other authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

X.L. is supported by the State Key Laboratory of Ophthalmology at the Zhongshan Ophthalmic Center, Sun Yat-sen University in Guangzhou, and the National Natural Science Foundation of China (grants 81330021, 81670855). P.C. is supported by the Federal Government of Belgium (Belgian Policy Society grant IUAP P7/03), long-term structural Methusalem funding by the Flemish Government, the Research Foundation Flanders (FWO-Vlaanderen), the Foundation Against Cancer (grants 2012-175 and 2016-078), Kom op tegen Kanker (Stand up to Cancer) from the Flemish Cancer Society, and an Advanced Grant from the European Research Council (ERC) (grant EU-ERC743074).

LITERATURE CITED

1. Änggård EE. 1990. The endothelium—the body's largest endocrine gland? *J. Endocrinol.* 127(3):371–75
2. Potente M, Carmeliet P. 2017. The link between angiogenesis and endothelial metabolism. *Annu. Rev. Physiol.* 79:43–66
3. Rafii S, Butler JM, Ding BS. 2016. Angiocrine functions of organ-specific endothelial cells. *Nature* 529(7586):316–25
4. Cahill PA, Redmond EM. 2016. Vascular endothelium—gatekeeper of vessel health. *Atherosclerosis* 248:97–109
5. Potente M, Gerhardt H, Carmeliet P. 2011. Basic and therapeutic aspects of angiogenesis. *Cell* 146(6):873–87
6. Carmeliet P, Jain RK. 2011. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473(7347):298–307

7. Eelen G, Cruys B, Welti J, De Bock K, Carmeliet P. 2013. Control of vessel sprouting by genetic and metabolic determinants. *Trends Endocrinol. Metab.* 24(12):589–96
8. Eelen G, de Zeeuw P, Simons M, Carmeliet P. 2015. Endothelial cell metabolism in normal and diseased vasculature. *Circ. Res.* 116(7):1231–44
9. Mather KJ. 2013. The vascular endothelium in diabetes—a therapeutic target? *Rev. Endocr. Metab. Disord.* 14(1):87–99
10. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, et al. 2013. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 154(3):651–63
11. De Bock K, Georgiadou M, Carmeliet P. 2013. Role of endothelial cell metabolism in vessel sprouting. *Cell Metab*. 18(5):634–47
12. Helmlinger G, Endo M, Ferrara N, Hlatky L, Jain RK. 2000. Formation of endothelial cell networks. *Nature* 405(6783):139–41
13. Uldry M, Thorens B. 2004. The SLC2 family of facilitated hexose and polyol transporters. *Pflügers Arch. Eur. J. Physiol.* 447(5):480–89
14. Yu P, Wilhelm K, Dubrac A, Tung JK, Alves TC, et al. 2017. FGF-dependent metabolic control of vascular development. *Nature* 545(7653):224–28
15. Vizán P, Sánchez-Tena S, Alcarraz-Vizán G, Soler M, Messeguer R, et al. 2009. Characterization of the metabolic changes underlying growth factor angiogenic activation: identification of new potential therapeutic targets. *Carcinogenesis* 30(6):946–52
16. Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquière B, et al. 2014. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab*. 19(1):37–48
17. Israelsen WJ, Dayton TL, Davidson SM, Fiske BP, Hosios AM, et al. 2013. PKM2 isoform-specific deletion reveals a differential requirement for pyruvate kinase in tumor cells. *Cell* 155(2):397–409
18. Christofk HR, Vander Heiden MG, Wu N, Asara JM, Cantley LC. 2008. Pyruvate kinase M2 is a phosphotyrosine-binding protein. *Nature* 452(7184):181–86
19. Hitosugi T, Kang S, Vander Heiden MG, Chung TW, Elf S, et al. 2009. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. *Sci. Signal.* 2(97):ra73
20. Boeckel JN, Derlet A, Glaser SF, Luczak A, Lucas T, et al. 2016. JMJD8 regulates angiogenic sprouting and cellular metabolism by interacting with pyruvate kinase M2 in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 36(7):1425–33
21. Gupta V, Bamezai RN. 2010. Human pyruvate kinase M2: a multifunctional protein. *Protein Sci. Publ. Protein Soc.* 19(11):2031–44
