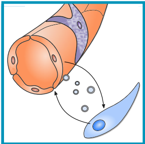
Physiol Rev 98: 3–58, 2018
Published November 22, 2017; doi:10.1152/physrev.00001.2017

ENDOTHELIAL CELL METABOLISM

Guy Eelen, Pauline de Zeeuw, Lucas Treps, Ulrike Harjes, Brian W. Wong, and Peter Carmeliet

Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, KU Leuven, Leuven, Belgium; and Laboratory of Angiogenesis and Vascular Metabolism, Center for Cancer Biology, VIB, Leuven, Belgium



Eelen G, de Zeeuw P, Treps L, Harjes U, Wong BW, Carmeliet P. Endothelial Cell Metabolism. *Physiol Rev* 98: 3–58, 2018. Published November 22, 2017; doi: 10.1152/physrev.00001.2017.—Endothelial cells (ECs) are more than inert blood vessel lining material. Instead, they are active players in the formation of new blood vessels (angiogenesis) both in health and (life-threatening) diseases. Recently, a new concept arose by which EC metabolism drives angiogenesis in parallel to well-established angiogenic growth factors (e.g., vascular endothelial growth factor). 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3-driven glycolysis generates energy to sustain competitive behavior of the ECs at the tip of a growing vessel sprout, whereas carnitine palmitoyltransferase 1a-controlled fatty acid oxidation regulates nucleotide synthesis and proliferation of ECs in the stalk of the sprout. To maintain vascular homeostasis, ECs rely on an intricate metabolic wiring characterized by intracellular compartmentalization, use metabolites for epigenetic regulation of EC subtype differentiation, crosstalk through metabolite release with other cell types, and exhibit EC subtype-specific metabolic traits. Importantly, maladaptation of EC metabolism contributes to vascular disorders, through EC dysfunction or excess angiogenesis, and presents new opportunities for anti-angiogenic strategies. Here we provide a comprehensive overview of established as well as newly uncovered aspects of EC metabolism.

I.	**INTRODUCTION**	**3**
II.	**ANGIOGENESIS: GENERAL...**	**3**
III.	**ENDOTHELIAL CELL...**	**6**
IV.	**COMPARTMENTALIZATION OF...**	**14**
V.	**CHANGES IN METABOLISM DURING...**	**17**
VI.	**ENDOTHELIAL CELL HETEROGENEITY,...**	**18**
VII.	**ENDOTHELIAL CELL METABOLISM IN...**	**20**
VIII.	**ENDOTHELIAL CELL METABOLISM...**	**30**
IX.	**METABOLIC MEMORY OF ENDOTHELIAL...**	**34**
X.	**METABOLIC CROSSTALK**	**36**
XI.	**THERAPEUTIC OPPORTUNITIES...**	**36**
XII.	**CONCLUDING SUMMARY**	**39**

I. INTRODUCTION

Even though cellular metabolism has been studied for over a century, endothelial cell (EC) metabolism has been receiving growing attention only during the last few years. Blood vessel forming ECs display a remarkable behavioral plasticity; while quiescent for years, ECs can switch almost instantaneously to an activated, highly proliferative, and migratory state in response to growth factor stimuli, primarily through vascular endothelial growth factor (VEGF) signaling (436). It has long been overlooked if this angiogenic switch (angiogenesis is the broad term for the formation of new blood vessels) is reflected by a metabolic switch and if so whether the altered metabolism is a key driver or merely a subsequent bystander adaptation. Recent papers on glycolysis and fatty acid oxidation (FAO) in ECs reveal that metabolism drives vessel sprouting in parallel to well-established growth factor-based (genetic) signaling (114, 481). These seminal findings have paved the way towards a more in-depth understanding of EC metabolism, which gains further importance in light of limited overall successes of growth factor-centric therapies in treating pathological angiogenesis (38, 151, 583). Indeed, the endothelium, either by dysfunctionality or by excessive vessel sprouting, can be at the origin of devastatingly lethal disorders (145). Proof-of-principle studies demonstrate how targeting EC metabolism can be exploited as an alternative for growth factor-based approaches, with an advantageous reduction in resistance and escape mechanisms [as they occur for example in tumor vasculature upon anti-VEGF treatment (70); see sect. VIII]. This review aims to provide emerging insights in various aspects of EC metabolism both in health and disease and discusses our current knowledge on intricate topics such as heterogeneity and compartmentalization of EC metabolism and metabolic crosstalk between ECs and other cell types. Thorough understanding of metabolic programming of ECs in quiescent versus angiogenic state and in normal developmental and physiological angiogenesis versus dysfunctional and pathological angiogenesis promises to offer novel opportunities for future EC metabolism-centric therapeutics.

II. ANGIOGENESIS: GENERAL PRINCIPLES AND CONCEPTS

The vasculature is a truly remarkable organ. It is one of the first functional organs to form during embryogenesis and matures into a closed cardiovascular system to conduct

0031-9333/18 Copyright © 2018 the American Physiological Society

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

blood flow through an intricate network of large- to medium-size vessels extending into micrometer-size capillaries, adding up to an astonishing 90,000 km in total length in adults (436, 588). Apart from some exceptions (e.g., cartilage and cornea are avascular), all tissues rely on blood vessels for a continuous supply of nutrients and oxygen, and on lymphatic vessels to drain and filter interstitial fluids. In addition, blood vessels take part in controlling systemic pH and temperature homeostasis and in mediating immune responses (reviewed in Ref. 588).

During early embryo development, a primitive vascular plexus is formed in a process termed vasculogenesis. In brief, mesodermal angioblasts (EC progenitors) aggregate to form primitive vessel-like endothelial tubes lacking mural cell coverage (167, 424) **(FIGURE 1)**. The hemangioblast, a precursor shared by ECs and hematopoietic cells, has also been proposed as another source to form endothelium during development (reviewed in Ref. 565). Subsequent extensive remodeling and growth of the primary plexus occurs through different mechanisms of vessel formation such as vessel splitting (intussusception) and vessel sprouting (generally known as angiogenesis). Vessel splitting or intussusceptive growth expands the capillary bed literally by "splitting" a capillary into two adjacent vessels. The opposite walls of the capillary project into the capillary lumen and have their ECs contact each other to locally form an endothelial bilayer, which is then holed by reorganization of intracellular junctions. Pericytes and myofibroblasts cover the resulting hollow transcapillary pillar, which increases in circumference to split the capillary in two parallel vessels (341) **(FIGURE 1)**.

Sprouting angiogenesis, whereby new capillaries sprout from a preexisting vessel, is the more frequently studied and better-understood form of vessel formation, and typically entails a series of highly orchestrated processes driven by identified pro-angiogenic stimuli. Driving this phenomenon is the surrounding tissue's need for oxygen and nutrients, which incites production of VEGF, fibroblast growth factors (FGFs), and other pro-angiogenic stimuli by nonvascular cells (70). Upon reaching the existing vessel, VEGF binds to its receptor VEGF receptor 2 (VEGFR2 also known as Flk1 or KDR) on ECs and, locally, the preexisting vessel "relaxes" to allow a new vessel sprout to arise: endothelial cell-cell contacts untighten, pericytes detach, and the basement membrane is broken down. The formation of the new sprout has been studied recently intensely in the context of retinal angiogenesis and occurs in a highly coordinated manner (reviewed in Ref. 436) whereby one EC, the one that was exposed to the highest VEGF level, is selected to become the "tip cell" and to migrate and guide the new sprout towards the very source of the growth factor (567). While migrating, the sprout needs to elongate by multiplying the number of "stalk cells" following immediately behind the tip cell **(FIGURE 2*A*)**.

Even though originating from the same preexisting vessel, tip and stalk cells in the nascent vessel differ both functionally and morphologically. Tip cells have numerous filopodia and protrusions corresponding to their highly motile behavior, whereas stalk cells have relatively few filopodia (192). Tip versus stalk specification is highly dynamic and as such allows selection of the fittest EC for the tip position to lead the sprout at every given moment. ECs compete for

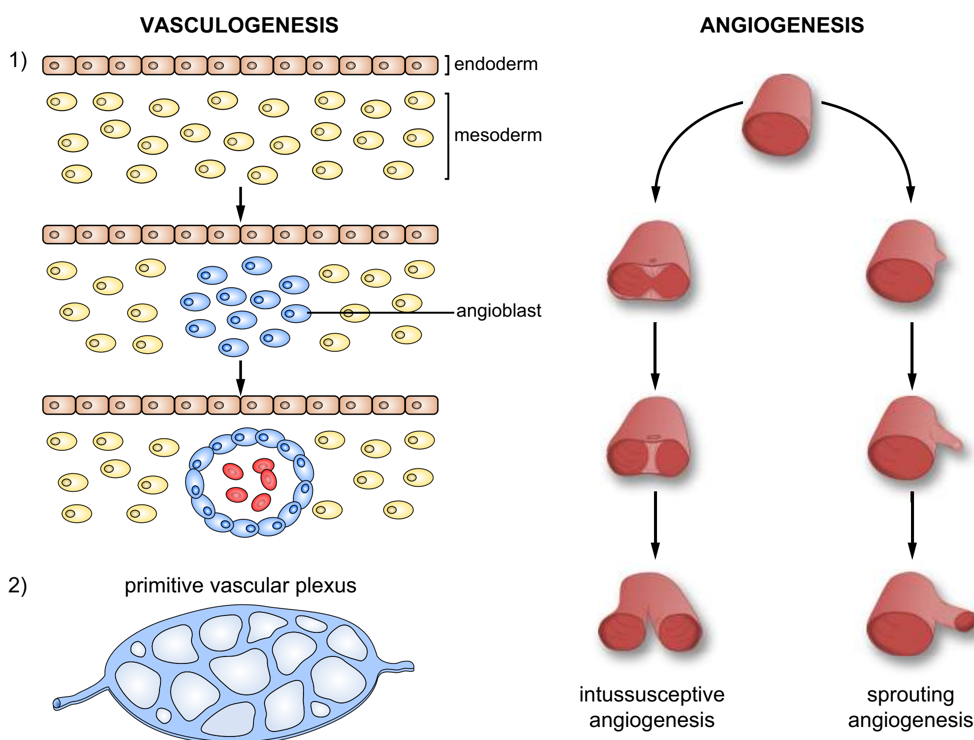


FIGURE 1. General concepts in angiogenesis: formation of a vascular plexus. During vasculogenesis, mesodermal EC progenitors (angioblasts) cluster to form vessel-like endothelial tubes (*1*) which eventually form a primitive vascular plexus (*2*). This primitive plexus subsequently undergoes substantial remodeling by vessel intussusception whereby a preexisting capillary splits in two adjacent vessels or by sprouting angiogenesis whereby a new capillary sprouts of a preexisting vessel.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

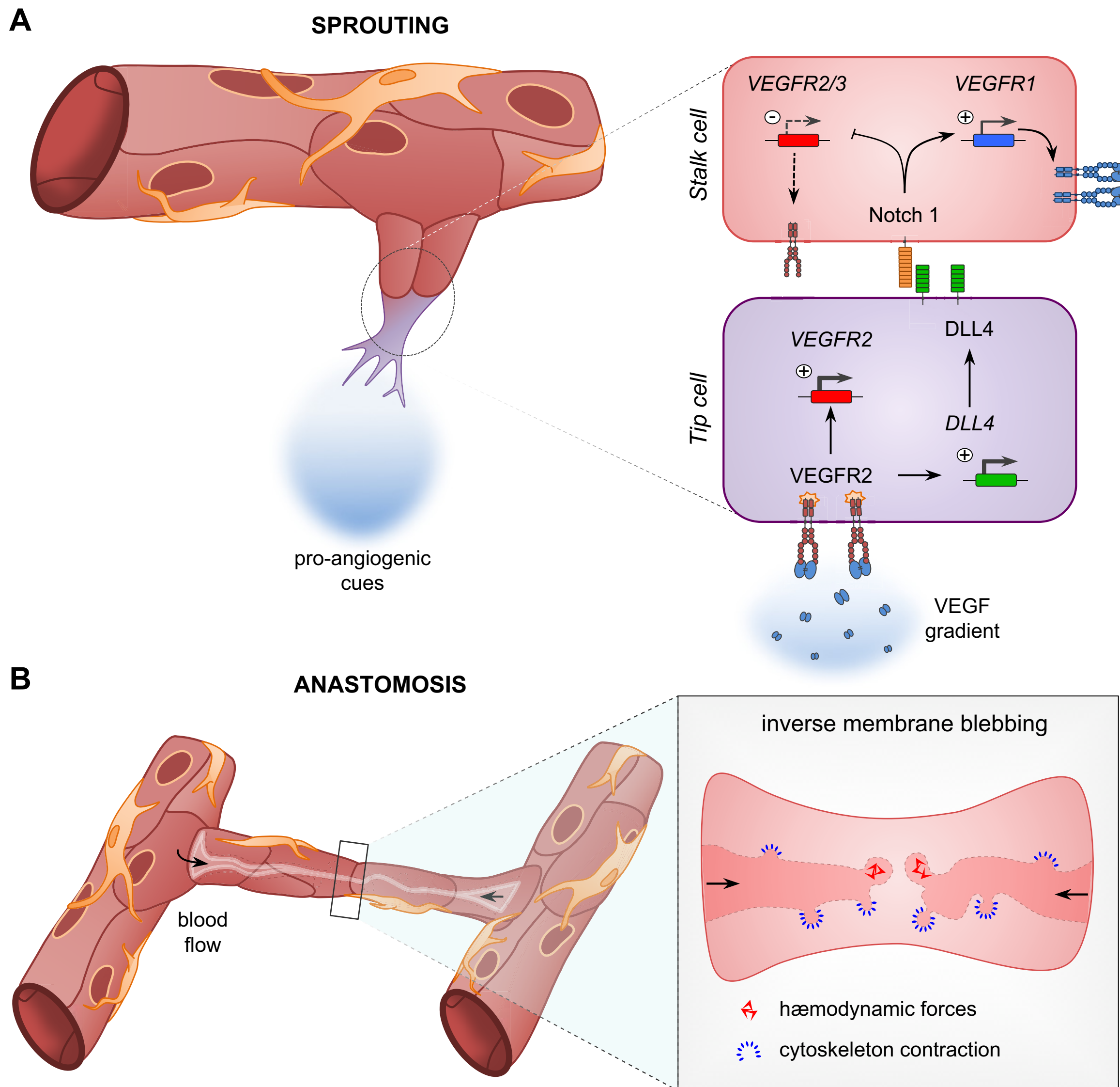


FIGURE 2. General concepts in angiogenesis: tip versus stalk specification and anastomosis. *A*: in sprouting angiogenesis, a pro-angiogenic growth factor gradient induces tip and stalk cell formation in a preexisting vessel to form a new sprout. In the tip cell, VEGF binds and activates its receptor VEGFR2, which induces Dll4 expression. In the neighboring ECs, Dll4 binds Notch receptors, which drive expression of the "decoy" VEGF receptor 1 (VEGFR1) while reducing VEGFR2 expression. Ultimately, this increases the VEGFR1/VEGFR2 ratio and lowers EC responsiveness to VEGF, causing the EC to adopt a stalk cell phenotype. Other genetic signals are involved in tip/stalk specification but are not included in the figure for reasons of clarity. *B*: newly formed sprouts from neighboring vessels meet and fuse through a process termed anastomosis. To allow blood flow, a fully functional, interconnected lumen needs to form in the new sprout; the hemodynamic force of the blood flow itself can cause lumen expansion by causing inverse blebbing of the ECs' apical membrane. VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; Dll4, Delta like 4.

the tip cell position in a process termed "tip cell overtaking" and "EC shuffling" (17, 255). Intriguingly, the tip cell itself maintains neighboring ECs in a stalk cell phenotype by expressing the Notch ligand Delta-like 4 (Dll4). In adjacent ECs, Dll4 binds Notch receptors, causing the Notch intracellular domain (NICD) to be released and to drive expression of the high-affinity/low-activity "decoy" VEGF receptor 1 (VEGFR1 also known as Flt1) (179) while reducing VEGFR2 expression. The increased VEGFR1/VEGFR2 ratio lowers EC responsiveness to VEGF and imposes a stalk cell phenotype (432) **(FIGURE 2A)**. Continuous "cell shuffling" at the utmost leading end of the sprout allows the EC with the lowest VEGFR1/VEGFR2 ratio (this is the fittest EC in terms of VEGF sensing) to obtain the tip position (17, 255). The shuffling causes continuous changes in cellular neighboring interactions, disrupting the Dll4-Notch lateral-inhibition feedback mechanism. However, in the new sprout, Dll4 levels fluctuate dynamically in individual cells, leading to a salt-and-pepper organization along the sprout of $Dll4^{high}$ and $Dll4^{low}$ ECs. This pattern guarantees that for every given tip-stalk configuration, the correct between-neighbors Dll4-Notch signaling can take place to support

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

the differential behavior between two neighboring ECs (255, 549). Based on findings that tip cell overtaking rates were not altered upon VEGF addition or Dll4-Notch signaling inhibition (17), a recent computational modeling study proposes that tip cell overtaking is a side effect arising from normal stochastic motion of ECs during sprout formation. The VEGF-Dll4-Notch signaling then ensures that the EC that has randomly acquired the tip position indeed adopts tip cell behavior, rather than dictating which EC takes the tip position (43).

Expression of Dll4 itself is under transcriptional control of the Tel-CtBP repressor complex, which is disassembled from the Dll4 promoter by a VEGF stimulus to allow a transient rise in Dll4 levels (467). The Wnt-β-catenin signaling pathway, another important player in angiogenesis (reviewed in Ref. 456), can also affect Dll4 transcription (94). Next to Dll4, Jagged1 is another Notch ligand involved in tip versus stalk specification; in vitro data show how Jagged1 opposes Dll4 by acting as a negative regulator of Notch activity and promotes EC proliferation and sprouting (37). In addition, the PTEN (phosphatase and tensin homolog) tumor suppressor is crucial to establish the Notch-mediated stalk cell proliferation arrest and to procure normal vessel density and patterning in vivo (490). The VEGF-Dll4-Notch signaling cascade is a major control mechanism of tip-stalk specification, but other signaling axes codetermine this complex mechanism in vessel sprouting and patterning (reviewed in Refs. 149, 279, 436, 445).

The more mature, cobblestone-like "phalanx cells" (360) connect the growing sprout with the preexisting vessel and start forming a lumen to allow blood flow. To form a closed system, neighboring sprouts meet and their tip cells fuse (anastomosis); macrophages, through pro-angiogenic factors they secrete, act as linkers between two anastomosing tip cells (161). A key event after anastomosis is the development of an interconnected lumen to allow functional blood flow. A number of different mechanisms have been suggested to drive lumen formation and extension (reviewed in Ref. 41). Recent findings show that the hemodynamic force of the blood flow itself can drive lumen formation in the newly forming sprout, by a mechanism termed "inverse blebbing"; blood flow pressure stimulates the apical membrane of ECs to form bleblike deformations causing local contraction of actomyosin, allowing lumen expansion (190) (**FIGURE 2*B***). A fully functional vessel is finally established and stabilized by the recruitment of platelet-derived growth factor (PDGF) receptor β (PDGFRβ) expressing perivascular cells through excretion of PDGF-B by the ECs (180).