22. Wilhelm K, Happel K, Eelen G, Schoors S, Oellerich MF, et al. 2016. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* 529(7585):216–20
23. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, et al. 2015. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler. Thromb. Vasc. Biol.* 35(1):137–45
24. Niimi K, Ueda M, Fukumoto M, Kohara M, Sawano T, et al. 2017. Transcription factor FOXO1 promotes cell migration toward exogenous ATP via controlling P2Y1 receptor expression in lymphatic endothelial cells. *Biochem. Biophys. Res. Commun.* 489(4):413–19
25. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. 2008. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell Metab*. 7(1):11–20
26. Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029–33
27. Jongkind JF, Verkerk A, Baggen RG. 1989. Glutathione metabolism of human vascular endothelial cells under peroxidative stress. *Free Radic. Biol. Med.* 7(5):507–12
28. Ghesquière B, Wong BW, Kuchnio A, Carmeliet P. 2014. Metabolism of stromal and immune cells in health and disease. *Nature* 511(7508):167–76
29. Lorenzi M. 2007. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Exp. Diabetes Res.* 2007:61038
30. Pandolfi PP, Sonati F, Rivi R, Mason P, Grosveld F, Luzzatto L. 1995. Targeted disruption of the housekeeping gene encoding glucose 6-phosphate dehydrogenase (G6PD): G6PD is dispensable for pentose synthesis but essential for defense against oxidative stress. *EMBO J.* 14(21):5209–15

31. Wells L, Vosseller K, Hart GW. 2001. Glycosylation of nucleocytoplasmic proteins: signal transduction and O-GlcNAc. *Science* 291(5512):2376–78
32. Vosseller K, Sakabe K, Wells L, Hart GW. 2002. Diverse regulation of protein function by O-GlcNAc: a nuclear and cytoplasmic carbohydrate post-translational modification. *Curr. Opin. Chem. Biol.* 6(6):851–57
33. Croci DO, Cerliani JP, Dalotto-Moreno T, Méndez-Huergo SP, Mascanfroni ID, et al. 2014. Glycosylation-dependent lectin-receptor interactions preserve angiogenesis in anti-VEGF refractory tumors. *Cell* 156(4):744–58
34. Rahimi N, Costello CE. 2015. Emerging roles of post-translational modifications in signal transduction and angiogenesis. *Proteomics* 15(2–3):300–9
35. Luo B, Soesanto Y, McClain DA. 2008. Protein modification by O-linked GlcNAc reduces angiogenesis by inhibiting Akt activity in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 28(4):651–57
36. Ngoh GA, Facundo HT, Zafir A, Jones SP. 2010. O-GlcNAc signaling in the cardiovascular system. *Circ. Res.* 107(2):171–85
37. Hülsmann WC, Dubelaar ML. 1988. Aspects of fatty acid metabolism in vascular endothelial cells. *Biochimie* 70(5):681–86
38. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, et al. 2015. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 520(7546):192–97
39. Kerner J, Hoppel C. 2000. Fatty acid import into mitochondria. *Biochim. Biophys. Acta* 1486(1):1–17
40. Elmasri H, Ghelfi E, Yu CW, Traphagen S, Cernadas M, et al. 2012. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: role of stem cell factor/c-kit pathway. *Angiogenesis* 15(3):457–68
41. Elmasri H, Karaaslan C, Teper Y, Ghelfi E, Weng M, et al. 2009. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB J.* 23(11):3865–73
42. Harjes U, Bridges E, McIntyre A, Fielding BA, Harris AL. 2014. Fatty acid-binding protein 4, a point of convergence for angiogenic and metabolic signaling pathways in endothelial cells. *J. Biol. Chem.* 289(33):23168–76
43. Harjes U, Kalucka J, Carmeliet P. 2016. Targeting fatty acid metabolism in cancer and endothelial cells. *Crit. Rev. Oncol. Hematol.* 97:15–21
44. Iso T, Maeda K, Hanaoka H, Suga T, Goto K, et al. 2013. Capillary endothelial fatty acid binding proteins 4 and 5 play a critical role in fatty acid uptake in heart and skeletal muscle. *Arterioscler. Thromb. Vasc. Biol.* 33(11):2549–57
45. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, et al. 2010. Vascular endothelial growth factor B controls endothelial fatty acid uptake. *Nature* 464(7290):917–21
46. Dijkstra MH, Pirinen E, Huusko J, Kivelä R, Schenkwein D, et al. 2014. Lack of cardiac and high-fat diet induced metabolic phenotypes in two independent strains of *Vegf-b* knockout mice. *Sci. Rep.* 4:6238
47. Kivelä R, Bry M, Robciuc MR, Räsänen M, Taavitsainen M, et al. 2014. VEGF-B-induced vascular growth leads to metabolic reprogramming and ischemia resistance in the heart. *EMBO Mol. Med.* 6(3):307–21
48. Davies BSJ, Goulbourne CN, Barnes RH 2nd, Turlo KA, Gin P, et al. 2012. Assessing mechanisms of GPIHBP1 and lipoprotein lipase movement across endothelial cells. *J. Lipid Res.* 53(12):2690–97
49. Patella F, Schug ZT, Persi E, Neilson LJ, Erami Z, et al. 2015. Proteomics-based metabolic modeling reveals that fatty acid oxidation (FAO) controls endothelial cell (EC) permeability. *Mol. Cell. Proteom.* 14(3):621–34
50. Wong BW, Wang X, Zecchin A, Thienpont B, Cornelissen I, et al. 2017. The role of fatty acid β-oxidation in lymphangiogenesis. *Nature* 542(7639):49–54
51. Huang H, Vandekeere S, Kalucka J, Bierhansl L, Zecchin A, et al. 2017. Role of glutamine and interlinked asparagine metabolism in vessel formation. *EMBO J.* 36(16):2334–52
52. Kim B, Li J, Jang C, Arany Z. 2017. Glutamine fuels proliferation but not migration of endothelial cells. *EMBO J.* 36(16):2321–33
53. Newsholme P, Procopio J, Lima MM, Pithon-Curi TC, Curi R. 2003. Glutamine and glutamate—their central role in cell metabolism and function. *Cell Biochem. Funct.* 21(1):1–9
54. Krützfeldt A, Spahr R, Mertens S, Siegmund B, Piper HM. 1990. Metabolism of exogenous substrates by coronary endothelial cells in culture. *J. Mol. Cell. Cardiol.* 22(12):1393–404

55. DeBerardinis RJ, Cheng T. 2010. Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. *Oncogene* 29(3):313–24
56. Kucharzewska P, Welch JE, Svensson KJ, Belting M. 2010. Ornithine decarboxylase and extracellular polyamines regulate microvascular sprouting and actin cytoskeleton dynamics in endothelial cells. *Exp. Cell Res.* 316(16):2683–91
57. Matsunaga T, Weihrauch DW, Moniz MC, Tessmer J, Warltier DC, Chilian WM. 2002. Angiostatin inhibits coronary angiogenesis during impaired production of nitric oxide. *Circulation* 105(18):2185–91
58. Harrison DG. 1997. Cellular and molecular mechanisms of endothelial cell dysfunction. *J. Clin. Investig.* 100(9):2153–57
59. Morris SM Jr. 2009. Recent advances in arginine metabolism: roles and regulation of the arginases. *Br. J. Pharmacol.* 157(6):922–30
60. Palmer RM, Ashton DS, Moncada S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333(6174):664–66
61. Kovamees O, Shemyakin A, Eriksson M, Angelin B, Pernow J. 2016. Arginase inhibition improves endothelial function in patients with familial hypercholesterolaemia irrespective of their cholesterol levels. *J. Intern. Med.* 279(5):477–84
62. Villalba N, Sackheim AM, Nunez IA, Hill-Eubanks DC, Nelson MT, et al. 2017. Traumatic brain injury causes endothelial dysfunction in the systemic microcirculation through arginase-1-dependent uncoupling of endothelial nitric oxide synthase. *J. Neurotrauma* 34(1):192–203
63. Amelio I, Cutruzzola F, Antonov A, Agostini M, Melino G. 2014. Serine and glycine metabolism in cancer. *Trends Biochem. Sci.* 39(4):191–98
64. Tibbetts AS, Appling DR. 2010. Compartmentalization of mammalian folate-mediated one-carbon metabolism. *Annu. Rev. Nutr.* 30:57–81
65. Mozaffarian D. 2016. Natural *trans* fat, dairy fat, partially hydrogenated oils, and cardiometabolic health: the Ludwigshafen Risk and Cardiovascular Health Study. *Eur. Heart J.* 37(13):1079–81
66. Libby P, Bornfeldt KE, Tall AR. 2016. Atherosclerosis: successes, surprises, and future challenges. *Circ. Res.* 118(4):531–34