III. ENDOTHELIAL CELL METABOLISM DRIVING ANGIOGENESIS

The following paragraphs aim to provide a comprehensive overview of normal (i.e., under nondiseased circumstances) EC metabolism confined to glucose, amino acids (AAs), and fatty acids (FAs), the three major substrates for energy and biomass production in ECs, as they have been most extensively studied (for schematic overview, see **FIGURE 3**). Even though they have greatly enhanced our insights into EC metabolism, most of the data discussed originate from ECs (often derived from specific vascular beds; see sect. VI for more details on EC heterogeneity) cultured in vitro in two-dimensional monolayers, which do not fully match the plethora of in vivo three-dimensional environmental cues and which have been shown to lead to differences in cellular metabolism in other cell types (112). Additionally, the traditional culture media in which ECs are grown fail to mimic the in vivo metabolite composition and might substantially rewire the EC's metabolism (67). Still, phenotyping of mice lacking the metabolic target specifically in ECs, employed only since very recently, supports the basic relevance of the in vitro findings. Future in vivo EC metabolic studies (see

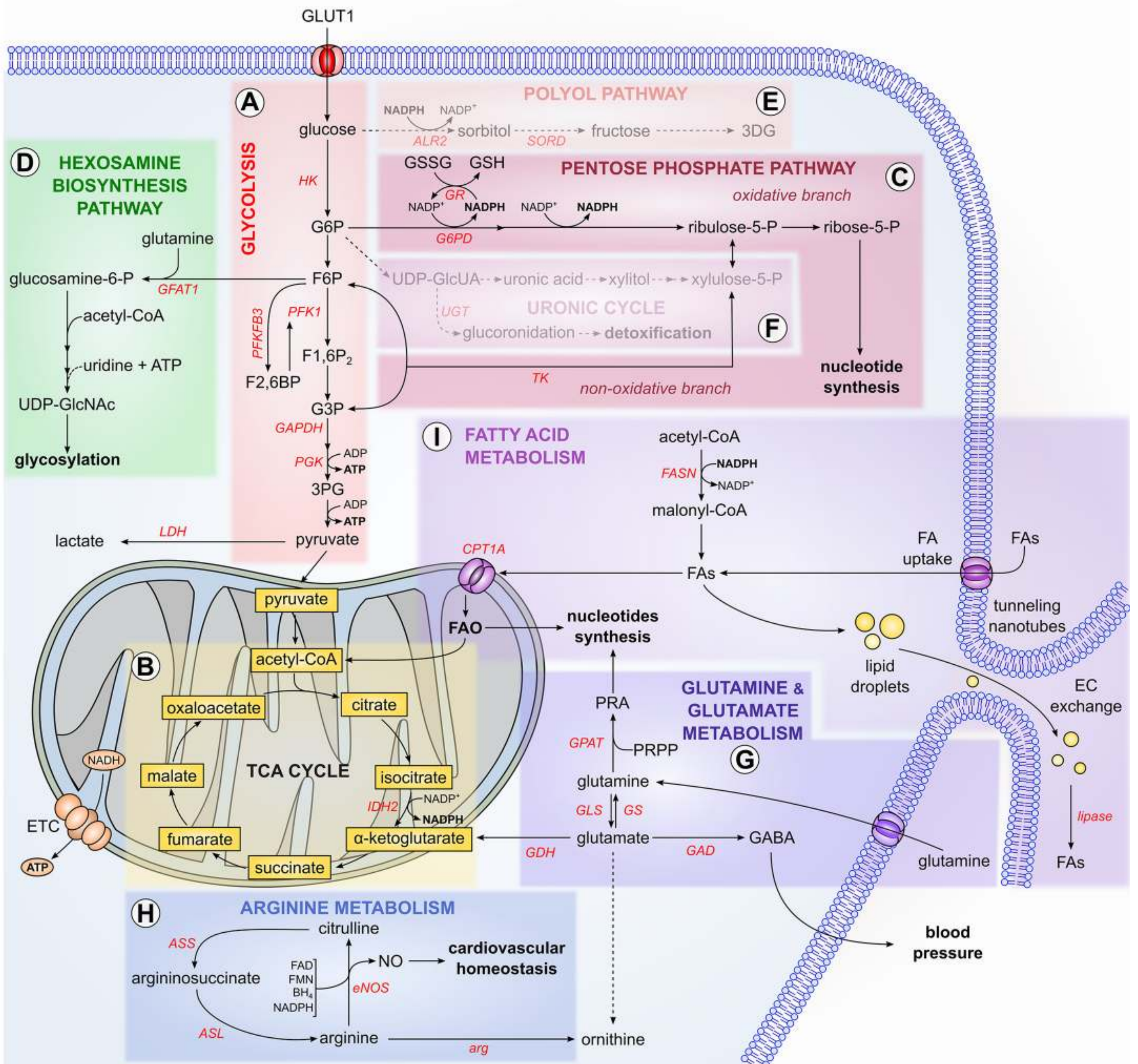
FIGURE 3. General overview of metabolic pathways in healthy ECs. Schematic representation of metabolic pathways in normal ECs (*A*, glycolysis; *B*, TCA cycle; *C*, pentose phosphate pathway; *D*, hexosamine biosynthesis pathway; *E*, polyol pathway; *F*, uronic cycle; *G*, glutamine and glutamate metabolism; *H*, arginine metabolism; *I*, fatty acid metabolism); to prevent overcrowding of the figure, not all individual steps in each pathway are shown. For the same reason, one-carbon metabolism, the mevalonate pathway, and cysteine-to-H_2S metabolism are omitted from this figure and described in detail in **FIGURES 10 AND 11**. Pathways with minimal activity in healthy ECs have lower opacity in the figure. 3DG, 3-deoxyglucosone; 3PG, 3-phosphogylcerate; α-KG, α-ketoglutarate; ALR2, aldose reductase 2; ARG, arginase; ASL, argininosuccinate lyase; ASS, argininosuccinate synthase; BH_4, tetrahydrobiopterin; CoA, coenzyme A; CPT1a, carnitine palmitoyltransferase 1a; DHAP, dihydroxyacetone phosphate; eNOS, endothelial nitric oxide synthase; ETC, electron transport chain; F1,6P_2, fructose-1,6-bisphosphate; F2,6BP, fructose-2,6-bisphosphate; F6P, fructose-6-phosphate; FA, fatty acid; FAD, flavin adenine dinucleotide; FAO, fatty acid oxidation; FASN, fatty acid synthase; FMN, flavin mononucleotide; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; glucosamine-6-P, glucosamine-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GABA, γ-aminobutyric acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GAD, glutamic acid decarboxylase; GDH, glutamate dehydrogenase; GFAT1, glutamine fructose-6-phosphate amidotransferase; GLS, glutaminase; GPAT, glutamine phosphoribosylpyrophosphate amidotransferase; GR, glutathione reductase; GlucN6P, glucosamine-6-phosphate; GLUT1, glucose transporter 1; GS, glutamine synthetase; GSH, reduced glutathione; GSSG, oxidized glutathione; HK, hexokinase; IDH2, isocitrate dehydrogenase 2; LDH, lactate dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OAA, oxaloacetate; PGK, phosphoglycerate kinase; PFK1, phosphofructokinase-1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PRA, 5-phosphoribosyl-1-amine; PRPP, 5-phosphoribosyl-1-pyrophosphate; ribose-5-P, ribose-5-phosphate; ribulose-5-P, ribulose-5-phosphate; ROS, reactive oxygen species; SORD, sorbitol dehydrogenase; TCA, tricarboxylic acid; THF, tetrahydrofolate; TK, transketolase; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine; UDP-GlcUA, UDP-glucuronic acid; xylulose-5-P, xylulose-5-phosphate.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

sect. XI) will further reinforce, complement, or adjust these data.

A. Glucose Metabolism: All About Glycolysis?

ECs prefer not to maximize energy (ATP) production by shunting all glucose they take up into oxidative phosphorylation (OXPHOS) but rely on glycolysis instead **(FIGURE 3*A*)** (114, 291). Indeed, in rat coronary microvascular ECs, ~98% of glucose is being metabolized to lactate while only a mere 0.04% was oxidized in the tricarboxylic acid (TCA) cycle **(FIGURE 3*B*)** (291). Similarly, in human umbilical vein ECs (HUVECs), glycolytic flux (~1.5 μmol glucose$\cdot$h$^{-1}\cdot$mg protein^{-1}) was estimated to be >200-fold higher than glucose oxidation fluxes (114). This is in line with the relatively small mitochondrial volume in ECs (209) but seemingly contradicts the assumption that ECs could directly use the high oxygen levels in blood (e.g., arterial ECs are exposed to a partial oxygen pressure of ~100 mmHg; Ref. 148). Selecting glycolysis over oxidative phosphorylation lowers the net ATP yield per mole glucose (approximately 20-fold lower), but might offer ECs a number of payoffs. First, high glycolysis rates sustain lactate production, which functions as a pro-angiogenic signaling molecule (243, 471, 506, 557). Second, reactive oxygen species (ROS, typically gen-



Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

erated by oxidative phosphorylation in ECs) are kept at a minimum, whereas amounts of oxygen available for transfer to surrounding cells and tissues are maximally preserved since they are not consumed by oxidative phosphorylation. Third, reliance on glycolysis preconditions ECs for sprouting into an avascular, hypoxic environment where interstitial glucose levels are not rate-limiting but oxygen levels are (58, 188). In addition, when external glucose is nonlimiting, high glycolysis rates can be even quicker in producing ATP than oxidative phosphorylation. As such, a direct comparison between pulmonary microvascular ECs (PMVECs; highly glycolytic, rapid growth) and pulmonary arterial ECs (PAECs, more oxidative, slower growth) revealed twofold higher total cellular ATP content in PMVECs versus PAECs in spite of twofold higher oxygen consumption rates in the latter (423). Finally, high glycolysis rates sustain macromolecule synthesis by feeding glucose carbons into glycolytic side branches (see below in this section).

VEGF stimulation causes ECs to elevate their glycolytic flux and to increase expression levels of the glucose transporter 1 [GLUT1/SLC2A1; by activating phosphoinositide 3-kinase (PI3K)-Akt signaling (627)] and of glycolytic enzymes, such as lactate dehydrogenase-A (LDH-A) and the bifunctional 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) (114, 423, 430). Whereas glycolytic cell types often express the monocarboxylate transporter 4 (MCT4) for adequate efflux of glycolysis-derived lactate (429), it remains to be substantiated if VEGF induces MCT4 expression/activity in ECs. Besides the glycolytic upregulation in response to VEGF, the effects of other key angiogenic growth factors promoting EC growth, such as for example angiopoietin-1, on glycolytic metabolism are not well documented at present and warrant future research. Conversely, Dll4 reduces PFKFB3 protein levels and lowers glycolytic flux in HUVECs (114). PFKFB3 is a glycolysis regulator and uses its kinase activity (which is increased 700-fold over its phosphatase activity) to generate fructose-2,6-bisphosphate (F2,6BP), which allosterically activates the rate-limiting glycolytic enzyme phosphofructokinase-1 (PFK1). Even though genetic silencing or chemical inhibition of PFKFB3 only partially (by 40%) reduces glycolytic flux, probably inherent to its function as a regulator of glycolysis rather than a truly rate-limiting enzyme, it suffices to substantially impair in vitro EC sprouting as well as in vivo vessel branching and outgrowth (114, 483, 608). Additional compelling evidence for a prominent role of PFKFB3 in vessel sprouting derives from loss of tip cell behavior in PFKFB3-silenced HUVECs, even when genetically primed to acquire tip cell position by simultaneous Notch knock-down (114). Conversely, PFKFB3 overexpression overrules the pro-stalk signal from simultaneous NICD overexpression (114). Importantly, similar findings were obtained when mosaically overexpressing PFKFB3 and/or NICD selectively in ECs in zebrafish embryos, i.e., NICD transgene expressing ECs were usually observed at the stalk position, while ECs expressing both the NICD and PFKFB3 transgene moved to the tip position (114). Furthermore, computational modeling, confirmed by experimentation, on tip-stalk behavior in EC sprouting, predicted that glycolytic ATP is indeed a driver for EC rearrangements in the sprout by enhancing filopodia formation and by decreasing intercellular adhesion (102). Furthermore, the computational approach accurately predicted that distorted EC rearrangements in the sprout caused by elevated VEGF stimulation (as in pathological angiogenesis; see sect. VIII) can be normalized by PFKFB3 inhibition (102). Not only the growing sprout but the fully lumenized, more mature, and quiescent endothelium too relies on tight PFKFB3 regulation. Indeed, the laminar shear stress, exerted on the quiescent endothelium by blood flow, induces the flow-responsive transcription factor Krüppel-like factor 2 (KLF2) to repress PFKFB3 expression through binding to a KLF2-binding site in the PFKFB3 promoter (126). The resulting reduced glycolysis rate, together with lower glucose uptake but also reduced mitochondrial content, ensures a metabolically quiescent state in the mature endothelium.

B. Glycolytic Side Branches: Pentose Phosphate Pathway and Hexosamine Synthesis

Glucose-6-phosphate (G6P), the first glycolytic intermediate after glucose, can shunt into the pentose-phosphate pathway (PPP) **(FIGURE 3*C*)** to be oxidized, albeit for pentose production for anabolic nucleotide and nucleic acid synthesis rather than for catabolic purposes (461). G6P dehydrogenase (G6PD) is the rate-limiting enzyme of the irreversible oxidative branch (oxPPP) that generates ribulose-5-phosphate, whereas transketolase (TK) rate-limits the reversible nonoxidative branch (non-oxPPP) in which ribose-5-phosphate (a substrate for nucleotide synthesis) is generated and, when the reaction occurs in the reverse direction, the glycolytic intermediates fructose-6-phosphate (F6P) and glyceraldehyde-3-phosphate (G3P) are produced. Inhibiting either of both enzymes lowers EC viability and migration (563, 638). The oxPPP produces NADPH, a crucial cofactor for (endothelial) nitric oxide synthase [(e)NOS]-driven nitric oxide (NO) synthesis (see below in this section). Additionally, NADPH is required for lipid and nucleotide synthesis, and used by glutathione reductase to convert oxidized glutathione disulfide (GSSG) to reduced glutathione (GSH), thereby controlling intracellular redox homeostasis (reviewed in Refs. 84, 425, 489).

Bovine aortic ECs overexpressing G6PD have higher NADPH levels, retain GSH levels, and as a result better withstand oxidative insult. Additionally, these cells display increased eNOS activity (313). The resulting increase in NO availability might partially underlie the enhanced angiogenic behavior in G6PD-overexpressing ECs upon VEGF treatment. Conversely, G6PD-silencing reduces migration, prolifera-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

tion, and tube formation in response to VEGF (312). Additionally, VEGFR2, Akt, and eNOS (tyrosine) phosphorylation are induced upon G6PD overexpression by mechanisms requiring further elucidation, while being reduced by G6PD silencing, possibly further contributing to the above findings (312). Reciprocally, VEGF induces G6PD tyrosine phosphorylation and plasma membrane localization/activity in an Src tyrosine kinase-dependent manner (411). Of note, aortic rings from G6PD-deficient "Pretsch" mice, having only ~15% residual G6PD activity in homozygotes (437) and ~32% remaining NADPH levels (as measured in the kidneys) (609), show reduced vessel outgrowth in comparison with control animals (312).

One step further down the glycolytic pathway, fructose-6-phosphate (F6P) can be shunted into the hexosamine biosynthesis pathway (HBP) (**FIGURE 3*D***). The HBP is often considered a "nutrient sensing pathway" given the requirement of glucose, glutamine, acetyl-CoA, uridine, and ATP to form the end product UDP-*N*-acetylglucosamine [UDP-GlcNAc; at ±40 nmol/g tissue, the second most abundant nucleotide form in the organism after ATP (±100 nmol/g tissue) (117)] of which the GlcNAc group is used in *O*- and *N*-linked protein glycosylation (26). Glutamine:fructose-6-phosphate aminotransferase 1 (GFAT1) is the first and rate-limiting enzyme in the pathway and condenses glutamine and F6P into glucosamine-6-P. GFAT1 was shown to be under transcriptional control of the spliced X-box binding protein 1 (XBP1s) and as such links the HBP with endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) (576). Activating transcription factor 4 (ATF4), another UPR effector, induces GFAT1 expression upon glucose shortage and amino acid deprivation (76). By virtue of its nutrient sensing role, the HBP could be of vital importance in ECs when sprouting into avascular, nutrient-scarce surroundings. Nevertheless, the precise metabolic role of the HBP in ECs, beyond glycosylating and thereby sustaining proper functioning of key angiogenic regulators like VEGFR2 and Notch (37, 551), remains unknown and is likely highly contextual. For instance, supplying HUVECs and EA.hy926 ECs with glucosamine increases protein *O*-GlcNAc-ation and reduces EC migration and vascular tube formation, while reducing glycosylation by overexpressing *O*-GlcNAcase has opposite effects (335). If and how exactly these findings relate to the effect of glucosamine on the PPP flux and subsequently on eNOS activity (see below in this section) or other angiogenic factors, or to the role of glycosylation as a modifier of the "histone code," thereby translating nutrient status into chromatin dynamics (117) (see sect. IX), remains to be determined.

2-Deoxy-D-glucose (2-DG) is best known as a glycolytic inhibitor: hexokinase phosphorylates 2-DG, which then competitively inhibits phosphoglucose isomerase, the next enzyme in the glycolytic pathway. Additionally, as a mannose-mimetic, 2-DG also reduces *N*-glycosylation by competing with mannose to build dolichol-pyrophosphate-linked oligosaccharides, precursors for *N*-glycosylation (reviewed in Ref. 299). Low-dose 2-DG treatment disturbs endothelial *N*-glycosylation and reduces EC proliferation, migration, and in vitro capillary formation through VEGFR2 hypoglycosylation and ER stress resulting in reduced ERK and Akt levels (287, 369). EC-specific deletion of Nogo-B-interacting protein (NgBr), involved in the synthesis of dolichol (the anchor for oligosaccharides for protein glycosylation), causes embryonic vascular development defects with an underlying reduction in glycosylation of VEGFR2, CD31, and VE-cadherin (421), thus highlighting the importance of dolichol synthesis for angiogenesis.

C. Other Glucose Metabolism Pathways

Under physiological conditions, ~3% of glucose is diverted into the polyol pathway (**FIGURE 3*E***), a two-step pathway in which cytosolic aldose reductase (ALR2) reduces glucose to sorbitol (at the expense of NADPH and ultimately GSH redox cycling) and sorbitol dehydrogenase (SORD) subsequently converts sorbitol to fructose (331, 347). Fructose, also a common source of sugar in natural foods, is a precursor of the noxious and highly reactive dicarbonyl compound 3-deoxyglucosone (3-DG) (260), which contributes to the nonenzymatic production of highly toxic advanced glycation end products (AGEs; see sect. VII) (577). Whether fructose can be metabolized to intermediates entering glycolysis, as occurs in hepatocytes (281), is unknown. Interestingly, an in vitro study indicated ALR2-related enzyme expression in rat retinal capillary ECs; however, sorbitol levels were barely detectable under basal conditions (618). These results are in line with the low baseline activity of the endogenous polyol pathway in ECs. Yet, in diabetic and atherogenic conditions, the deregulation of the polyol pathway accounts for disease progression, as discussed in section VII.

The uronic/glucuronate pathway (**FIGURE 3*F***) is another alternative glucose metabolism pathway, accountable for ~5% of the total glucose catabolized per day in humans (68). Instead of generating ATP, it uses G6P to produce UDP-glucose (used in glycogen synthesis or galactose metabolism), which is subsequently oxidized to UDP-glucuronic acid (UDP-GlcUA) (3, 68). Through a stepwise process, UDP-GlcUA is converted to uronic acid, xylitol, and xylulose-5-phosphate to finally generate ribulose-5-phosphate, the end product of the oxPPP (see above in this section) (3). Moreover, the xylulose-5-phosphate can be converted back to F6P by TK and as such connects to the HBP or to glycolysis. Alternatively, UDP-GlcUA is used in glucuronidation, a detoxification pathway involving the UDP-glucuronyltransferase (UGT)-mediated covalent binding of glucuronic acid to drugs, xenobiotics, and endogenous compounds including bilirubin, bile acids, fatty acids (FAs) and steroid hormones (468). UGT isoforms are expressed in human

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

brain microvascular ECs, thereby contributing to the protection of the brain to toxic substances (408). Interestingly, UGT expression was also increased in a primary culture of epileptic brain ECs, suggesting the induction of a protective program upon pathological drivers (193).

D. Oxidative Metabolism: Too Few Mitochondria?

The dogmatic view on the mitochondrion as the cell's "powerhouse" does not fully reconcile with the EC's glycolytic nature. ECs rely only minimally on mitochondrial OXPHOS for ATP generation (114). Concurrently, endothelial mitochondrial volume is only 2–6% of the total cellular volume, whereas 28 and 32% have been reported for hepatocytes and cardiomyocytes, respectively (reviewed in Ref. 529). Still, mitochondria in ECs are not dysfunctional and remain fully coupled to ATP synthesis, implying normal flow through the electron transport chain (ETC) (129, 288). As such, and notwithstanding a low respiratory capacity, ECs triple their oxygen consumption upon switching from quiescence to proliferation during angiogenesis, although it remains to be determined for which purposes exactly this increase in respiration is used. This allows the mitochondrial uncoupling benzoquinone embelin to establish an anti-angiogenic effect in tumor xenografts and wound healing in vivo (101). Furthermore, ECs display the Crabtree effect, whereby lower glucose levels (~1 mM) cause mitochondrial respiration to increase, with opposite effects (growth inhibition and reduced respiration) in high glucose (e.g., 25 mM) (288, 291).

Beyond an obvious function in cellular metabolism, mitochondria in ECs are increasingly being recognized for their role in signaling events. Whereas the focus has mainly been on mitochondrial ROS as a signaling element, other mitochondrial factors may play a role, although this requires further study. For instance, coupling factor 6 (CF6) is a component of mitochondrial ATP synthase but is also present on the EC surface and released into the circulation. Even though this event is not merely a consequence of cellular damage (suggestive instead of an active transport system, Ref. 405), plasma CF6 levels might be indicative of EC dysfunction (see sect. VII) and inflammation (404, 406). The role of other mitochondria-derived signaling factors in ECs has been reviewed in References 265, 584.

Compared with ECs in other vascular beds, the mitochondrial volume in cerebrovascular ECs (CECs) at the blood-brain barrier (BBB) is almost double (401). The BBB is a highly dynamic yet selective interface between the blood and the central nervous system and rigorously safeguards neuronal environment homeostasis by controlling influx and efflux of substances to and from the brain. CECs turn to aerobic respiration to meet the energetic demands for sustaining multiple transport systems; additionally, it has been hypothesized that oxidative stress from exposure to xenobiotic substances might be counteracted by the reducing equivalents generated through oxidative metabolism (104). Strikingly, shear stress-mediated induction of an endothelial BBB phenotype in vitro causes human brain microvascular ECs to have higher expression levels of TCA cycle enzymes, whereas glycolytic genes are downregulated, a finding that requires further study in light of the above (negligible) role of mitochondria in ECs (104). In vivo as well as in vitro, mitochondrial crisis in CECs severely compromises BBB function (127). Finally, heightened expression of miR-34a in CECs inhibits mitochondrial OXPHOS causing BBB breakdown probably through lowering of mitochondrial cytochrome C expression (59).

E. Amino Acid Metabolism

Amino acids (AAs) are traditionally classified as nonessential (NEAA) or essential (EAA), based on the organism's own ability of de novo synthesizing the AA or the need for dietary uptake, respectively. NEAAs can become conditionally essential under specific developmental or pathophysiological conditions; furthermore, whether an AA is essential or not differs between species. Apart from constituting building blocks for proteins, AAs can enter the TCA cycle as keto acids after transaminase-mediated removal of the amino group.

1. Glutamine and glutamate

Glutamine (single letter code: Q) and its deamidated derivative glutamate (single letter code: E) are both NEAAs, although glutamine becomes conditionally essential during severe trauma, and during chemo- or radiotherapy when excessive consumption outruns its synthesis (531). While glutamine is the most abundant extracellular AA (with physiological levels around 0.65 mM of the total ~2.5 mM free AA concentration in plasma), glutamate is the most abundant intracellular AA (at reported concentrations ranging from 2 to 20 mM) (390). Both glutamine and glutamate are readily being oxidized by microvascular ECs (up to 68 $nM \cdot h^{-1} \cdot mg\ protein^{-1}$ under glucose deprivation) and, together with alanine, rank in the class of high oxidation rate AAs (>35 $nM \cdot h^{-1} \cdot mg\ protein^{-1}$) (291). In HUVECs, ~30% of the TCA carbons is glutamine-derived, a share comparable to glycolysis- and FA-derived carbon (481). Radioactive flux measurements from the early 1990s have even suggested that glutamine oxidation, together with palmitate oxidation, would be the main energy source in liver ECs (507), although it is unclear whether this finding is restricted to liver ECs, given that other studies documented opposite findings, at least in relation to the role of palmitate as energy source (see below in this section). Glutamate enters the TCA cycle via a single enzymatic conversion [to α-ketoglutarate; catalyzed by glutamate dehydrogenase (GDH)], whereas glutamine needs one additional prior en-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

zymatic conversion into glutamate [catalyzed by glutaminases (GLS)] **(FIGURE 3G)**. Alanine requires three enzymatic conversions (to pyruvate by alanine aminotransferase, pyruvate to acetyl-CoA by pyruvate dehydrogenase and finally to citrate by citrate synthase-mediated condensation of acetyl-CoA and oxaloacetate) before entering the TCA cycle. In addition, in ECs the above enzymes are highly active; GLS activity for example is 20-fold higher in bovine pulmonary ECs than in cell types with established high glutaminolysis rates such as lymphocytes (308), although this finding requires additional confirmation in other EC subtypes and using different techniques. GLS inhibition causes HUVECs to adopt a senescence-like phenotype (550), further underscoring the role of EC glutamine metabolism. Most recently, endothelial glutamine metabolism was shown to be crucial for vessel sprouting in angiogenesis both in vitro and in vivo (using EC-specific knockout mice for the rate limiting enzyme GLS). Blocking endothelial glutamine metabolism perturbs tip/stalk dynamics and lowers EC proliferation. Mechanistically, glutamine in ECs contributes to TCA cycle anaplerosis and drives asparagine synthesis through asparagine synthetase (ASNS) activity, which by itself proves indispensable for vessel sprouting (242).

Mammalian cells generally express two different GLS isozymes, kidney-type GLS1 and liver-type GLS2, possibly serving two distinct functions. While glutamine carbons are driven into the TCA cycle by GLS1, GLS2 shunts glutamine carbon and nitrogen into glutathione for redox homeostasis. In cancer cells at least, this duality in function is reflected by a difference in transcriptional regulation: c-MYC controls GLS1 expression, whereas GLS2 expression is p53 driven (184, 241, 590). Whether a similar functional difference between GLS1 and GLS2 exists in ECs is not known at present, but, strikingly, in the mouse retinal microvasculature, tip cells display increased GLS2 expression in comparison with stalk cells (513).

Glutamine supplies the EC's central metabolism with carbon but also with nitrogen. Glutamine's γ-nitrogen is crucial for nucleotide synthesis and is incorporated into both the purine and the pyrimidine ring structure. In the purine nucleobases adenine and guanine, the nitrogens on position 3 and 9 are donated by glutamine, and guanine's amino group is additionally derived from glutamine. Glutamine phosphoribosylpyrophosphate amidotransferase catalyzes the rate-limiting step in purine synthesis by transferring NH_3 released from glutamine onto 5-phosphoribosyl-1-pyrophosphate (PRPP) to form 5-phosphoribosyl-1-amine (PRA); the amino group introduced (on position 9) will form the *N*-glycosidic bond of the nucleotide. 5′-Phosphoribosylformylglycinamidine synthetase uses glutamine to introduce the nitrogen at position 3. In de novo pyrimidine synthesis, cytosolic carbamoyl phosphate synthetase II introduces glutamine's amide nitrogen at position 3 of the uracil, cytosine, and thymine nucleobases (reviewed in Ref. 95). Interestingly, a recent elaborate labeling study in non-small cell lung cancer cell lines revealed that glutamine, exhibiting the highest consumption rates together with glucose, primarily contributes to protein production, whereas the majority of cellular carbon mass originates from AAs other than glutamine that display significantly lower consumption rates (239). These data imply that high consumption rates of a given substrate do not necessarily correlate with their use for cell carbon mass production; whether this holds true specifically for ECs too remains to be determined.

The GFAT-mediated rate-limiting step in the HBP **(FIGURE 3D)** condenses F6P and glutamine's amino group into glucosamine-6-phosphate (GlcN6P) (505). In rat coronary microvascular ECs, increasing doses of glutamine have been reported to increase GFAT activity and GlcN6P levels. Increasing glucose levels (as precursor for F6P) similarly resulted in higher GlcN6P production, which was completely abrogated upon full glutamine deprivation. In ECs, the amount of glutamine used in the HBP has been estimated to account for only 0.3% of total glutamine metabolized (596). Of note, this value might be context-dependent given that GFAT activity can be >10-fold higher in cultured than in freshly isolated ECs (possibly reminiscent of a differentiation effect on GFAT) (596).

Both human umbilical venous and aortic ECs express glutamic acid decarboxylase (GAD) to produce γ-aminobutyric acid (GABA) from glutamate (487) **(FIGURE 3G)**. Strikingly, GAD inhibition reduces cellular ATP levels, and GABA itself increases pyruvate oxidation and fatty acid oxidation. The mechanism behind the increased oxidative fluxes is not known yet. However, GABA dose-dependently increases palmitate uptake by mitochondria, thereby enhancing fatty acid oxidation (487). Even though the exact role of EC-produced GABA is not clear, it protects the endothelium from ROS and has anti-inflammatory effects on ECs. Possibly even more important, GABA reduces blood pressure, most likely in an autonomic ganglia-dependent way (487).

2. Arginine and urea cycle intermediates

Even though the liver is the main site for arginine metabolism and is generally believed to be the only tissue to express a complete urea cycle, arginine and urea cycle intermediates play important roles in ECs as well **(FIGURE 3H)**. Much like glutamine, arginine is a conditionally EAA with plasma concentrations ranging from 0.095 to 0.250 mM (530) and intracellular levels varying between 0.1 and 0.8 mM in cultured ECs and 2 and 4 mM in freshly isolated ECs (reviewed in Ref. 346). In ECs, arginine is mainly competed for as a substrate by either arginase or by (e)NOS. Hydrolysis of arginine by arginases yields urea and ornithine, which can subsequently be used for polyamine, glutamate, or proline synthesis. Mammalian arginase comes in two different iso-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

forms (coded for by two genes located on different chromosomes) displaying tissue-specific expression and distinct subcellular localization (258). Arginase I (arg I) is cytoplasmic and abundantly expressed by hepatocytes where it catalyzes the final step of the urea cycle. In contrast, arginase II (arg II) is a mitochondrial protein expressed in a wider variety of cell types. Both arg I- and arg II-derived ornithine can be used in polyamine synthesis, whereas additionally, arg II-derived ornithine can be used for glutamate and proline synthesis in a urea cycle-independent manner. HUVECs express both arg I and II, but specific isoform expression in ECs differs between species (detection of arg I and II isoforms in ECs from human, rat, mouse, pig, and cow origin has been reviewed in Ref. 379) and the specific role of either isoform in EC subtypes requires further study.

On the other hand, NOS converts arginine to citrulline, thereby producing NO and as such directly competing with arginases for arginine availability. In addition to this obvious substrate competition, N^{ω}-hydroxy-L-arginine (L-NOHA), an intermediate in the NOS reaction, potently inhibits arginase activity (51, 108). In mammals, three different NOS isozymes exist, sharing 50–60% protein homology: neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and endothelial (eNOS). nNOS is constitutively expressed in neurons, whereas eNOS is predominantly expressed in ECs. Under basal conditions, iNOS is normally not expressed, yet different stimuli including immunologic and inflammatory cues can induce iNOS expression in various cell types (174, 450). iNOS further differs from the other NOS isoforms in calmodulin binding (required for NOS activity): nNOS and eNOS require increased levels of Ca^{2+} for calmodulin binding, whereas high-affinity iNOS-calmodulin binding occurs independently of Ca^{2+} (174). nNOS and iNOS are predominantly cytosolic, whereas eNOS can be both cytosolic or localized in membrane caveolae (22). nNOS, iNOS, and eNOS all require NADPH, tetrahydrobiopterin (BH_4), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) as cofactors for their activity (174). nNOS-derived NO is involved in synaptic plasticity as well as the control of blood pressure and can act as a neurotransmitter, whereas iNOS-derived NO controls various inflammation-linked symptoms and is causal in blood pressure falls upon septic shock (174).

eNOS-derived NO is widely known for its role as an important vasorelaxant and for sustaining cardiovascular homeostasis and vasoprotection (175). However, under certain (pathological) conditions (see sects. VII and VIII), NOS activity goes awry and the enzyme loses its ability to convert arginine into citrulline. In this "uncoupled" state, an electron from NADPH is being donated to molecular oxygen to produce superoxide (O_2^-) instead of NO (599), which impacts cardiovascular function. As such, eNOS uncoupling is a source of oxidative stress as it produces O_2^-, which yields peroxynitrite ($ONOO^-$) upon reaction with residual NO or can induce oxidation of the eNOS cofactor BH_4 into the biologically inactive dihydrobiopterin (BH_2). Both actions ultimately reduce overall NO bioavailability. Although fascinating and intensely studied, the mechanism of action of (e)NOS and its regulation (e.g., through posttranslational modifications) are beyond the scope of this review and have been excellently reviewed elsewhere (25, 332, 443, 464). From an EC metabolism point of view, it should be noted that in ECs, fatty acid synthase (FAS)-mediated de novo lipid synthesis is required to sustain eNOS palmitoylation (palmitate is a lipid product), membrane association, and activity (579). Furthermore, glutamine and glucosamine (derived from glutamine through GFAT activity, see above in this section) inhibit endothelial NO production by lowering PPP activity-coupled NADPH production, required for eNOS activity (595). The highly increased GFAT expression in cultured ECs warrants thoughtful interpretation of these findings. Both cationic (ornithine, lysine, homoarginine) and neutral AAs (glutamine, leucine, serine) in the extracellular environment were found to lower endothelial NO production in the EA.hy926 EC line by reducing intracellular arginine levels (through a combination of reduced arginine uptake and increased arginine efflux) (267). Finally, NO itself might have a crucial role in keeping EC mitochondrial respiration at a low rate by inhibiting cytochrome *c* oxidase (56, 86).

Through an abbreviated urea cycle, arginine can be resynthesized from citrulline by the consecutive action of the urea cycle enzymes argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL) with argininosuccinate as an intermediate metabolite. In ECs, this citrulline to arginine flux has been estimated to vary between 0.7 and 1.9 nmol arginine produced$\cdot 10^6$ cells$^{-1} \cdot$h^{-1} (reviewed in Ref. 346) and serves to maintain arginine levels in spite of continuous NO release (372). The relevance of this recycling mechanism is underscored by reduced endothelial NO production and significantly elevated blood pressure in ASL-deficient mice and humans (156).

3. Branched-chain amino acids

With a characteristic branch in their aliphatic side chains, branched chain amino acids (BCAAs) make up a distinct class of AAs. Of the proteinogenic BCAAs, leucine and valine in particular play an unexpected role in EC metabolism. Leucine induces GFAT activity [possibly by increasing mammalian target of rapamycin (mTOR)-mediated GFAT protein synthesis (reviewed in Ref. 625)] to produce more glucosamine, which, as mentioned above, lowers NO production from arginine (595, 625). In several cell types, leucine was found to allosterically activate GDH, thereby enhancing glutamine catabolism (reviewed in Refs. 319, 621); whether the same occurs in ECs remains undetermined. Valine, or more in particular its catabolic intermediate 3-hydroxyisobutyrate (3-HIB), is crucial for transendothelial FA transport. Indeed, muscle-secreted 3-HIB promotes

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

transendothelial FA transport, thereby increasing FA uptake and accumulation in muscle with insulin resistance as a final outcome (257). Whether 3-HIB also affects FA oxidation within ECs remains unknown. Finally, deletion of the large neutral amino acid transporter 1 (LAT1/SLC7A5) from ECs at the BBB perturbs the normal AA profile in the brain, lowering leucine and isoleucine levels in particular, thereby causing aberrant cap-dependent mRNA translation and leading to autism spectrum disorder-related neurobehavioral alterations (533). The above-mentioned role for leucine as a GDH activator seems less involved in this phenomenon as brain glutamate levels were only minimally affected.

F. Fatty Acid Metabolism

In general, FAs enter the cell by passive diffusion (flip-flop; referring to the complete reorientation of the FA carboxyl head group when moving from the outer to the inner plasma membrane leaflet) or by fatty acid translocase (FAT)/CD36-mediated transfer (facilitated diffusion) (221, 352) **(FIGURE 3I)**. Very-long-chain FAs are preferentially being transported by fatty acid transport protein (FATP) 1 or other FATPs. For an in-depth overview of cellular FA uptake mechanisms and related controversies, the following review is suggested (201). Their low aqueous solubility (estimated 1–10 nM; Ref. 566) causes FAs to be mostly protein (or membrane) bound. Therefore, in the cytoplasm, FAs are either free or rely on binding to FA binding proteins (FABPs) for transportation to their destination. Expression of FAT/CD36, FATP3 and 4, and FABP3, 4, and 5 has been demonstrated in ECs (reviewed in Ref. 221).

bFGF and VEGF-VEGFR2 signaling induces FABP4 expression in ECs, the latter in a Dll4-Notch signaling dependent manner (152, 153, 220). Loss of FABP4 expression impairs EC proliferation and migration and increases apoptosis susceptibility. Angiogenic sprouting is hampered in ex vivo aortic rings from FABP4$^{-/-}$ mice (152, 153). Furthermore, FABP4 silencing causes ECs to heighten their fatty acid oxidation (FAO) rate, presumably to remove unbound FAs (which lost their FABP4 “chaperone”) (219, 602). In such conditions, increased FAO leads to ROS production, presumably by enhancing mitochondrial respiration (219). Whereas FABP4 is predominantly expressed in microvascular endothelium, FABP5 expression is seen in both micro- and macrovascular ECs, but seems not to be induced by VEGF-A or bFGF. Like FABP4, FABP5 silencing reduces EC proliferation and chemotactic migration but, unlike FABP4 silencing, confers resistance to apoptotic cell death (630), suggesting contextual roles for these FA binding chaperones. In FA consuming tissues (e.g., muscle and heart), FABP4 and FABP5 in capillary ECs transport FAs across the endothelium and towards the tissue (252). In vitro, FATP3 and FATP4 expression in ECs is induced by muscle-released VEGF-B, suggesting a VEGF-B-mediated transendothelial transport of FAs into muscle tissues. Notwithstanding a reduced FA uptake and accumulation in heart and muscle in VEGF-B$^{-/-}$ mice (215, 216), the possible implications for insulin resistance (and its possible treatment by targeting the VEGF-B axis) remain debated (276). Of note, to ensure their own uptake of FAs, muscle and fat cells release lipoprotein lipase (LPL), which hydrolyzes triglyceride-rich lipoproteins, in the capillary lumen. Capillary ECs aid in the transendothelial FA transport by facilitating transport of LPL from these parenchymal cells to the capillary lumen (113).

Once FAs are taken up, they can either be fueled into catabolic processes or esterified and stored. Lipid droplets (LDs) are specialized intracellular organelles, in which excess lipids are stored. They contain triglycerides and sterol esters, thereby providing a reservoir for energy fuel and membrane synthesis (538). The abundance of intracellular LDs increases in response to increasing amounts of extracellular FAs, indicating that exogenous FAs, when in excess, are incorporated into LDs (340) that can potentially be accessed via lipolytic enzymes in times of nutrient scarcity. ECs contain LDs (21, 219, 340) and express hormone-sensitive lipase, a key enzyme required for the lipolytic breakdown of triglycerides from LDs (219). Furthermore, nonadjacent ECs can be connected by thin membrane bridges, which form tunneling nanotubes (TNT). TNTs permit intercellular exchange of signals or various components such as ions, proteins, and organelles **(FIGURE 3I)**. Interestingly, LDs have been shown to be a cargo of TNTs, and the extent of LD transport via TNT was increased in response to VEGF (21). Although the functional significance is yet to be validated, this observation provides evidence for a potential nutrient exchange between ECs under a pro-angiogenic cue.