67. Gimbrone MA Jr., García-Cardeña G. 2016. Endothelial cell dysfunction and the pathobiology of atherosclerosis. *Circ. Res.* 118(4):620–36
68. Davignon J, Ganz P. 2004. Role of endothelial dysfunction in atherosclerosis. *Circulation* 109(23):III27–32
69. Kawashima S, Yokoyama M. 2004. Dysfunction of endothelial nitric oxide synthase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 24(6):998–1005
70. Napoli C, Ignarro LJ. 2007. Polymorphisms in endothelial nitric oxide synthase and carotid artery atherosclerosis. *J. Clin. Pathol.* 60(4):341–44
71. Baum C, Johannsen SS, Zeller T, Atzler D, Ojeda FM, et al. 2016. ADMA and arginine derivatives in relation to non-invasive vascular function in the general population. *Atherosclerosis* 244:149–56
72. Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. 2015. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. *Atherosclerosis* 239(1):61–66
73. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. 1999. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 99(24):3092–95
74. Pope AJ, Druhan L, Guzman JE, Forbes SP, Murugesan V, et al. 2007. Role of DDAH-1 in lipid peroxidation product-mediated inhibition of endothelial NO generation. *Am. J. Physiol. Cell Physiol.* 293(5):C1679–86
75. Dowsett L, Piper S, Slaviero A, Dufton N, Wang Z, et al. 2015. Endothelial dimethylarginine dimethylaminohydrolase 1 is an important regulator of angiogenesis but does not regulate vascular reactivity or hemodynamic homeostasis. *Circulation* 131:2217–25
76. Bogdanski P, Suliburska J, Szulinska M, Sikora M, Walkowiak J, Jakubowski H. 2015. L-arginine and vitamin C attenuate pro-atherogenic effects of high-fat diet on biomarkers of endothelial dysfunction in rats. *Biomed. Pharmacother.* 76:100–6
77. Loscalzo J. 2003. Adverse effects of supplemental L-arginine in atherosclerosis: consequences of methylation stress in a complex catabolism? *Arterioscler. Thromb. Vasc. Biol.* 23(1):3–5

78. Menghini R, Casagrande V, Cardellini M, Ballanti M, Davato F, et al. 2015. FoxO1 regulates asymmetric dimethylarginine via downregulation of dimethylaminohydrolase 1 in human endothelial cells and subjects with atherosclerosis. *Atherosclerosis* 242(1):230–35
79. Tsuchiya K, Tanaka J, Shuiqing Y, Welch CL, DePinho RA, et al. 2012. FoxOs integrate pleiotropic actions of insulin in vascular endothelium to protect mice from atherosclerosis. *Cell Metab.* 15(3):372–81
80. Xiao Y, Huang W, Zhang J, Peng C, Xia M, Ling W. 2015. Increased plasma S-adenosylhomocysteine-accelerated atherosclerosis is associated with epigenetic regulation of endoplasmic reticulum stress in apoE$^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* 35(1):60–70
81. Wu S, Gao X, Yang S, Meng M, Yang X, Ge B. 2015. The role of endoplasmic reticulum stress in endothelial dysfunction induced by homocysteine thiolactone. *Fundam. Clin. Pharmacol.* 29(3):252–59
82. Zhang H, Liu Z, Ma S, Zhang H, Kong F, et al. 2016. Ratio of S-adenosylmethionine to S-adenosylhomocysteine as a sensitive indicator of atherosclerosis. *Mol. Med. Rep.* 14(1):289–300
83. Rabelo LA, Ferreira FO, Nunes-Souza V, da Fonseca LJ, Goulart MO. 2015. Arginase as a critical prooxidant mediator in the binomial endothelial dysfunction-atherosclerosis. *Oxidative Med. Cell. Longev.* 2015:924860
84. Steppan J, Nyhan D, Berkowitz DE. 2013. Development of novel arginase inhibitors for therapy of endothelial dysfunction. *Front. Immunol.* 4:278
85. de Bruin RG, van der Veer EP, Prins J, Lee DH, Dane MJ, et al. 2016. The RNA-binding protein quaking maintains endothelial barrier function and affects VE-cadherin and β-catenin protein expression. *Sci. Rep.* 6:21643
86. Novodvorsky P, Chico TJ. 2014. The role of the transcription factor *KLF2* in vascular development and disease. *Prog. Mol. Biol. Transl. Sci.* 124:155–88