With accessible FA sources in their immediate vicinity (i.e., in the blood) and equipped with various FA uptake mechanisms, it remains enigmatic why ECs express the key enzymes for de novo FA synthesis. Indeed, ECs express acetyl-CoA carboxylase and FA synthase (FASN) required for conversion of acetyl-CoA into malonyl-CoA and production of long-chain FAs (e.g., palmitate) from acetyl- and malonyl-CoA, respectively (reviewed in Ref. 221). Whether endothelial FA synthesis is used to generate lipids for membrane or signaling is not known, but endothelial FA synthesis might serve additional purposes. Indeed, FASN silencing reduces posttranslational palmitoylation and subsequent membrane localization of eNOS (see above in this section). As a result, EC sprouting (in aortic rings) is compromised while EC permeability is increased (579). Given the number of possible palmitoylation targets in ECs (580), it is tempting to speculate that FASN silencing affects additional EC functions.

Endothelial FAO plays a rather unexpected role in vessel sprouting. EC-specific deletion of carnitine palmitoyltrans-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

ferase 1a (CPT1a), which imports FAs into mitochondria and thereby rate-limits FAO flux, decreases EC proliferation (not migration) and causes sprouting defects both in vitro and in vivo (481). Even though ECs have been shown to increase their FAO flux to cope with nutrient scarcity (e.g., glucose deprivation) in an AMP-activated protein kinase (AMPK)-dependent way (107), the observed sprouting defect is unlikely to be caused by energy distress given the minimal contribution (~5%) of FAO to total cellular ATP production in ECs (114, 481). By virtue of its indirect contribution to cellular NADPH production (reviewed in Ref. 220), endothelial FAO could also sustain redox homeostasis; however, CPT1a-silenced ECs do not display significant levels of redox imbalance nor oxidative stress (483). Strikingly, and contrasting cancer cell types that mainly rely on glucose and glutamine as carbon sources for TCA cycle intermediates (555), stable isotope tracing in ECs revealed that the TCA cycle intermediate citrate incorporated comparable amounts of carbons from palmitate as from glucose and glutamine (481). FAO in ECs regulates biomass synthesis and more in particular deoxyribonucleotide (dNTP) production required for DNA synthesis during EC proliferation **(FIGURE 3*I*)**. Notably, this process does not rely on net replenishment (anaplerosis), but palmitate contributes to sustaining TCA cycling for biomass synthesis in conjunction with (an)other anaplerotic substrate(s) (480). Hence, endothelial CPT1a silencing depletes dNTP levels without affecting RNA levels, even though [^{14}C]palmitate labeling showed incorporation of palmitate carbons into ribonucleotides for RNA synthesis; most probably, RNA salvage pathways sustain RNA homeostasis upon CPT1a silencing. Protein synthesis is not affected by blocking FAO either, likely again because of compensatory mechanisms. Remarkably, however, the dNTP synthesis defect was not compensated by glucose or glutamine metabolism, possibly implying specific usage of carbons from different nutrients for particular cellular processes in ECs. Also, ECs turn out to be rather unique in the use of the above metabolic pathway; out of an array of different (normal and malignant) cell types assayed, only fibroblasts show a similar use of FAO for DNA synthesis (481). One could wonder why other (especially rapidly proliferating) cells do not use this pathway given that 1 molecule palmitate can contribute 16 carbons, whereas glucose and glutamine only have a 6- and 5-carbon backbone to contribute, respectively. Perhaps, ECs sprouting into the avascular tissue rely on internal lipid sources when other nutrients become more scarce.

IV. COMPARTMENTALIZATION OF ENDOTHELIAL CELL METABOLISM

From the unicellular to multicellular level, all organisms display a complex structural and functional organization. Organs, tissues, cell types, and subcellular organelles exemplify different layers of functional specification and compartmentalization. Interestingly, spatial organization also occurs at the metabolite level, where metabolic pathways are organized in specific intracellular compartments.

A. Glycolytic Compartmentalization

When vessels start to sprout, quiescent ECs become highly motile and face increased energy demands to support the ATP-consuming process of actin remodeling by the contractile force generating actomyosin ATPase (105). In quiescent ECs, glycolytic enzymes are detectable primarily in the perinuclear cytosol (114). In contrast, when these ECs become motile and start to form lamellipodia and filopodia to migrate, they relocate their glycolytic machinery also to these projecting cell extensions (114). In agreement, biosensing techniques reveal high levels of ATP at membrane ruffles in lamellipodia of motile ECs, which disappear upon PFKFB3 silencing, thus indicating a glycolytic origin (114). Bulky mitochondria on the other hand are excluded from these thin and motile structures, most likely because of size incompatibility (reviewed in Refs. 114, 144). Poisoning mitochondrial respiration does not affect EC sprouting, underscoring that highly localized glycolytic but not mitochondrial ATP generation primarily drives EC motility (114).

Moreover, PFKFB3 coimmunoprecipitates with β-actin and is enriched in the filamentous (F-) actin fraction in migrating versus quiescent ECs (114). Given that it is a conserved mechanism, the ability to bind glycolytic enzymes to actin during migration provides organisms (cells) an evolutionary advantage (303). By connecting with the cytoskeleton, and particularly with F-actin, glycolytic enzymes cluster into "glycolytic hubs" **(FIGURE 4*A*)**. Such multienzymatic complexes (also known as metabolons) offer several advantages: *1*) increased catalytic efficiency by channeling intermediary metabolites from one enzyme to the next neighbor, *2*) increased kinetic constant, *3*) enhanced coordination of enzymatic actions in the same pathway, *4*) retention of potentially toxic metabolic intermediates, *5*) circumvention of enzymatic inhibition or degradation, and *6*) prevention of ATP depletion from other cellular subcompartments (11, 71, 356). As such, in erythrocytes and skeletal muscle, the association with F-actin increases the flux through aldolase and promotes the tetrameric conformation of PFK (454, 569). Furthermore, increasing glycolysis-derived ATP production on the same spot of high demand prevents the cell from suffering global (possibly life-threatening) ATP depletion. Noteworthy, in mammary epithelial MCF10A cells, insulin disrupts the actin cytoskeleton and thereby releases aldolase from its F-actin-bound state. This PI3K signaling-driven mechanism procures sufficient levels of cytosolic aldolase and glycolytic activity to meet the cell's demand for macromolecule synthesis during increased growth and possibly involves fueling the non-oxPPP for biomass synthesis (240). If and to which extent a similar mechanism is also present in ECs remains to be determined.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

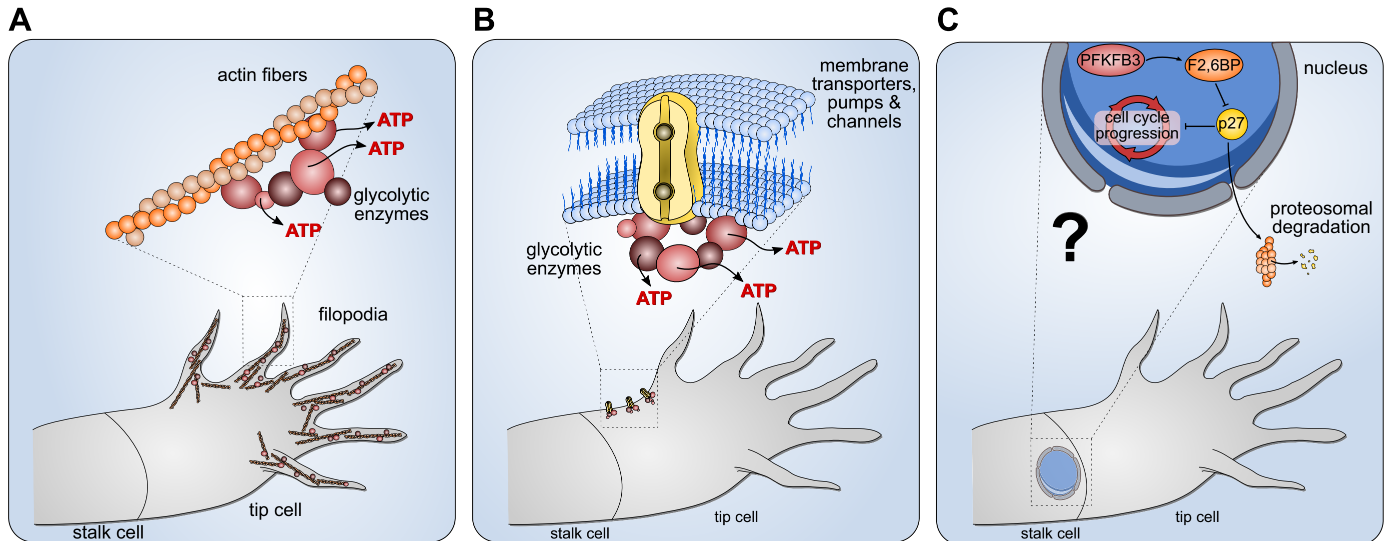


FIGURE 4. Glycolytic compartmentalization in ECs. Glycolytic enzymes and glycolytic activity in quiescent ECs are primarily located in perinuclear regions (not shown in figure) but additionally compartmentalize with highly specific cellular structures or components to locally generate ATP or to perform other functions. *A*: motile ECs colocalize glycolytic enzymes such as PFKFB3 with F-actin fibers in migratory structures like filopodia and lamellipodia. This allows rapid and on-the-spot generation of the energy required by these structures to drive cellular movement and bypasses the need for mitochondria that are too large to fit inside these specialized structures. *B*: membrane-located transporters, pumps, and channels require ATP to function. This ATP can be delivered in situ by colocalizing glycolytic enzymes. *C*: in other cell types, PFKFB3 is also present in the nucleus to locally produce F2,6BP which enhances phosphorylation and subsequent proteasomal degradation of the Cdk inhibitor $p27^{Cip/Kip}$; as a result, cellular proliferation is increased. Whether this also occurs in ECs remains unknown. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; F2,6BP, fructose-2,6-bisphosphate; p27, cyclin-dependent kinase inhibitor 1B.

Compartmentalization of glycolytic enzymes is not a sole privilege of the actin cytoskeleton. Fast axonal transport of vesicles in neurons requires large amounts of ATP to fuel the molecular motors (dynein, kinesin) that drive vesicle movement (632). Intriguingly, this process too does not rely on mitochondrial respiration but requires the activity of the glycolytic enzyme GAPDH. In a huntingtin-dependent manner, GAPDH associates with the vesicles and provides on-board energy (ATP) for vesicle transport (632). Interestingly, in ECs, glycolytic ATP is required for VE-cadherin endocytosis and thus remodeling of cell-cell junctions (66). Whether a similar compartmentalization of glycolytic enzymes occurs on VE-cadherin containing endocytic vesicles in ECs remains to be determined. Glycolytic hubs also coexist with and directly supply ATP to membrane ion transporters and pumps (Ca^{2+}-ATPase and Na^+-K^+-ATPase), exchangers (Na^+/H^+), and channels (ATP-sensitive K^+) in vascular and other cell types (reviewed in Ref. 120) (**FIGURE 4*B***). The above data suggest that mitochondrial oxidative phosphorylation is crucial to maintain long-term steady-state metabolism, whereas local, and short-term, high-energy demands are met by compartmentalized glycolysis (155, 500).

In addition to its lamellipodial compartmentalization, PFKFB3 has also been shown to localize in the nucleus. Two specific carboxy-terminal lysines (K472; K473) are involved in PFKFB3′s nuclear localization. Even though this nuclear PFKFB3 generates F2,6BP within the nucleus and activates PFK1, it does not lead to an increase in glycolytic flux (613, 614). Instead, F2,6BP enhances cyclin-dependent kinase (Cdk)-mediated phosphorylation of the Cdk inhibitor $p27^{Cip/Kip}$ (a potent blocker of G_1-to-S cell cycle phase transition) leading to its proteasomal degradation; as a result, cellular proliferation is increased (613). F2,6BP within the nuclear compartment reflects the rate of glucose uptake and might thus translate glucose availability into cell proliferation. Whether nuclear F2,6BP is also important for the proliferation control of glycolysis-addicted ECs remains unknown (**FIGURE 4*C***). One could hypothesize that, in line with the high expression of PFKFB3 in malignant cells (79), tumor-derived ECs (see sect. VIII) in particular may have high nuclear PFKFB3 expression to connect glycolytic flux to high cell proliferation rates.

B. The Arginine Paradox: A Matter of Compartmentalization?

As outlined above, eNOS-mediated production of NO makes arginine metabolism a highly studied topic, mostly because of its direct link with the pathophysiology of atherosclerosis (see sect. VII). Also, endothelial arginine metabolism features an intriguing yet controversial compartmentalization. In order of abundance, eNOS predominantly localizes to specific microdomains of the plasma

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

membrane (including caveolae and lipid rafts), the *cis*-Golgi and then the cytosol, nucleus, and mitochondria (77). The same ranking holds for the enzyme's activity with a 9- to 10-fold increased activity in caveolae over the EC plasma membrane, and a 7-fold increased activity in plasma membrane fraction over the cytosolic fraction (493). Further underscoring the location-activity relationship is the reduction in NO production in intact HEK 293 cells expressing a palmitoylation- and myristoylation-defective eNOS mutant (abolishing its sequential localization to Golgi and caveolae), while this mutant proved catalytically competent in cell-free assays (491). Intriguingly, in ECs, eNOS interacts at the plasma membrane with the arginine transporter CAT-1 (SLC7A1) and with ASS and ASL, both enzymes involved in recycling arginine from citrulline (reviewed in Ref. 77). This is suggestive of another endothelial metabolite channeling (*cfr* glycolysis), whereby substrate (arginine) delivery from extracellular sources (CAT-1), conversion of substrate to end product (NO by eNOS), and recycling of end product (NO) into substrate (arginine by ASS, ASL) are spatially clustered.

Arginases on the other hand compete with eNOS for arginine and are mainly localized in the cytosol (arg I) or mitochondria (arg II), distant from the major site of eNOS activity (258, 380). Despite evidence showing decreased NO production upon arginase overexpression and, conversely, induction of NO synthesis after arginase inhibition, this seemingly logical one to one equation gets troubled when one takes substrate concentrations and enzyme kinetics into account. ECs in culture have intracellular arginine levels ranging from 0.1 to 0.8 mM, which would amply suffice to guarantee near-maximal eNOS activity given eNOS' high arginine affinity (K_m = 1–5 μM). Arginase has a rather low affinity for arginine (K_m = 1–20 mM); simple comparison of the K_m values precludes efficient competition between both enzymes for the substrate they share. A possible explanation for the above competition has been proposed to relate to the higher catalysis rate (V_{max}) of arginase than eNOS (475); similarly, in activated macrophages arginase has a higher V_{max} than iNOS (378). The above numbers reveal another feature of endothelial arginine/eNOS metabolism, commonly known as the "arginine paradox": even though under physiological conditions, eNOS would be fully saturated with intracellular arginine levels, both in vitro and in vivo data show that increasing extracellular arginine further induces NO production.

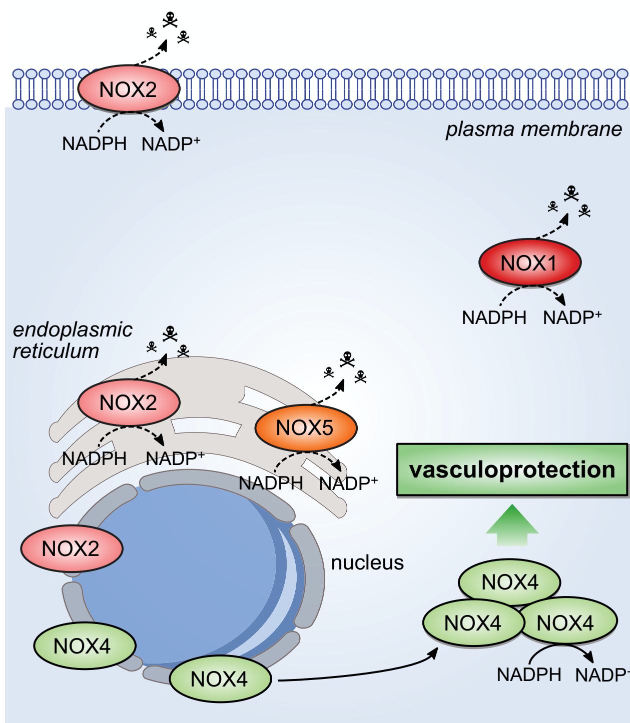
C. Compartmentalization of Reducing Power

NADPH provides reducing power in different cellular compartments, yet the NADPH pool is rather limited, certainly in light of the high fluxes through pathways that use it [±25% of all reactions listed in the KEGG database involve the use of NAD(P)H (403)]. Remarkably, none of the NAD(P)H forms is known to be transported across intracellular membranes, which suggests a highly optimized compartmental organization (392, 434). Two distinct subcellular pools of NADPH have been documented: cytosolic NADPH regenerated by the oxPPP and the mitochondrial fraction (434). While not fully established yet, NAPDH compartmentalization is likely to occur in ECs too. A recently developed method with deuterium (^{2}H)-labeled metabolites to trace hydrogen in compartmentalized reactions that use NADPH as a cofactor, would allow more detailed analysis of the different NADPH pools in ECs (315).

Further evidence for NADPH compartmentalization in ECs derives from the finding that the endothelial NADPH oxidase (NOX) system is compartmentalized. NOXs use NADPH to produce ROS, and the human vasculature expresses isoforms NOX1, NOX2, NOX4 (predominant isoform in ECs), and NOX5, which are sources of ROS in the vessel wall (130, 367). NOX expression is induced by an armamentarium of signals including proinflammatory cytokines, growth factors (i.e., VEGF, thrombin), hypoxia, hyperglycemia (see sect. VII), elevated FFA levels, and athero-prone-perturbed shear stress (see sect. VII) (123, 204, 244, 248, 367, 492, 504). While NOX1, 2, and 5 increase oxidative stress and reduce NO bioavailability and promote EC dysfunction (see sect. VII), NOX4-produced H_2O_2 can have a vasoprotective role by maintaining endothelial function (see sect. VII and **FIGURE 5**) (300). It is not entirely uncommon for moderate endothelial ROS levels to have a beneficial rather than a detrimental effect in angiogenesis. The pro-angiogenic VEGF stimulus uses ROS as signaling molecules downstream of VEGFR2, a phenomenon involving PI3K and Rac1 activity as upstream regulators (89). ROS can also mediate angiogenesis by VEGF-independent mechanisms, for example, through the oxidation of lipids, thereby generating pro-angiogenic species (e.g., ω-carboxyethylpyrrole), or by activating ataxia-telangiectasia mutated kinase, which promotes pathological angiogenesis (275). Under physiological conditions, NOX2 is mainly expressed in the membranes of the nucleus and ER at relatively low levels, to contribute to intracellular redox signaling pathways (130). In contrast, under diseased conditions, NOX2 mainly associates in clusters at the plasma membrane, generating excess levels of (extracellular) superoxides (130) (**FIGURE 5**). In normoxic human pulmonary arterial ECs, NOX4 mainly resides in the nucleus; strikingly, hyperoxia-induced motility and tube formation in these cells depends on NOX4-generated ROS and is accompanied by increased levels of cytoplasmic NOX4 (427).

Whether metabolite channeling is broadly conserved and generally applies to metabolic pathways is currently unknown, but is definitely important to better apprehend the fine-tuned regulations of metabolic fluxes in ECs. However, setting up the required and relevant in vitro models to study this aspect of EC metabolism is a highly challenging endeavor (138).

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.



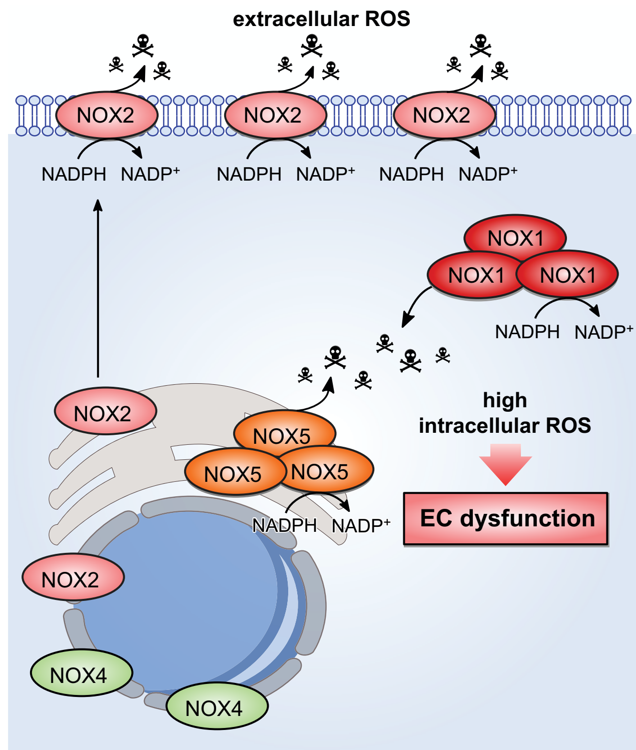


FIGURE 5. Compartmentalization of NOX activity in ECs. Schematic and simplified overview of the role and subcellular localization of different NADPH oxidases (NOX) in ECs under normal conditions (*left panel*) and pathological conditions (*right panel*). Note that, in contrast to other NOXs, NOX4 activity can have vasculoprotective actions. More details on the role of NOXs in ECs are discussed in the main text. NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase; ROS, reactive oxygen species, represented in the figure by skull and crossbones.

V. CHANGES IN METABOLISM DURING ENDOTHELIAL CELL DIFFERENTIATION

During vertebrate development, after fertilization, the vascular system is one of the first organ systems to form, ensuring rapid delivery of nutrients and oxygen to tissues in the growing embryo. In contrast to angiogenesis, which involves the formation of new blood vessels from preexisting ones as a result of the balance of pro- and anti-angiogenic signals, vasculogenesis occurs from the de novo formation of a primary capillary plexus from mesodermal cell precursors (see sect. II).

Changes in cellular metabolism of stem cells have been described to be modulated in a number of ways: *1*) extrinsic signals such as growth factors and cytokines or intrinsic signals such as transcription factors and signaling pathways can direct changes in cell cycle and differentiation, leading to changes in metabolic demand (i.e., for biosynthesis of building blocks) and metabolic flux (i.e., signal-mediated activation of metabolic pathways); *2*) differentiation leads to parallel changes in metabolic enzyme expression/activity, leading to metabolic rewiring reflected by altered metabolic demands of differentiated cells; and *3*) changes in metabolic flux lead to alterations in the activation of signaling pathways, epigenetic modifications, and the availability of metabolites as substrates or "signals" (5).

Embryonic and induced pluripotent stem cells have been reported to have higher rates of glycolysis compared with more differentiated cells such as cardiomyocytes and fibroblasts. It is believed that stem cells are preferentially glycolytic due to an immature mitochondrial network, with few mitochondria and reduced oxidative capacity (157, 438).

ECs and the vascular system develop from the mesoderm. Soon after gastrulation, signals from the visceral endoderm pattern the underlying mesoderm to form the mammalian extraembryonic yolk sac (35, 564). As described above, VEGFR2 is a key signaling receptor in ECs (402), and VEGFR2-positive mesodermal progenitor cells can serve as vascular progenitors (617). Metabolic traits of progenitors during differentiation need further characterization, but seem to be dynamic. VEGFR2-positive mesodermal progenitor cells have lower oxygen consumption rates than their VEGFR2-negative counterparts, and temporarily undergo suppression of (glucose) metabolism during mesoderm differentiation (647). Of note, a more oxidative-prone metabolism has been described to sustain embryonic stem cell differentiation (619). It is not clear if and to what extent

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

this also occurs during differentiation towards ECs which, as mentioned above, are highly glycolytic and rely on PFKFB3-driven glycolysis for generation of ATP (114). This would mean that in the further differentiation from the mesoderm lineage to ECs, the cellular metabolism switches back from oxidative to glycolysis. Furthermore, the exact transcriptional regulators that control this effect during differentiation remain to be determined. However, key regulators of endothelial function might be prime candidates such as the VEGF-VEGFR2 signaling axis (114, 128) in proliferating ECs and the Dll4-Notch (268) and FoxO1-MYC (586) signaling pathways in quiescent ECs.

Human and mouse embryonic stem cells can be differentiated to ECs in vitro (199, 314). Furthermore, arterial versus venous EC differentiation is possible (509), as well as differentiation to lymphatic ECs (323) (see sect. VI). These in vitro models would be useful to determine the exact molecular controls for the changes in EC metabolism during their differentiation.

VI. ENDOTHELIAL CELL HETEROGENEITY, REFLECTED BY DIFFERENCES IN METABOLISM?

ECs are found throughout all tissues of the body and are comprised of three main systems: arterial (delivering oxygen-rich blood and nutrients to tissue), venous (removing deoxygenated blood and waste products from tissues), and lymphatic (involved in the regulation of interstitial fluid balance, serving as a route for immune cell extravasation and regulation of intestinal lipid uptake via the lacteals) (2). The further differentiation into specialized arterial, venous, and lymphatic subtypes is driven by a combination of environmental, mechanical, and transcriptional factors (348). EC metabolism is emerging as a determining factor in these processes too.

Arteriovenous differentiation is controlled in part by Notch signaling (540), which has also been reported to modulate EC metabolism (268). While a molecular signature of key transcription factors involved in arterial specification (Prdm16, ROX2, NKX2.3, EMX2, MSX1, Sox17, Hey2, Aff3) was recently reported, transcriptomic data suggest increased expression of the glucose transporters GLUT1/GLUT3 in human umbilical arterial ECs (15). However, these arterial ECs have been reported to have a similar glycolytic flux as their venous EC (114, 591) counterparts and, conversely, PAECs have even been shown to have lower glucose consumption rates than PMVECs (see sect. III) (423). These seemingly contradictory findings might further exemplify the metabolic heterogeneity of ECs residing in different tissues. FAO flux may be higher in arterial than in venous ECs (481) but exactly which signals regulate increased FAO flux in arterial ECs remains unknown. Possible candidates involve other key factors and phenomena regulating arterial EC phenotypes such as flow/shear-stress as well as vasoactive and growth factor/signaling interactions with smooth muscle cells (SMCs) (i.e., PDGF-B/D, PDGFRβ, Ang1/Tie2, S1P, transforming growth factor-β, Notch, Eph/ephrin, connexin) (324). In other cell types, loss of Prdm16 results in mitochondrial fragmentation and decreased oxidative phosphorylation (88, 124, 334), but the exact link between Prdm16 and cellular metabolism in ECs remains largely unstudied.

As mentioned above, specification into tip or stalk cells also entails differences in EC metabolism (**FIGURE 6A**). Lowering glycolytic flux through PFKFB3 silencing compromises both tip cell competitiveness and stalk cell proliferation

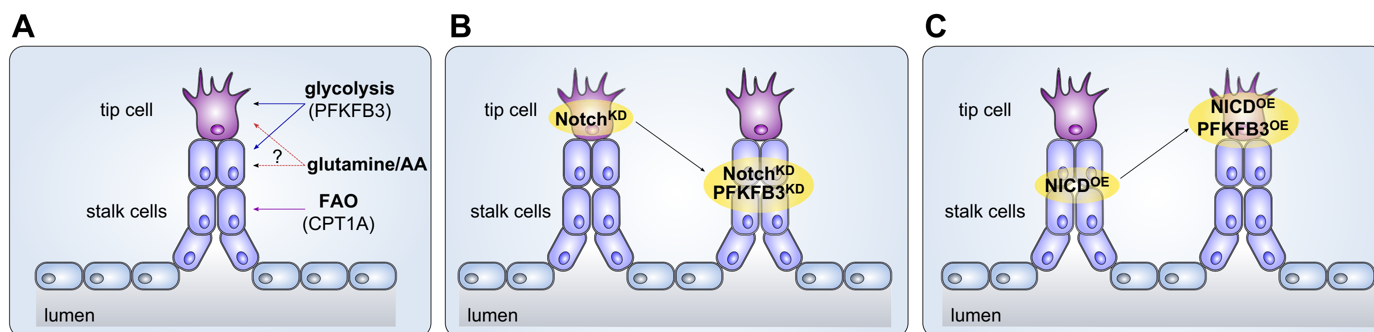


FIGURE 6. Metabolic heterogeneity in tip versus stalk cells. *A*: endothelial tip and stalk cells display differences in metabolic wiring. PFKFB3-driven glycolysis sustains both tip cell competitiveness and stalk cell proliferation, whereas CPT1a-mediated FAO is crucial for stalk cell proliferation but not for tip cell behavior. If and how other metabolic substrates like glutamine (or AAs by extension) drive either tip or stalk cell behavior, or both, requires further study. *B*: EC metabolism can override genetic tip versus stalk cues. ECs that have been genetically instructed to adopt tip cell behavior by silencing of Notch revert to a stalk cell phenotype upon simultaneous silencing of PFKFB3. *C*: conversely, overexpression of PFKFB3 provokes tip cell behavior, even in ECs that receive a strong genetic pro-stalk cue from NICD overexpression. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; AA, amino acids; FAO, fatty acid oxidation; CPT1a, carnitine palmitoyltransferase 1a; KD, knock-down; OE, overexpression; NICD, Notch intracellular domain.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

(114). Remarkably, the defect in tip cell behavior is maintained under a strong pro-tip cell cue created by simultaneous Notch knock-down (**FIGURE 6B**). Conversely, ECs that were instructed to assume stalk cell behavior by overexpression of NICD regained tip cell behavior upon overexpression of PFKFB3, even in vivo (114) (**FIGURE 6C**). Inhibition of endothelial FAO (see sect. III) selectively affects stalk cell proliferation without impeding tip cell behavior (481). These data imply the existence of a tip versus stalk metabolic signature acting in parallel or even overriding genetic instructions. Whether and how metabolic pathways, other than glycolysis and FAO, complete this metabolic signature needs further research (**FIGURE 6A**). Nearly nothing is known about how amino acid metabolism, glutamine metabolism in particular, differs between tip and stalk cells. In T-ALL cells, Notch signaling supports growth by promoting glutamine metabolism and injection of glutamine-derived carbons into the TCA cycle (227). Whether Notch, as a pro-stalk cue, has similar effects on glutamine metabolism in ECs is not fully understood. In case tip and stalk ECs feature different glutamine metabolism-related traits, it will be of key importance to determine if glutamine metabolism and genetic signals co-define the tip-stalk identity (as is the case for PFKFB3).

Lymphatic EC (LEC) differentiation in vertebrates occurs primarily from venous ECs (VECs) (12), although some lymphatic beds develop from non-venous EC origins (278, 351, 391, 511), and is mainly driven by VEGF-C/VEGFR3 signaling and the transcription factors Sox18 (177) and prospero homeobox protein 1 (Prox1) (238, 431, 585, 624). Lymphatic biology is less well studied than blood vascular biology and only most recently was the first characterization of LEC metabolism performed (591). LECs have higher FAO flux compared with venous or other vascular ECs, and the VEGF-C/VEGFR3 signaling axis is important in the activation of FAO (591). Furthermore, Prox1, a transcription factor which is essential for LEC differentiation and maintenance of LEC identity (431), binds the CPT1a (rate-controlling in FAO, see sect. III) promoter. The resulting upregulation in FAO is required for Prox1-mediated VEC-to-LEC differentiation through the regulation of lymphatic gene expression, particularly VEGFR3 (591). Strikingly, this Prox1-mediated increase in FAO affects LEC differentiation and function, not via the regulation of energy or redox homeostasis, but rather through the production of acetyl-CoA, which fuels epigenetic modifications (histone acetylation) preferentially at lymphatic genes over blood vascular genes (591). This specificity stems from the interaction of the histone acetyltransferase p300 with Prox1, which specifically binds promoters of genes involved in lymphangiogenesis. These novel findings extend the dogmatic view that only transcription and growth factors regulate VEC-to-LEC differentiation, and now show that Prox1 not only functions as a transcription factor solely driving pure lymphangiogenic genes, but is also driving FAO and acetyl-CoA production to sustain histone acetylation of its target genes (591) (**FIGURE 7**). Since histone acetylation makes the promoter region of these target genes more accessible to transcription factors, Prox1 enhances its own transcriptional effect to induce LEC differentiation by "hijacking" FAO metabolism through this epigenetic mechanism.

Even though the role of epigenetics in EC differentiation was previously recognized [for example, histone demethylation at the VEGFR2 and VE-cadherin promoter in the differentiation of embryonic stem cells to ECs (597)], the involvement of specific metabolic pathways herein remains unclear. As such, the above findings provide evidence for FAO-mediated regulation of histone acetylation in cellular differentiation, whereas glucose metabolism and other pathways have been previously shown to impact histone acetylation in other cell types (306, 362, 382, 582). Although LECs have lower levels of glycolytic flux compared with VECs or other blood vascular EC types (591), the role of glycolysis and other metabolic pathways in LEC activation and function remains to be explored.

Of note, LECs also utilize FAO to regulate nucleotide synthesis for proliferation (591), akin to blood vascular ECs (see sect. III). Also, the effect of FAO to upregulate VEGFR3 in LECs renders these cells more responsive to the promigratory effect of VEGF-C (342), the ligand activating VEGFR3, possibly explaining why silencing of CPT1a impairs LEC migration. These combined effects may explain why selective loss of CPT1a in LECs causes lymphatic defects during embryonic development and why pharmacological blockade of CPT1 by etomoxir treatment inhibits pathological injury-induced lymphangiogenesis (591). The therapeutic implications of these seminal findings are discussed in more detail further below.

Investigation of the metabolic characteristics of other organ-specific vascular beds is still in its infancy, although one would expect a degree of vascular specialization in accordance with the differences in local microenvironments of such specialized tissues as the heart, brain, liver, kidneys, etc. Indeed, transplantation of "generic" ECs into various tissues results not only in engraftment, but these ECs also acquire some features of organotypic ECs (393), raising the question whether organ-specific features may also alter the metabolic properties of these specialized ECs. For example, it was recently demonstrated that Meox2/Tcf15 heterodimers program heart capillary endothelium for cardiac FA uptake (93), which is important in regulating the transport of FFAs and very-low-density lipoprotein (VLDL) across the endothelium to provide FAs for cardiac energy metabolism. Future efforts directed towards the characterization and understanding of the metabolic characteristics of organotypic EC types could pave the way for tailored therapeutics in specific vascular beds.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

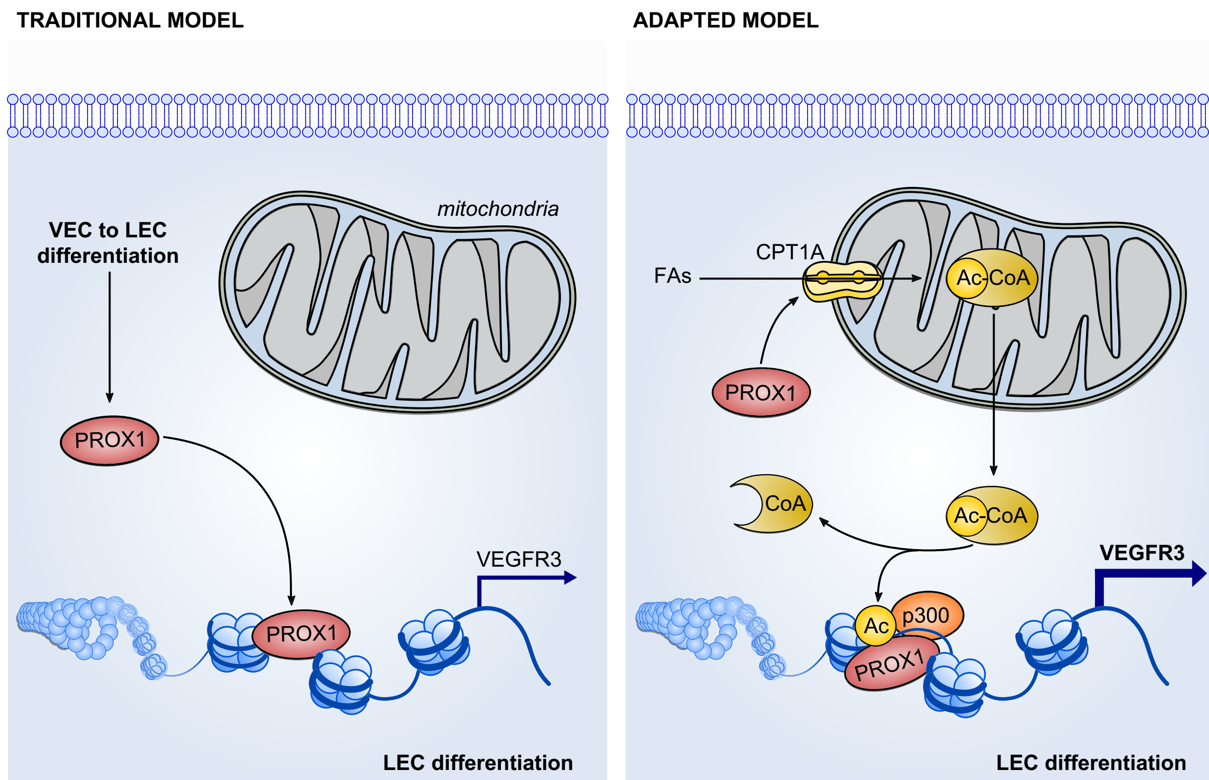


FIGURE 7. Novel role for fatty acid oxidation in differentiation of lymphatic ECs. The dogmatic view on lymphangiogenesis states that at the onset of VEC to LEC differentiation, the transcription factor Prox1 induces the expression of typical lymphangiogenic genes such as VEGFR3. In light of recent findings, this traditional model has been adapted and refined. The binding of Prox1 in the promoter region of lymphangiogenic genes involves an interaction between Prox1 and the histone acetyltransferase p300 to acetylate histones and remodel chromatin. The required acetyl groups are supplied by acetyl-CoA which derives from CPT1a-mediated fatty acid oxidation. Prox1 directly induces the expression of CPT1a to enhance fatty acid oxidation flux. As such, the transcription factor Prox1 teams up with central metabolism to drive lymphatic differentiation. VEC, venous endothelial cell; LEC, lymphatic endothelial cell; Prox1, prospero homeobox protein 1; VEGFR3, vascular endothelial growth factor receptor 3; FAs, fatty acids; CPT1a, carnitine palmitoyltransferase 1a; Ac, acetylated; Ac-CoA, acetyl coenzyme A.

VII. ENDOTHELIAL CELL METABOLISM IN VASCULAR DISORDERS: ENDOTHELIAL CELL DYSFUNCTION

In healthy individuals, the endothelium maintains vascular tone, structure, and homeostasis. In contrast, EC dysfunction results in the development of several pathologies, including diabetes and atherosclerosis (a frequent complication of diabetes). Metabolic aberrations in ECs can mediate vascular complications associated with the pathogenesis of the above-mentioned disorders.

A. Diabetes and the Endothelium

Diabetes has a high and incessantly increasing prevalence of 8.8% globally (in adults, in 2015) (250a). Diabetes is hallmarked by hyperglycemia [excessive levels of blood glucose, attributable to a deficiency of (type 1) or resistance to (type 2) insulin], which contributes to EC dysfunction. The EC dysfunction arises early and plays a key role in the pathogenesis of diabetes-associated micro- and macrovasculopathies. Moreover, it causes the acceleration of atherosclerosis (33, 61, 479, 603) (see below in this section). While diabetic EC dysfunction tilts the physiological balance towards vasoconstrictive, proinflammatory, and prothrombotic effects, the resulting vascular disease phenotype can be paradoxically tainted by either excessive or impaired angiogenesis (282, 285, 350, 604). Excessive angiogenesis contributes to diabetic retinopathy, early stage nephropathy, and atherosclerotic plaque destabilization. Insufficient angiogenesis, on the other hand, plays a role in macrovascular disorders (coronary artery disease, peripheral vascular disease, and ischemic stroke), impaired wound healing and skin ulcers (diabetic foot ulcers), embryonic vasculopathies, and transplant rejection in diabetic subjects. Lastly, peripheral neuropathy is a microvasculopathy linked to reduced nutritive blood flow (secondary to diabetes) to the sensory nerve fibers (512).