87. Loyer X, Potteaux S, Vion AC, Guerin CL, Boulkroun S, et al. 2014. Inhibition of microRNA-92a prevents endothelial dysfunction and atherosclerosis in mice. *Circ. Res.* 114(3):434–43
88. Tanweer O, Wilson TA, Metaxa E, Riina HA, Meng H. 2014. A comparative review of the hemodynamics and pathogenesis of cerebral and abdominal aortic aneurysms: lessons to learn from each other. *J. Cerebrovasc. Endovasc. Neurosurg.* 16(4):335–49
89. McCormick ML, Gavrila D, Weintraub NL. 2007. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. *Arterioscler. Thromb. Vasc. Biol.* 27(3):461–69
90. Fan LM, Douglas G, Bendall JK, McNeill E, Crabtree MJ, et al. 2014. Endothelial cell-specific reactive oxygen species production increases susceptibility to aortic dissection. *Circulation* 129(25):2661–72
91. Wasserman DH, Wang TJ, Brown NJ. 2018. The vasculature in prediabetes. *Circ. Res.* 122(8):1135–50
92. Hamilton SJ, Watts GF. 2013. Endothelial dysfunction in diabetes: pathogenesis, significance, and treatment. *Rev. Diabet. Stud.* 10(2–3):133–56
93. Kolluru GK, Bir SC, Kevil CG. 2012. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Int. J. Vasc. Med.* 2012:918267
94. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, et al. 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321(7258):405–12
95. Eelen G, de Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. 2018. Endothelial cell metabolism. *Physiol. Rev.* 98(1):3–58
96. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, et al. 2002. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 105(14):1656–62
97. Liu J, Wang C, Liu F, Lu Y, Cheng J. 2015. Metabonomics revealed xanthine oxidase-induced oxidative stress and inflammation in the pathogenesis of diabetic nephropathy. *Anal. Bioanal. Chem.* 407(9):2569–79
98. Shevalye H, Lupachyk S, Watcho P, Stavniichuk R, Khazim K, et al. 2012. Prediabetic nephropathy as an early consequence of the high-calorie/high-fat diet: relation to oxidative stress. *Endocrinology* 153(3):1152–61
99. Sasaki N, Yamashita T, Takaya T, Shinohara M, Shiraki R, et al. 2008. Augmentation of vascular remodeling by uncoupled endothelial nitric oxide synthase in a mouse model of diabetes mellitus. *Arterioscler. Thromb. Vasc. Biol.* 28(6):1068–76

100. Su Y, Qadri SM, Hossain M, Wu L, Liu L. 2013. Uncoupling of eNOS contributes to redox-sensitive leukocyte recruitment and microvascular leakage elicited by methylglyoxal. *Biochem. Pharmacol.* 86(12):1762–74
101. Tang X, Luo YX, Chen HZ, Liu DP. 2014. Mitochondria, endothelial cell function, and vascular diseases. *Front. Physiol.* 5:175
102. Ceolotto G, Gallo A, Papparella I, Franco L, Murphy E, et al. 2007. Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. *Arterioscler. Thromb. Vasc. Biol.* 27(12):2627–33
103. Wang XR, Zhang MW, Chen DD, Zhang Y, Chen AF. 2011. AMP-activated protein kinase rescues the angiogenic functions of endothelial progenitor cells via manganese superoxide dismutase induction in type 1 diabetes. *Am. J. Physiol. Endocrinol. Metab.* 300(6):E1135–45
104. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, et al. 2006. Activation of AMP-activated protein kinase reduces hyperglycemia-induced mitochondrial reactive oxygen species production and promotes mitochondrial biogenesis in human umbilical vein endothelial cells. *Diabetes* 55(1):120–27
105. Li FY, Lam KS, Tse HF, Chen C, Wang Y, et al. 2012. Endothelium-selective activation of AMP-activated protein kinase prevents diabetes mellitus-induced impairment in vascular function and reendothelialization via induction of heme oxygenase-1 in mice. *Circulation* 126(10):1267–77
106. Dinkova-Kostova AT, Abramov AY. 2015. The emerging role of Nrf2 in mitochondrial function. *Free Radic. Biol. Med.* 88(Part B):179–88