The integrity of the endothelium is also highly dependent on endothelial repair and regeneration, which are partly co-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

determined by migration and proliferation of surrounding mature vessel wall-resident ECs. Circulating bone marrow-derived endothelial progenitor cells (EPCs) may also contribute to endothelial regeneration and mitigate EC dysfunction (20, 142, 233, 453). Diabetic patients present with reduced numbers of circulating EPCs, which are linked to reduced endothelial regeneration and to the severity of peripheral vascular disease and as such may serve as a biomarker of vasculopathy in diabetic patients (146, 158, 202, 247, 330, 466, 536). Rather than hyperglycemia, (pre-diabetic) insulin resistance (see below in this section) is suggested to be the culprit in adversely affecting EPC-mediated repair (103, 385, 447).

The time and severity of exposure to hyperglycemia highly influences the progression of diabetes-associated vasculopathies (10, 263). In early intervention studies, reduction of hyperglycemia decreases the development and progression of micro- and macrovascular complications (388a, 514, 549a, 593). However, the efficacy of glycemic control in patients with long-standing hyperglycemia is less pronounced for macrovascular complications, and very stringent glycemic control (via insulin treatment) can even aggravate cardiovascular disease (1, 4, 136, 183). This indicates that in long-term diabetic patients, besides an aberrant metabolic memory (see sect. IX), additional factors such as insulin resistance and dyslipidemia may also play a dominant role in the development of diabetic vasculopathies. Here below, we focus on the effects of hyperglycemia and insulin resistance on EC metabolism and how this leads to EC dysfunction (**FIGURE 8**). Many of the reports discussed below are observations from in vitro experiments; while these have shed light on underlying mechanisms, in vivo verification is necessary in the prospect of clinical translation.

B. Hyperglycemia

1. Hyperglycemia and cytosolic/mitochondrial ROS

Hyperglycemia-induced increases in endothelial cytosolic ROS levels mainly derive from NADPH oxidases (NOXs; see sect. IV) via the protein kinase C (PKC)-dependent pathway (130, 213), from endothelial xanthine oxidase (27, 118, 359, 465) and from eNOS uncoupling (see sect. IV). Both veins and arteries of diabetic coronary artery bypass patients show increased levels and activity of NOX protein subunits (p22phox, p67phox, p47phox), which are normalized by PKC inhibition (213). Endothelial xanthine oxidase, one of the two isoforms of xanthine oxidoreductase (a key enzyme in purine metabolism) is another enzymatic source of ROS linked to diabetic vascular disease, causing vascular inflammation and atherogenesis (27, 118, 359, 465). A recent metabonomics study confirmed that hyperglycemia-induced elevated levels of xanthine oxidase and perturbed purine metabolism promote diabetic nephropathy in streptozotocin (STZ)-treated diabetic rats (326).

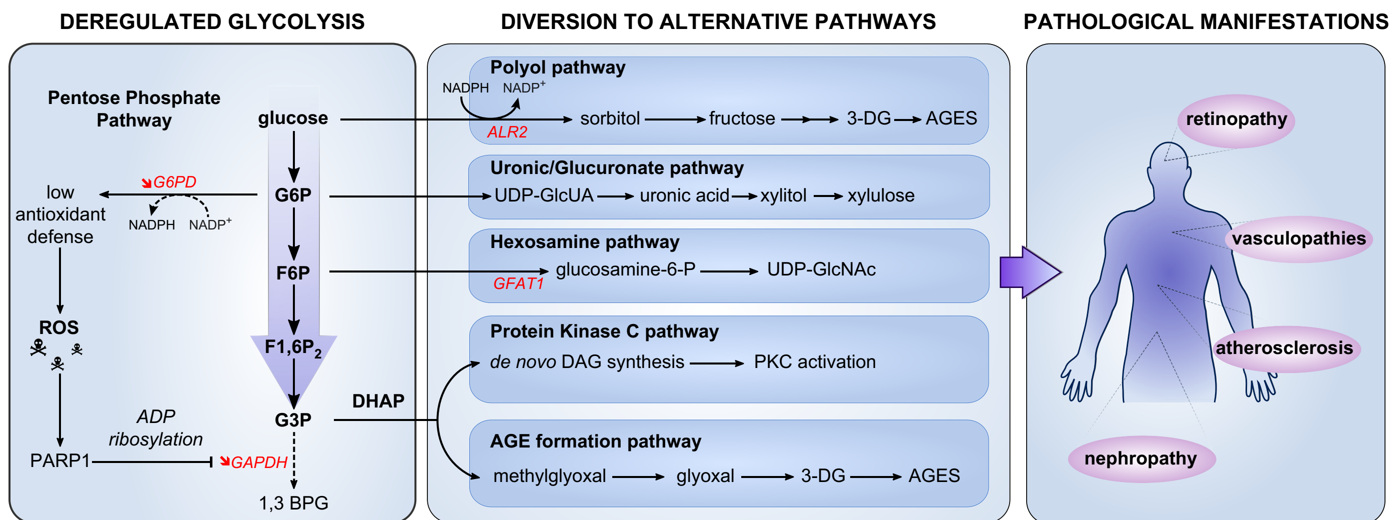


FIGURE 8. Pathological glycolytic side branches in hyperglycemic dysfunctional ECs. In diabetic ECs, hyperglycemia-induced ROS accumulation (for example caused by impairment of G6PD-mediated PPP flux) causes glycolysis to stall at GAPDH. Upstream glycolytic intermediates pile up and are diverted into pathological glycolytic side branches giving rise to further increases in ROS levels and the formation of noxious AGEs all contributing to the different vascular disease manifestations. 1,3BPG, 1,3-bisphosphoglycerate; 3-DG, 3-deoxyglucosone; ALR2, aldose reductase 2; AGES, advanced glycation end products; DAG, diacylglycerol; DHAP, dihydroxyacetone phosphate; F1,6P_2, fructose-1,6-bisphosphate; F6P, fructose-6-phosphate; G3P, glyceraldehyde-3-phosphate; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAT1, glutamine fructose-6-phosphate amidotransferase; glucosamine-6-P, glucosamine-6-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; PARP1, poly-ADP-ribose polymerase 1; PKC, protein kinase C; UDP-GlcUA, UDP-glucuronic acid; UDP-GlcNAc, uridine diphosphate *N*-acetylglucosamine.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

Likewise, eNOS uncoupling was shown to contribute to EC dysfunction in diabetic mouse models (63, 476, 523), and to mediate peripheral neuropathy in Zucker diabetic rats (496). The increase in eNOS uncoupling-derived oxidative stress in diabetic vessels was alleviated by BH_4 supplementation (213). Mechanisms contributing to eNOS uncoupling-derived oxidative stress in diabetes include interrupted metabolism of arginine and cofactors BH_4 and NADPH, resulting in their decreased availability, endothelial insulin resistance (203, 217, 251, 446, 498), aberrant *O*-glycosylation (24, 134, 165), increased levels of advanced glycation end products (AGEs) (515, 516), and hyperglycemia-induced restriction of PPP flux (310, 638) (see below in this section; **FIGURE 8**).

High intracellular glucose levels in ECs also cause mitochondriopathy featuring dysfunctional mitochondrial biogenesis and autophagy (causing damaged mitochondria to pile up), impaired mitochondrial function, and increased mitochondrial fission (reviewed in Refs. 420, 469, 495). In general, increased fission is associated with mitochondrial ROS production, while reducing mitochondrial DNA content and the activity of metabolic processes, including the ETC and ATP synthesis. The hyperglycemia-induced mitochondrial fission is driven by rho-associated protein kinase (ROCK)-1-mediated dynamin-related protein-1 (Drp1) recruitment to the mitochondria, previously established as a key event in fission (572). Since healthy mitochondria in ECs are presumably important for Ca^{2+} homeostasis, to generate physiological ROS levels, and to initiate apoptosis (with energy production relying primarily on glycolysis), dysfunctional mitochondria may promote EC dysfunction or apoptosis through Ca^{2+} overload or by exacerbating oxidative stress (529).

In health, damaging insults, such as oxidative stress, are often counterbalanced by protective (antioxidant) mechanisms to maintain vascular homeostasis; these are especially important for quiescent ECs that are continuously exposed to a high oxygen environment in the peripheral blood. The metabolic regulators peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), AMP-activated protein kinase (AMPK), and NF-E2-related factor-2 (Nrf2) are candidates to counter hyperglycemia-induced ROS. For instance, NO mediates ROS protection via upregulation of PGC1α (50, 325), and PGC1α regulates mitochondrial antioxidant defenses in ECs (552). However, the effects of high glucose on these molecular orchestrators are complex and contextual, since high glucose induces endothelial PGC1α expression, blunting activation of eNOS, and impairing EC migration and vasculogenesis; moreover, the antioxidant N-acetylcysteine (NAC) blocks the induction of PGC1α (478).

Pharmaceutical activation of AMPK in ECs can alleviate oxidative stress in hyperglycemic ECs. For instance, rosiglitazone (PPARγ agonist that activates AMPK) reduces NOX activity in hyperglycemic ECs (72). Activation of AMPK by metformin or 5-aminoimidazole-4-carboxamide-riboside (AICAR) also alleviates oxidative stress by inducing mRNA expression of manganese superoxide dismutase (MnSOD; with mitochondrial antioxidant function) (574) and inducing mitochondrial biogenesis via PGC1α (294), although it remains unclear how the latter process alleviates oxidative stress (possibly via replacing damaged ROS-producing mitochondria with new healthy ones). This overall beneficial effect has been confirmed in vivo: EC-specific AMPK activation prevented diabetes-induced EC dysfunction (317).

Nrf2 is considered to play a prominent role in the maintenance of structural and functional integrity of mitochondria, and to be a principal regulator of cellular redox homeostasis (125). In ECs exposed to hyperglycemia, Nrf2 regulates the activity of antioxidant enzymes such as glyoxalase I (GLO1; key enzyme in detoxifying methylglyoxal, see below in this section) (611). There is even evidence of cross talk between Nrf2 and AMPK resulting in the prevention of cardiomyopathy in diabetic mouse models (373, 575, 639). Despite these promising findings, outcomes of clinical trials using antioxidant therapies have been rather disappointing (309).

2. Hyperglycemia and diversion of glycolytic intermediates into alternative metabolic pathways

In the nucleus, elevated levels of ROS and reactive nitrogen species contribute to the activation of the DNA repair mechanism enzyme poly(ADP-ribose) polymerase 1 (PARP1) upon hyperglycemia-induced DNA single-strand break formation, resulting in subsequent inhibition of GAPDH through PARP1-dependent ribosylation and intracellular NAD^+ depletion (119, 133, 135). GAPDH is a key glycolytic enzyme whose activity is essential for maintaining glycolytic flux. As such, GAPDH inhibition stalls glycolysis, and accumulated upstream glycolytic intermediates subsequently divert into several alternative metabolic pathways (**FIGURE 8**) known to be altered in ECs by glucotoxicity: *1*) the polyol pathway, *2*) the glucuronate cycle, *3*) the HBP, and *4*) the glycation pathway. Competitive PARP1 antagonists, inhibiting PARP1 activity in aortic ECs, can abolish the hyperglycemia-induced activation of each of these pathways (133). Therefore, oxidative stress is often considered an initial event in EC dysfunction, which also fits with the notion of hyperglycemia-induced Nrf2-dependent transcription of antioxidant enzymes and its prevention of diabetes-associated vascular complications.

Excess glucose, not being metabolized because of stalled glycolysis, is diverted into the polyol pathway to produce sorbitol and fructose, which promotes nonenzymatic generation of noxious AGEs (195, 347, 479, 577). In HUVECs, high concentrations of fructose also provoke a prothrombotic phenotype (85). Overexpression of an ALR2 trans-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

gene in ECs from diabetic mouse models promotes atherosclerosis, whereas its inhibition reduces ROS levels and EC proliferation (396, 527, 556, 612). In the *db/db* mouse model of diabetes, the early events in diabetic retinopathy were linked to increased ALR2 levels (82).

Theoretically, elevated G6P levels can feed into the glucuronate cycle, but comprehensive studies on the glucuronate cycle in diabetic ECs are lacking. However, increased glucose metabolism via glucuronate and L-xylulose has been reported in diabetic murine models and patients, resulting in diabetes-associated renal dysfunction (68, 547, 589). Inhibitors targeting the enzyme L-xylulose reductase, catalyzing a NADPH-linked reduction of L-xylulose into xylitol, have been proposed for the treatment of long-term complications in diabetes (68).

F6P overload causes increased flux into the HBP, where it is converted into GlcN6P and subsequently into UDP-GlcNAc by GFAT (see sect. III). Under physiological conditions, UDP-GlcNAc is crucial for reversible posttranslational protein *O*-glycosylation; however, under hyperglycemic conditions, aberrant protein glycosylation results in altered protein activity, EC dysfunction, and accelerated pathogenesis of diabetes-associated atherosclerosis (165, 343, 349, 501). Aberrant *O*-glycosylation deregulates endothelial insulin signaling by obstructing key phosphorylation sites on insulin receptor substrate (IRS)-1 and eNOS, thereby impairing eNOS activity and interfering with NO synthesis (24, 134, 165). Lastly, expression of *O*-GlcNAcase, which reverses protein *O*-glycosylation, is decreased in coronary ECs from diabetic mice (343).

In a similar fashion, other accumulated glycolytic intermediates, i.e., G3P and dihydroxyacetone phosphate (DHAP), are diverted towards the production of highly reactive dicarbonyl compounds such as methylglyoxal, glyoxal, and 3-DG in the glyoxalase system. These reactive aldehydes modify (through glycation) DNA and (cytosolic and mitochondrial) proteins, resulting in the nonenzymatic formation of toxic AGEs. Also, excess G3P and DHAP fuel de novo diacylglycerol (DAG) synthesis, which subsequently activates PKC, resulting in vascular abnormalities via increased levels of NOXs and eNOS uncoupling (111, 248, 250). DAG-dependent PKC activation occurs under conditions of excessive circulating levels of glucose and FFAs.

As end products of dysfunctional EC metabolism, elevated levels of AGEs promote oxidative stress in the endothelium via several detrimental mechanisms (see sect. III), further exacerbating diabetes-associated vasculopathies. First, they modify intracellular protein signaling after posttranslational modifications of lysine and arginine residues of proteins (459). Second, they render the endothelium dysfunctional by altering collagen-type proteins through protein cross-linking, causing decreased vessel elasticity, cell adhesion, and matrix-matrix interaction (357). Additionally, AGEs can adversely affect other cells types, mediated at least in part through interaction of AGEs with their receptor (RAGE; present on several cell types) and subsequent PKC and nuclear factor-kappa B (NFκB) activation (357, 626). While NFκB promotes endothelin-1-mediated vasoconstriction and inflammation (441), elevated levels of PKC can disturb insulin signaling pathways and glucose metabolism in skeletal muscle cells (370) or result in the dephosphorylation of PDGFRβ and subsequent apoptosis of perivascular mural pericytes (191). Notably, loss of pericyte coverage, resulting in leaky endothelium, is one of the earliest signs of diabetic retinopathy (494).

In ECs, methylglyoxal is considered to be the main reactive aldehyde formed under hyperglycemic conditions (52, 497). Elevated methylglyoxal levels in diabetic patients are linked to diabetes-associated vasculopathies (365, 387, 397, 488). In vitro, methylglyoxal triggers eNOS uncoupling; however, the exact mechanism remains poorly defined (515). It was recently suggested that methylglyoxal exposure may also lead to alterations in EC redox status, via the inhibition of NADPH-generating enzymes, thereby depleting GSH levels and intensifying overall oxidative stress (377). Rats overexpressing GLO1, a key enzyme detoxifying methylglyoxal (regulated by Nrf2; see above in this section), showed signs of reduced endothelial glycative and oxidative stress and alleviated EC dysfunction (262). Of note, normalization of glucose levels in diabetic patients did not entirely prevent elevation of methylglyoxal levels and subsequent diabetic vasculopathies, and progressive ROS production and AGE accumulation persisted after glucose normalization (73, 171), begging for a better understanding of EC metabolism perturbation in diabetes.

3. Hyperglycemia and restricted PPP flux

Hyperglycemia has also been reported to impair G6PD-mediated entry of glucose into the PPP (638), resulting in reduced availability of NADPH. As NADPH is a critical cofactor of eNOS, this ultimately results in increased oxidative stress and decreased NO bioavailability (310). Restoring the oxPPP flux, possibly through activation of G6PD, could promote NADPH and GSH redox cycling and is therefore an attractive target to reduce overall oxidative stress in the context of hyperglycemia. Noteworthy, under hyperglycemic conditions and subsequent stalled glycolysis, increasing the activity of the non-oxPPP enzyme TK with benfotiamine treatment diverts excess glycolytic triosephosphates (F6P and G3P) into the non-oxPPP, rather than into the alternative toxic metabolic pathways (discussed above). Benfotiamine treatment prevented diabetic nephropathy in STZ-treated rats (23, 218, 610).

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

C. Insulin Resistance

Since inhibition of hyperglycemia-induced oxidative stress, pharmacologically or via endogenous antioxidant mechanisms, prevents the hyperglycemia-associated activation of the above-mentioned pathways, oxidative stress is considered to be a key initial event in EC dysfunction. However, apart from hyperglycemia, insulin resistance has recently emerged as another major precursor of atherothrombotic events and poor cardiovascular outcome (34, 414). Insulin resistance is the prediabetic state of glucose intolerance (early stages of diabetes type 2) and is characterized by impaired insulin sensitivity and hyperinsulinemia (state of increased secretion and decreased clearance of insulin). Previously, insulin resistance was primarily linked to target tissues like liver, skeletal muscle, and adipose tissue, but emerging evidence indicates that insulin resistance also occurs in ECs (417).

While insulin signaling stimulates glucose uptake in target tissues mainly via intracellular trafficking of GLUT4, ECs display insulin-independent glucose uptake via GLUT1 (other GLUTs are less abundantly expressed in ECs) and intraendothelial glucose levels are therefore independent of insulin sensitivity (266). Furthermore, ECs do not downregulate GLUT1 expression in response to high extracellular glucose concentrations, causing glucose concentrations in ECs to reflect glucose levels in the blood, which can explain why ECs are particularly susceptible to hyperglycemia. Still, there exists extensive literature on EC dysfunction secondary to insulin resistance in vascular endothelium (132, 181, 223, 283, 292, 320, 452, 559). While hyperglycemia-induced oxidative stress is now generally accepted to trigger vascular complications in both diabetes type 1 and 2, endothelial insulin resistance may explain the incidence of EC dysfunction predating the onset of diabetes type 2 (in the absence of hyperglycemia). Yet, targets of insulin signaling in ECs and their metabolism remain incompletely understood.

Insulin signals via MAPK- and PI3K-dependent pathways with different downstream effector proteins in the endothelium versus other target tissues (168, 375). The insulin-induced MAPK signaling (the "mitogenic/pro-atherogenic arm of insulin signaling"), which stimulates inflammation, vasoconstriction, and apoptosis, is not affected when ECs become insulin resistant (261). As a counter balance, PI3K-Akt signaling (the "metabolic arm" of insulin signaling) activates eNOS, resulting in NO-dependent vasodilation (633). Obesity-associated endothelial insulin resistance impairs eNOS activation, resulting in oxidative stress and reduced vasodilation and capillary recruitment (132, 139, 181, 261, 292). The latter causes a progressive decline of peripheral insulin distribution and subsequent glucose disposal in the skeletal muscle (292). As such, insulin resistance creates an imbalance between the metabolic and mitogenic/pro-atherogenic arms of insulin signaling (376), resulting in EC dysfunction with a subsequent net increase in vasoconstriction (hypertension and systemic insulin resistance) and a pro-inflammatory/thrombotic state (atherosclerosis) (16). Endothelial insulin resistance may therefore be causally linked to cardiovasculopathies preceding and following the onset of diabetes type 2 that are additionally exacerbated by the compensatory hyperinsulinemia (447, 545).