107. Xue M, Rabbani N, Momiji H, Imbasi P, Anwar MM, et al. 2012. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem. J.* 443(1):213–22
108. Brouwers O, Niessen PM, Miyata T, Østergaard JA, Flyvbjerg A, et al. 2014. Glyoxalase-1 overexpression reduces endothelial dysfunction and attenuates early renal impairment in a rat model of diabetes. *Diabetologia* 57(1):224–35
109. Mo C, Wang L, Zhang J, Numazawa S, Tang H, et al. 2014. The crosstalk between Nrf2 and AMPK signal pathways is important for the anti-inflammatory effect of berberine in LPS-stimulated macrophages and endotoxin-shocked mice. *Antioxid. Redox Signal.* 20(4):574–88
110. Paneni F, Costantino S, Cosentino F. 2015. Role of oxidative stress in endothelial insulin resistance. *World J. Diabetes* 6(2):326–32
111. Kaiser N, Sasson S, Feener EP, Boukobza-Vardi N, Higashi S, et al. 1993. Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. *Diabetes* 42(1):80–89
112. Wei X, Schneider JG, Shenouda SM, Lee A, Towler DA, et al. 2011. De novo lipogenesis maintains vascular homeostasis through endothelial nitric-oxide synthase (eNOS) palmitoylation. *J. Biol. Chem.* 286(4):2933–45
113. Wei X, Song H, Semenkovich CF. 2014. Insulin-regulated protein palmitoylation impacts endothelial cell function. *Arterioscler. Thromb. Vasc. Biol.* 34(2):346–54
114. Jang C, Oh SF, Wada S, Rowe GC, Liu L, et al. 2016. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat. Med.* 22:421–26
115. Khitan Z, Kim DH. 2013. Fructose: a key factor in the development of metabolic syndrome and hypertension. *J. Nutr. Metab.* 2013:682673
116. Cirillo P, Pellegrino G, Conte S, Maresca F, Pacifico F, et al. 2015. Fructose induces prothrombotic phenotype in human endothelial cells: a new role for "added sugar" in cardio-metabolic risk. *J. Thromb. Thrombolysis* 40:444–51
117. Jain RK. 2014. Antiangiogenesis strategies revisited: from starving tumors to alleviating hypoxia. *Cancer Cell* 26(5):605–22
118. Krock BL, Skuli N, Simon MC. 2011. Hypoxia-induced angiogenesis: good and evil. *Genes Cancer* 2(12):1117–33
119. Hida K, Maishi N, Sakurai Y, Hida Y, Harashima H. 2016. Heterogeneity of tumor endothelial cells and drug delivery. *Adv. Drug Deliv. Rev.* 99(Part B):140–47
120. Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, et al. 2016. Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. *Cancer Cell* 30(6):968–85

121. van Beijnum JR, Dings RP, van der Linden E, Zwaans BM, Ramaekers FC, et al. 2006. Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature. *Blood* 108(7):2339–48
122. Vegran F, Boidot R, Michiels C, Sonveaux P, Feron O. 2011. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-κB/IL-8 pathway that drives tumor angiogenesis. *Cancer Res.* 71(7):2550–60
123. Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, et al. 2015. A lactate-induced response to hypoxia. *Cell* 161(3):595–609
124. Wenes M, Shang M, Di Matteo M, Goveia J, Martin-Pérez R, et al. 2016. Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab.* 24:701–15
125. Rivera JC, Dabouz R, Noueihed B, Omri S, Tahiri H, Chemtob S. 2017. Ischemic retinopathies: oxidative stress and inflammation. *Oxidative Med. Cell. Longev.* 2017:3940241
126. Wong WL, Su X, Li X, Cheung CM, Klein R, et al. 2014. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Global Health* 2(2):e106–16
127. Shah GN, Price TO, Banks WA, Morofuji Y, Kovac A, et al. 2013. Pharmacological inhibition of mitochondrial carbonic anhydrases protects mouse cerebral pericytes from high glucose-induced oxidative stress and apoptosis. *J. Pharmacol. Exp. Ther.* 344(3):637–45
128. Dodson MW, Brown LM, Elliott CG. 2018. Pulmonary arterial hypertension. *Heart Failure Clin.* 14(3):255–69
129. Leopold JA, Maron BA. 2016. Molecular mechanisms of pulmonary vascular remodeling in pulmonary arterial hypertension. *Int. J. Mol. Sci.* 17(5):761