Forkhead box O (FoxO) proteins regulate endothelial insulin resistance. Indeed, endothelial deletion of the three major FoxO isoforms ($FoxO^{ECKO}$) attenuates endothelial insulin resistance, increases NO bioavailability, and protects low-density lipoprotein receptor knockout ($Ldlr^{-/-}$) mice from atherosclerosis (546) **(FIGURE 9)**. Downstream of the PI3K-Akt pathway, these FoxO proteins (encoded by FoxO1, FoxO3a, and FoxO4) inhibit insulin signaling and eNOS expression while enhancing iNOS expression upon oxidative stress, causing $ONOO^-$ production and EC dysfunction (284, 389, 528, 542, 546, 628). For these reasons, endothelial FoxO inhibition has been postulated as a potential therapeutic target to tackle endothelial insulin resistance and cardiovascular disease in diabetes type 2 patients (270, 452). However, subsequent experiments with $FoxO^{ECKO}$ mice demonstrated that, while increased levels of EC-derived NO have a favorable effect on EC dysfunction in $Ldlr^{-/-}$ mice (with advanced insulin resistance), they also impair insulin-dependent suppression of glucose production in hepatocytes of healthy mice (with early stage insulin resistance) (545). This suggests that the therapeutic value of vascular insulin sensitization via FoxOs may depend on the degree of metabolic disease or may only be beneficial for atherosclerosis-prone vessels (270). Furthermore, another reason for caution is that FoxO1 couples EC metabolic activity with vascular expansion and acts as a gatekeeper of endothelial quiescence by reducing glycolysis and mitochondrial respiration (586). Hence, EC-specific FoxO1 deletion results in EC hyperplasia and vessel enlargement (586). The FoxO1-induced reduction of endothelial oxidative metabolism may be a protective mechanism against excessive mitochondria-derived ROS in ECs, and therefore its inhibition (expected to increase ROS levels) may not directly benefit diabetic type 2 patients after all.

While hyperglycemia has been clearly connected to increased oxidative stress in ECs, the link between endothelial insulin resistance and oxidative stress is less well established to date. One possible mechanism relates to the increased circulating FFA levels, linked to obesity-associated dyslipidemia and insulin resistance in type 2 diabetics. Indeed, elevated FFA levels increase oxidative stress in ECs via NOX activation and eNOS inactivation, concomitant with PKC activation and de novo DAG synthesis (248, 273). DAG and PKC levels are elevated in diabetic animal models and contribute to diabetes-related vasculopathies, as excess levels of DAG negatively affect blood flow and vasodila-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

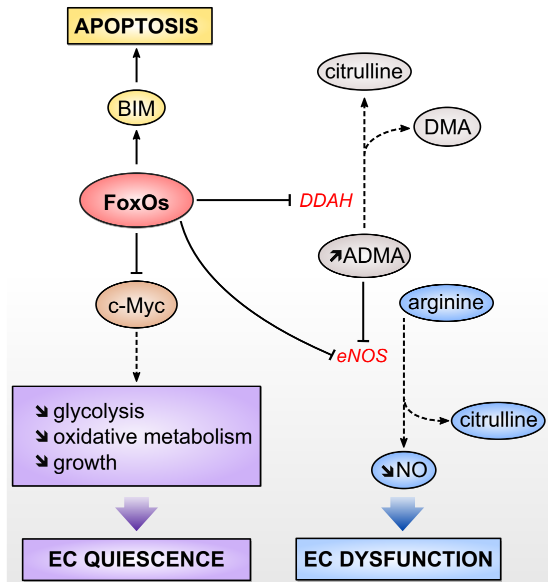


FIGURE 9. Different roles of FoxO transcription factors in ECs. Schematic representation of the dual role FoxO transcription factors can play in ECs, either as metabolic gatekeepers to sustain EC quiescence or, conversely, contributing to EC dysfunction. ADMA, asymmetrical dimethyl arginine; BIM, BCL2 like 11; DMA, dimethylamine; FoxO, forkhead box O; NO, nitric oxide.

tion, and increase fibrosis and vascular permeability (111, 248, 250). Another candidate possibly involved in endothelial insulin resistance-mediated oxidative stress and EC dysfunction is NOX2 (131, 181).

Systemic insulin induces endothelial FASN, required for eNOS palmitoylation and subsequent localization to caveolae where sources of arginine and BH_4 are optimal to produce NO (579). Diabetic animal models showed reduced endothelial FASN levels and decreased eNOS palmitoylation resulting in increased oxidative stress, inflammation, and impaired angiogenesis (579). Whether the diabetic vascular dysfunction can be rescued via FASN signaling remains unstudied. Nonetheless, insulin also regulates palmitoylation of proteins in ECs required for insulin-induced angiogenesis and EC migration (580). Proteomics could therefore be useful to identify novel insulin-dependent palmitoylation targets, possibly dysregulated by insulin resistance in diabetes, related to endothelial function and angiogenesis.

Lastly, endothelial insulin resistance can also lead to increased oxidative stress via eNOS uncoupling by interrupting the metabolism of the NO-precursor arginine and cofactor BH_4, resulting in their decreased availability (203, 217, 251, 446, 498). Thus endothelial insulin resistance and hyperglycemia can both influence EC dysfunction via oxidative stress, however, while hyperglycemia causes a variety of both micro- and macrovasculopathies, insulin resistance primarily promotes cardiovascular disorders, including atherosclerosis (562).

D. Atherosclerosis

Representing more than 80% of the cardiovascular deaths in 2013, atherosclerosis is another leading cause of morbidity in the Western society (383). EC dysfunction is a main contributor to this chronic disease, which develops in large and small arteries of various organs (198, 322). The pathogenesis of atherosclerosis is a multistep process: upon activation, ECs increase the expression of adhesion molecules [i.e., intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin], which promotes monocyte recruitment within the vascular wall. Macrophages derived from these monocytes engulf modified lipoproteins, accumulate intracellular cholesterol, and become foam cells (121, 322). The ECs on the other hand become progressively more dysfunctional, causing uncontrolled generation of toxic ROS with a concomitant drop in the production of the anti-atherogenic cues NO and hydrogen sulfide (H_2S), further exacerbating vascular perturbation and inflammation, increasing oxidative stress and driving overall progression of the disease.

E. eNOS Uncoupling and Pro-atherosclerotic Effect of ROS Generation

As discussed above, NO is a master switch in cardiovascular homeostasis by virtue of its vasorelaxant, antihypertensive, and antithrombotic properties. NO causes vascular smooth muscle cells (SMCs) to relax and prevents a number of proatherosclerotic events like platelet aggregation, SMC proliferation and migration, leukocyte adhesion, and oxidative stress (182, 259, 318, 543). In atherogenesis, when NADPH and BH_4 are limiting, eNOS becomes "uncoupled" and switches from NO production (atheroprotective) to generation of superoxide anions and $ONOO^-$, further amplifying eNOS uncoupling (36, 198) **(FIGURE 10)**.

In the $ApoE^{-/-}$ mouse model of atherosclerosis, supplementation with BH_4 indeed partially rescues the NO defect, decreases superoxide production, and improves endothelium-dependent vasodilation (36, 302). In ECs, BH_4 is generated via two pathways: de novo synthesis from GTP by GTP cyclohydrolase I (GTPCH-1) or regeneration from BH_2 by dihydrofolate reductase (DHFR). DHFR also converts dihydrofolate (DHF) to tetrahydrofolate (THF), thereby linking eNOS uncoupling to the folate one-carbon metabolism. Indeed, THF accepts one carbon from serine to generate 5,10-methylene-THF (meTHF) and glycine. Methylenetetrahydrofolate reductase (MTHFR) then reduces meTHF to 5-methyl-THF (mTHF), which gives a one-carbon unit to homocysteine to generate methionine (and to recycle THF) and the methyldonor *S*-adenosylmethionine (SAM) required for various methylation reactions (DNA, histone, other proteins) (36).

One peculiar target for methylation in this context is the eNOS substrate arginine itself. Arginine methylation yields

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

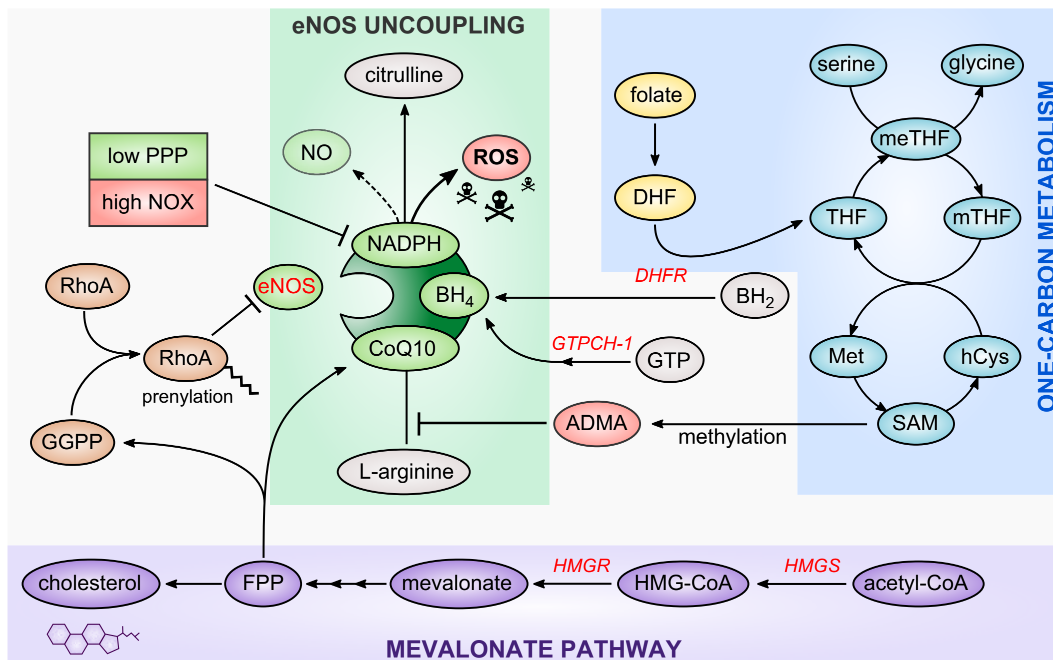


FIGURE 10. Metabolic aberrations in ECs in atherosclerosis. In atherosclerosis, ECs display prominent eNOS uncoupling leading to elevated ROS levels and reduced NO-mediated vasodilation. Underlying eNOS uncoupling are decreases in the levels of necessary cofactors (NADPH, CoQ10, BH_4) resulting from deregulated metabolic pathways or enzyme activities, and methylation of the eNOS substrate arginine (ADMA). Acetyl-CoA, acetyl-coenzyme A; ADMA, asymmetrical dimethyl arginine; BH_2, 7,8-dihydrobiopterin; BH_4, tetrahydrobiopterin; CoQ10, coenzyme Q10; DHF, dihydrofolate; DHFR, dihydrofolate reductase; eNOS, endothelial nitric oxide synthase; FPP, farnesylpyrophosphate; GGPP, geranylgeranyl pyrophosphate; GTPCH, GTP cyclohydrolase; hCYS, homocysteine; HMG-CoA, hydroxymethylglutaryl coenzyme A; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HMGS, 3-hydroxy-3-methylglutaryl-CoA synthase; MET, methionine; meTHF, 5,10-methylene-methyltetrahydrofolate; mTHF, 5-methyltetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; NOX, NADPH oxidase; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SAM, *S*-adenosylmethionine; THF, tetrahydrofolate.

asymmetric/symmetric dimethylarginines (ADMA, SDMA) (**FIGURE 10**). Unlike arginine, ADMA and SDMA cannot be used by eNOS to generate NO, but rather compete with arginine for NO production, representing another level of eNOS uncoupling (40). Oxidative stress enhances ADMA accumulation by inactivating the ADMA eliminating enzyme dimethyl-arginine dimethyl-aminohydrolase (DDAH) (253, 435). Being elevated in atherosclerotic patient sera (up to 10-fold) (44), ADMA and more precisely the arginine-to-ADMA ratio is considered a cardiovascular risk factor (28, 394). Of note, SDMA induces oxidative stress and increases superoxide anion production in glomerular kidney ECs (166). Clinically, plasma levels of SDMA positively correlate with mortality in patients with stable coronary heart disease (502).

FoxO1 in ECs increases levels of the eNOS inhibiting ADMA in vivo through downregulation of the ADMA degrading enzyme DDAH, thereby adding an additional layer of FoxO-mediated regulation of endothelial NO levels (see above and **FIGURE 9**) (368). Furthermore, in the aorta from $FoxO^{ECKO}/Ldlr^{-/-}$ mice, levels of NOXs are downregulated, suggesting a protective effect of FoxO ablation on the endothelium (546). Moreover, in cultured HUVECs, FoxO1 induces the expression of the proapoptotic molecule BIM (327). Even though these data are in favor of therapeutically inhibiting endothelial FoxO1 in atherosclerosis, this will be extremely challenging given the above-mentioned crucial role for FoxO1 in fine-tuning EC metabolism (586). Furthermore, complete inhibition of FoxO1 might be harmful given its role in regulating endothelial oxidative stress and decreasing eNOS levels. Instead, fine-tuning FoxO1 levels by targeting posttranslational modifications (i.e., acetylation) might be an option that requires however further study (57, 440).

In methyltransferase reactions, SAM is converted to *S*-adenosylhomocysteine (SAH), which is further hydrolyzed to homocysteine and adenosine by SAH hydrolase (SAHH). Hyperhomocysteinemia (HHcy) causes EC dysfunction and is an independent risk factor for cardiovascular disease by reducing the levels of available NO and *S*-nitrosylated proteins important for EC homeostasis (80, 517). While the exact mechanism involved is not fully understood, HHcy causes accumulation of homocysteine-thiolactone and immunogenic and toxic *N*-homocysteinylated proteins. These

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

two compounds might mediate the pro-atherogenic effect of HHcy by altering gene expression in ECs. According to transcriptome analysis in HUVECs, they mainly affect the expression of genes in one-carbon, sulfur amino acid and lipid metabolism, and in epigenetic/chromatin organization regulation (211). Several studies reveal that plasma SAH and homocysteine levels are associated with DNA hypomethylation in atherosclerosis and vascular disease (623, 644). In line with this, increased SAH levels, again via modulation of histone methylation, cause ER stress, which plays an important role in EC dysfunction by inducing oxidative stress and inflammation (598, 600, 601). Furthermore, homocysteine-mediated ROS production promotes EC death by disturbing the mitochondrial membrane potential (an early event in apoptosis) leading to caspase activation (539, 548, 620). Taken together, SAH and homocysteine levels may serve as sensitive and comprehensive biomarkers for EC dysfunction in atherosclerosis (635).

F. Mevalonate Pathway in ECs

In addition to one-carbon metabolism and folate/methionine cycling, the endothelial mevalonate or isoprenoid pathway (using acetyl-CoA to produce cholesterol) plays a significant part in atherogenesis (45, 160). In the mevalonate pathway, acetyl-CoA is first converted to 3-hydroxy-3-methylglutaryl (HMG)-CoA by HMG synthase, and then to mevalonate by HMG-CoA reductase (HMGR). Isoprenoid intermediates further downstream the pathway, such as geranylgeranylpyrophosphate (GGPP) and the cholesterol-backbone farnesylpyrophosphate (FPP), posttranslationally modify various proteins, including small GTPases such as RhoA and Rac1 (399). RhoA's activity to regulate cytoskeletal dynamics requires GGPP prenylation and subsequent EC plasma membrane localization, essential for EC homeostasis (236, 237). However, when active, RhoA also may contribute to EC dysfunction, in part by promoting the degradation of eNOS mRNA transcripts (301, 518) as well as by stimulating leukocyte recruitment and vascular inflammation during atherogenesis (374) **(FIGURE 10)**. Serum lipid-lowering statins inhibit HMGR and are widely used to reduce cholesterol levels. A non-lipid-lowering effect of statins involves the reduction of GGPP pools and the subsequent inhibition of RhoA activation, preventing excessive inflammation and degradation of eNOS transcripts. However, these beneficial effects (reduced RhoA prenylation) have only been observed at doses far above clinical applicability. Furthermore, statins also lower FPP levels required to produce the mitochondrial cofactor CoQ10 (involved in eNOS coupling) and can therefore have adverse effects on the endothelium (83, 384, 544) **(FIGURE 10)**.

G. NOX Control and NADPH Levels

As mentioned above, NADPH pools sustain eNOS coupling but can be depleted by ROS-generating NOXs **(FIGURE 10)**, which show prominent expressions in vascular diseases and are induced in atherosclerotic arteries exposed to turbulent blood flow (212, 249, 264, 472). Of the different isoforms expressed in ECs, NOX2 is a major source of ROS production (367). Its expression is upregulated in aortic endothelium of $ApoE^{-/-}$ mice and occurs before the emergence of atherosclerotic plaques, whereas loss of NOX2 expression attenuates ROS generation in ECs and monocytes and increases NO availability (264, 558). Interestingly, the polyphenol drug resveratrol decreases NOX2 expression in cerebromicrovascular ECs (541) and protects against mitochondrial-derived ROS by upregulating PGC1α (645) (see above in this section). Not all NOX-derived ROS are harmful; some can repress inflammation and lead to atheroprotection instead, as is the case for NOX4 (207, 300). Rather than indiscriminatively inhibiting all NOXs, the development of isoform-specific NOX inhibitors will be the key in optimizing clinical applicability (534). As such, the peptidic NOX2 selective inhibitor gp91ds-tat reverses insulin resistance-induced reduction of EC-mediated vasorelaxation (519). Yet, the important role of NOX2 in the innate immune system might hinder further clinical applications of this inhibitor (256).

H. Cysteine Metabolism and H_2S Production

The gaso-transmitter H_2S has long been considered to lack any physiological function and to be no more than a highly toxic and malodorous gas. In contrast, H_2S is an important cue in the regulation of EC and cardiovascular homeostasis by inducing blood vessel relaxation and cardioprotection (439). H_2S is produced by ECs and SMCs by three enzymes using cysteine: cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS), and the tandem of cysteine aminotransferase (CAT) and 3-mercaptopyruvate sulfurtransferase (3-MST). CSE is the predominant H_2S-producing enzyme in ECs and converts cysteine to pyruvate, ammonia, and H_2S (439, 571). CBS catalyzes two main reactions: first the direct transformation of cysteine to H_2S and homocysteine, and second the condensation of serine with the latter to generate cystathionine (571) **(FIGURE 11)**.