130. Thenappan T, Prins KW, Cogswell R, Shah SJ. 2015. Pulmonary hypertension secondary to heart failure with preserved ejection fraction. *Can. J. Cardiol.* 31(4):430–39
131. Rafikova O, Srivastava A, Desai AA, Rafikov R, Tofovic SP. 2018. Recurrent inhibition of mitochondrial complex III induces chronic pulmonary vasoconstriction and glycolytic switch in the rat lung. *Respir. Res.* 19(1):69
132. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, et al. 2016. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *J. Clin. Investig.* 126(9):3313–35
133. Brittain EL, Talati M, Fessel JP, Zhu H, Penner N, et al. 2016. Fatty acid metabolic defects and right ventricular lipotoxicity in human pulmonary arterial hypertension. *Circulation* 133(20):1936–44
134. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, et al. 1993. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N. Engl. J. Med.* 328(24):1732–39
135. Ghosh S, Gupta M, Xu W, Mavrakis DA, Janocha AJ, et al. 2016. Phosphorylation inactivation of endothelial nitric oxide synthesis in pulmonary arterial hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 310(11):L1199–205
136. Garrigue P, Bodin-Hullin A, Balasse L, Fernandez S, Essamet W, et al. 2017. The evolving role of succinate in tumor metabolism: an ^{18}F-FDG–based study. *J. Nuclear Med.* 58(11):1749–55
137. Zhang D, Wang Y, Shi Z, Liu J, Sun P, et al. 2015. Metabolic reprogramming of cancer-associated fibroblasts by IDH3α downregulation. *Cell Rep.* 10(8):1335–48
138. Dong L, Krewson EA, Yang LV. 2017. Acidosis activates endoplasmic reticulum stress pathways through GPR4 in human vascular endothelial cells. *Int. J. Mol. Sci.* 18(2):278
139. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, et al. 2014. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513(7519):559–63
140. Castegna A, Menga A. 2018. Glutamine synthetase: localization dictates outcome. *Genes* 9(2):108
141. Pisarsky L, Bill R, Fagiani E, Dimeloe S, Goosen RW, et al. 2016. Targeting metabolic symbiosis to overcome resistance to anti-angiogenic therapy. *Cell Rep.* 15(6):1161–74
142. Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, et al. 2014. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *PNAS* 111(33):12228–33
143. Becker JC, Andersen MH, Schrama D, thor Straten P. 2013. Immune-suppressive properties of the tumor microenvironment. *Cancer Immunol. Immunother.* 62(7):1137–48

144. Colombo M, Raposo G, Théry C. 2014. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* 30:255–89
145. Garcia NA, Moncayo-Arlandi J, Sepulveda P, Diez-Juan A. 2016. Cardiomyocyte exosomes regulate glycolytic flux in endothelium by direct transfer of GLUT transporters and glycolytic enzymes. *Cardiovasc. Res.* 109(3):397–408
146. Bergers G, Hanahan D. 2008. Modes of resistance to anti-angiogenic therapy. *Nat. Rev. Cancer* 8(8):592–603
147. Qu Q, Zeng F, Liu X, Wang QJ, Deng F. 2016. Fatty acid oxidation and carnitine palmitoyltransferase I: emerging therapeutic targets in cancer. *Cell Death Dis.* 7:e2226
148. Ashrafian H, Horowitz JD, Frenneaux MP. 2007. Perhexiline. *Cardiovasc. Drug Rev.* 25(1):76–97
149. Gross MI, Demo SD, Dennison JB, Chen L, Chernov-Rogan T, et al. 2014. Antitumor activity of the glutaminase inhibitor CB-839 in triple-negative breast cancer. *Mol. Cancer Ther.* 13(4):890–901
150. Jacque N, Ronchetti AM, Larrue C, Meunier G, Birsen R, et al. 2015. Targeting glutaminolysis has antileukemic activity in acute myeloid leukemia and synergizes with BCL-2 inhibition. *Blood* 126(11):1346–56
151. Ikeuchi H, Ahn YM, Otokawa T, Watanabe B, Hegazy L, et al. 2012. A sulfoximine-based inhibitor of human asparagine synthetase kills L-asparaginase-resistant leukemia cells. *Bioorg. Med. Chem.* 20(19):5915–27