The beneficial effects of H_2S on the vascular endothelium include *1*) inhibition of vascular inflammation, via repression of the NFκB pathway and the shear stress-induced binding of activated monocytes on vessel walls; *2*) increased oxidative stress defense by favoring the production of reduced glutathione and by inducing antioxidant gene expression (i.e., catalase, MnSOD); *3*) reduction of SMC proliferation; *4*) increased angiogenesis and EC self-repair processes; and *5*) reduced atherogenic modification of LDL and macrophage-derived foam cell formation (439, 571). Of interest, high-fat-fed mice with CSE deficiency suffer a higher risk to develop atherosclerotic lesions with high LDL and homocysteine levels, which is attenuated by treatment with the NaHS H_2S-donor (345, 605). Also in the context

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

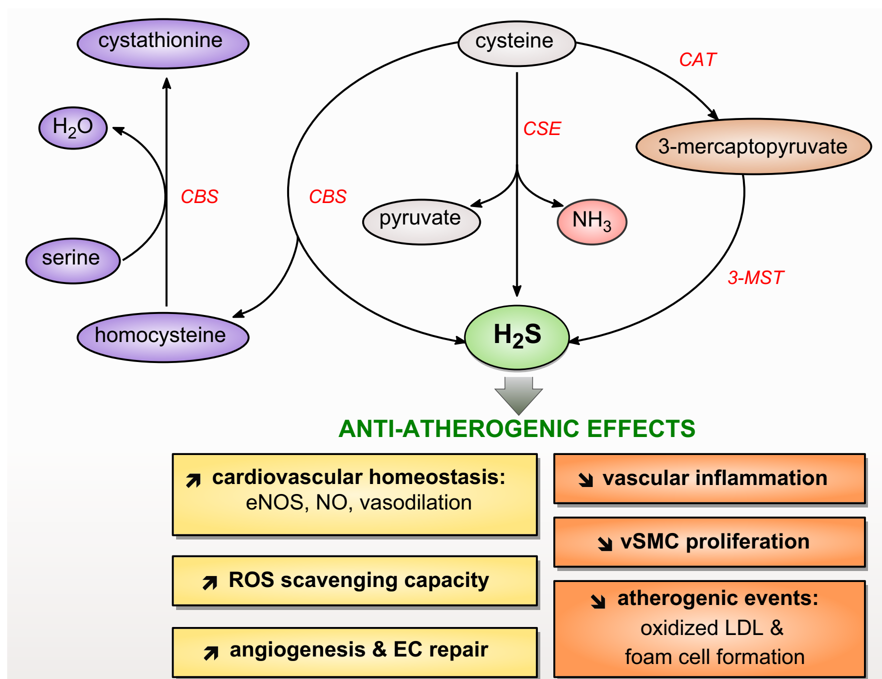


FIGURE 11. Endothelial cysteine to H_2S metabolism in atherosclerosis. The gasotransmitter H_2S is an important regulator of cardiovascular homeostasis and has anti-atherogenic effects. In ECs, H_2S can be produced from cysteine (via CSE or CAT and 3-MST activity) or in the condensation of cysteine and homocysteine to cystathionine (alternate action of CBS). The anti-atherogenic effects of H_2S are listed at the bottom of the image and are discussed in more detail in the main text. 3-MST, 3-mercaptopyruvate sulfurtransferase; CSE, cystathionine-γ-lyase; CBS, cystathionine-β-synthase; CAT, cysteine aminotransferase; eNOS, endothelial NO synthase; LDL, low-density lipoprotein; NH_3, ammonia; NO, nitric oxide; vSMC, vascular smooth muscle cell.

of atherosclerosis-associated HHcy, homocysteine is reported to inactivate CSE by homocysteinylation, further aggravating the disease (439, 571) **(FIGURE 11)**.

There is also a reciprocal interaction between H_2S and NO. Indeed, eNOS inhibition lowers H_2S-stimulated vasorelaxation, whereas CSE silencing abolishes NO-driven angiogenesis (91). This intertwined interaction is not fully understood, but is in part mediated by posttranslational modifications. By inducing eNOS *S*-sulfhydration, H_2S facilitates eNOS dimerization and stabilization, leading to NO production (13, 91). Interestingly, the H_2S-dependent sulfhydration of cysteine residues in pancreatic β-cells exposed to ER stress promotes aerobic glycolysis and reduces OXPHOS (185). Whether or not H_2S-driven sulfhydration contributes to metabolic reprogramming towards glycolysis in sprouting ECs too is not known yet.

I. Blood Flow Influencing EC Metabolism

Atherosclerotic plaques preferentially develop in areas subject to low and turbulent shear stress, whereas arteries exposed to uniform laminar shear stress are less prone to plaque formation (522). The effect of shear stress can be attributed to mechano-activated signaling pathways that instruct ECs to react to the external milieu but, interestingly, also impact EC metabolism. The transcription factor KLF2 is upregulated in ECs subject to atheroprotective laminar shear stress (422, 477). Genetic overexpression of KLF2 causes ECs to adopt a more quiescent behavior through several mechanisms, including increased VE-cadherin expression (KLF2 induces expression of the mRNA binding protein quaking, which enhances VE-cadherin mRNA translation) and tightening of the vascular barrier (115, 126), promotion of EC alignment in the flow axis (48), reduction of the pro-inflammatory state, and control of vascular homeostasis (395) **(FIGURE 12)**. Of particular interest is how KLF2 links the biomechanical stimulus of shear stress to EC glucose metabolism. Indeed, increased KLF2 expression reduces glucose uptake and glycolysis, in part by repressing PFKFB3′s promoter activity (126). Given that lowering glycolysis (by silencing PFKFB3) enhances EC quiescence (114), this KLF2-driven mechanism ensures that an adequate (lower) level of glycolytic flux sustains EC quiescence under laminar flow **(FIGURE 12)**.

In atheroprone regions, low shear stress and oxidized LDL (oxLDL) induce miR-92a expression to repress endothelial KLF2 and phosphatidic acid phosphatase type 2B (PPAP2B) expression (81, 296, 333, 594) **(FIGURE 12)**. Under normal atheroprotective flow, PPAP2B dephosphorylates lysophosphatidic acid (LPA) to prevent it from binding to its receptor (LPAR1) and inducing pro-inflammatory signaling (594). As such, EC-specific PPAP2B-deficient mice feature enhanced local and systemic inflammation associated with increased vascular permeability (412). Genome-wide association studies show that a single-nucleotide polymorphism (rs17114036) lowers PPAP2B expression in human ECs and is predictive of a higher risk for coronary artery disease independently of traditional risk factors, such as cholesterol and diabetes (484).

C-reactive protein (CRP) also participates in atherogenic effects of lipoproteins. Indeed, as shown in vitro in HUVECs, CRP increases the transcytosis of LDL across ECs, thereby enhancing LDL retention in vascular walls and

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

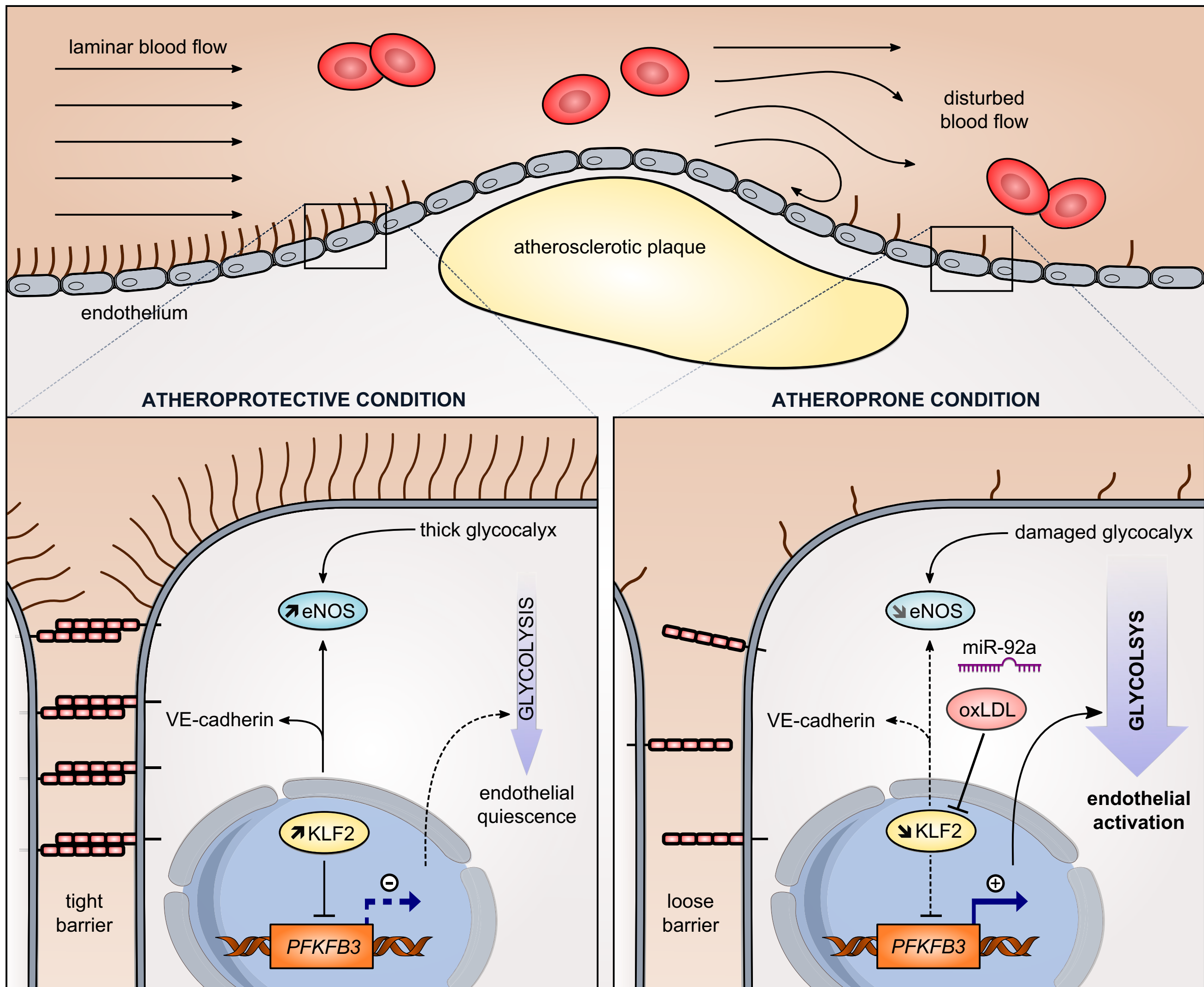


FIGURE 12. Impact of atheroprone versus atheroprotective flow on EC metabolism. Blood flow dynamics critically influence EC metabolism and behavior. Normal atheroprotective blood flow increases the activity of the flow-responsive transcription factor KLF2 which, in addition to increasing VE-cadherin expression and barrier function, transcriptionally represses PFKFB3 expression and lowers glycolytic rates to sustain EC quiescence. Atheroprone turbulent flow lowers KLF2 activity and VE-cadherin levels as well as releases the brake on PFKFB3 expression causing increased glycolysis and EC activation. Additionally, in atheroprone regions, low shear stress and oxLDL induce miR-92a expression to lower KLF2 levels. Finally, normal blood flow sustains a functional EC glycocalyx with protective function. However, atheroprone insults reduce thickness of this glycocalyx through mechanisms discussed in more detail in the main text. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3; KLF2, Krüppel-like factor 2; oxLDL, oxidized low-density lipoprotein; VE-cadherin, vascular endothelial cadherin; miR-92a, microRNA-92a.

ROS generation (42). Moreover, the pro-atherogenic effect of CRP has also been demonstrated in ApoE$^{-/-}$ mice (173). CRP therefore represents an important cardiovascular disease risk factor (154, 173, 254). Interestingly, the inflammatory cytokine IL-6 is a major inducer of CRP in ECs in vitro, in mice models of thrombosis, and in patients with cardiovascular disease (97, 460, 473). Furthermore, IL-1β can induce CRP levels, directly or indirectly via induction of IL-6 (62, 277). In atherosclerotic lesions, macrophages secrete IL-6 and IL-1β, dependently on glycolytic activity (499). Moreover, glycolytic enzymes such as the pyruvate kinase M2 (PKM2) also control IL-1β induction in macrophages (410). Therefore, targeting glycolysis in endothelium and/or in atherosclerotic-plaque infiltrating immune cells might offer new opportunities.

Another mechanism linking blood flow to EC metabolism relates to the endothelial glycocalyx layer (EGL), a thick gel-like layer composed of glycoproteins, sulfated proteoglycans (e.g., syndecan-1), and associated glycosaminoglycans (GAGs) at the luminal surface of ECs (110, 532). If intact, the EGL is atheroprotective; in ECs, the EGL constituent glypican-1 (GPC1) colocalizes with eNOS in caveolae and lipid rafts to mediate its activity in response to

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

shear stress (143). Moreover, the EGL tightens the endothelial barrier and limits vascular permeability and prevents thrombosis and vessel wall inflammation (through inhibition of respectively platelet and leukocyte adhesion to ECs) (110). Laminar shear stress promotes EGL deposition. In contrast, pro-atherosclerotic insults like hyperglycemia, ox-LDL, and ROS drive EGL damage and shedding (30, 280, 409, 503) (**FIGURE 12**). At the clinical level, patients with acute coronary syndrome display EGL damage with elevated levels of plasma syndecan-1, reminiscent of EGL shedding (371). Conversely, EGL degradation also promotes ROS generation, sustaining a vicious circle (295). Given that the EGL consists of glycoproteins and glycosaminoglycan chains (such as hyaluronan), its production is likely somehow linked to EC glucose metabolism. As discussed above, the HBP pathway produces UDP-GlcNAc for protein glycosylation. In addition, the GAG hyaluronan is made up by disaccharide units of glucuronic acid (GlcUA) and GlcNAc (122). Hyaluronan synthases use UDP-GlcUA and UDP-GlcNAc as rate-limiting substrates, synthesis of which is an energy-consuming process requiring glycolytic intermediates (560). Indeed, the uronic/glucuronate pathway is an alternative pathway for glucose oxidation that uses the glycolytic metabolite G6P to produce UDP-GlcUA, while the HBP uses the glycolytic intermediate (F6P) to produce UDP-GlcNAc (see section III and **FIGURE 3**). How ECs regulate these metabolic pathways to produce the EGL in response to atheroprotective/susceptible flow requires further study.

VIII. ENDOTHELIAL CELL METABOLISM IN VASCULAR DISORDERS: EXCESS ANGIOGENESIS

While diabetes and atherosclerosis are tainted by vascular complications as a result of early EC dysfunction, other life-threatening diseases including cancer and pulmonary arterial hypertension are mainly characterized by excessive angiogenesis, which likewise appears to be driven by endothelial metabolic aberrations.

A. Cancer

Excess angiogenesis in cancer is caused by *1*) aberrant angiogenic growth factor signaling due to gene mutations in cancer cells, *2*) angiogenic growth factor secretion by infiltrating immune cells and stromal cells, *3*) a pro-angiogenic make-up of the tumor microenvironment due to aberrant cancer cell metabolism, and *4*) dysfunctional blood vessels and amplification of hypoxia and low pH due to a lack of oxygen supply and waste removal.

Activation of oncogenes or inhibition of tumor suppressor genes (e.g., k-Ras, v-Src, v-Raf, and p53) leads to aberrant secretion of pro-angiogenic factors by cancer cells and consequently stimulates the growth of new blood vessels into the tumor (448). In addition, infiltrating platelets, immune cells, and stromal cells secrete factors that promote angiogenesis. As such, the tumor microenvironment is enriched with pro-angiogenic factors, including VEGF, bFGF, chemokines, and PDGF (581). Nutrient and oxygen deprivation contributes to the induction of tumor angiogenesis. Even tumors of <1 mm in size can show substantial levels of hypoxia (321). The subsequent stabilization of HIF-1α and induction of its target genes leads to further excessive secretion of VEGF and other angiogenesis promoting factors, and incites ECs to sprout and form new vessels (222). Once tumors grow to a diameter of 1–4 mm, hypoxia is reduced, because tumors become vascularized (321). Still, the excess production of pro-angiogenic signals leads to continuous stimulation of aberrant, even nonproductive tumor angiogenesis resulting in abnormal vessels. Pericytes, which promote maturation and vessel stabilization, associate with tumor endothelium but more loosely and to a far lesser extent than with normal endothelium (19, 176). The vessel walls of the tumor vasculature are spotted with gaps due to irregular shape and size of ECs, abnormal tight junction formation, and EC hyperproliferative activity and motility, leading to reduced blood flow and vessel perfusion. These properties amplify aggressive tumor growth and create a nutrient-deprived microenvironment, from where cancer cells attempt to escape; the leaky tumor vascular barrier facilitates cancer cell dissemination and metastatic spreading (137).

The metabolic profile of tumor tissues is most frequently characterized by low glucose concentrations (even as low as 0.12 mM) (234) and high lactate concentrations (as high as 40 mM) (568). The second most increased metabolite in tumor tissue is glutamate, derived from extracellular glutamine (205). Moreover, tumors are acidic, partly caused by higher glycolytic activity, in addition to higher general metabolic activity leading to increased CO_2 and H^+ production, and lack of metabolic waste removal due to insufficient blood perfusion (363). Most of these characteristics have been described to promote tumor angiogenesis. As such, VEGF transcription and/or secretion are increased by glucose deprivation and acidic pH (631, 636). Increased lactate stimulates angiogenic growth, since high intracellular lactate concentrations in ECs stimulate *1*) a pro-angiogenic, autocrine NFκB/IL8 loop (557) and *2*) normoxic stabilization of HIF-1α, thereby promoting VEGFR2 expression, VEGF-A secretion, and EC migration and sprouting (243, 506). This occurs through competition of lactate with 2-oxoglutarate, a cofactor for the prolyl hydroxylase domain protein 2 (PHD2); as a result, degradation of HIF-1α in normoxia is inhibited (116, 557). ECs import lactate via monocarboxylate transporter 1 (MCT1), expressed in tumor-associated vasculature (286), and can be targeted to reduce tumor angiogenesis.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

In general, tumor ECs (TECs) can display cytogenetic abnormalities (6, 229, 230) and have a different epigenetic profile (336) and gene expression signature than normal ECs (NECs) (336, 407, 486, 510, 553, 615). TECs are more angiogenic, more responsive to paracrine and autocrine pro-angiogenic signaling, have better survival strategies than NECs (14, 60, 298, 358, 615), and are often more resistant to chemotherapeutic and anti-angiogenic drugs (7, 9, 228, 231). Furthermore, TECs can be heterogeneous within one and the same cancer type (231, 232, 398). For example, TECs from high-metastatic melanomas have higher pro-angiogenic capacities than those from low-metastatic melanomas (398). Additionally and in contrast to those in low-metastatic melanoma, TECs from high-metastatic melanoma secrete biglycan, which further promotes metastasis (339). TECs can also adopt cancer cell-specific characteristics through horizontal DNA transfer (147); they can share certain genomic aberrations with cancer cells (641, 646) or even derive directly from cancer stem-like cells, as shown for glioblastoma (458).

TEC metabolism has long remained unchartered territory. Several features point towards a hyperactive glycolytic metabolism of TECs: *1*) their overstimulated angiogenic behavior, *2*) the partial resemblances they bear to cancer cells at least in certain aspects (147, 458, 646), and *3*) the observation that they overexpress the glucose transporter GLUT1 (486). RNA sequencing analyses of TECs isolated from B16 melanoma-infested livers shows that TECs and NECs have distinct metabolic gene signatures (66), with the TEC metabolic gene signature indeed confirming a hyperglycolytic phenotype leading to increased glucose-dependent biomass production, which is further validated by in vitro studies involving [^{14}C]glucose and [^{13}C]glucose tracing techniques (66). EC-specific heterozygous deletion of the glycolytic activator PFKFB3 (PFKFB3$^{+/\Delta EC}$) in tumor-bearing mice reduces the hyperglycolysis in TECs, even though not more than by 15–20%. While primary tumor size is not affected, cancer cell intravasation and metastatic spread are substantially reduced. Strikingly, tumor vessels in PFKFB3$^{+/\Delta EC}$ mice are enlarged and better covered with pericytes, display better perfusion dynamics, and have increased VE-cadherin junctions than in controls, all hallmarks of a normalized vasculature. Indeed, morphologically, PFKFB3$^{+/\Delta EC}$ tumor vessels appear regular and orderly formed, in contrast to the typically chaotic appearance of tumor vasculature in control mice (66) **(FIGURE 13)**.

Mechanistically, glycolysis inhibition reduces the amount of ATP required for VE-cadherin endocytosis, leaving more VE-cadherin at the plasma membrane and thereby increasing endothelial barrier integrity (66). Furthermore, lowering glycolytic flux decreases intracellular lactate levels and attenuates NFκB signaling (66), thereby counteracting the lactate-dependent, pro-angiogenic signaling loop described above (557). This decrease in NFκB signaling lowers expression of cancer cell adhesion molecules in TECs, preventing cancer cells from transmigrating (66). Of note, glycolysis inhibition in pericytes renders these mural cells more quiescent and adhesive by upregulating N-cadherin levels, allowing them to better cover and support newly formed vasculature favoring tumor vessel normalization (66).

Importantly, tumor vascularization can also occur via non-angiogenic mechanisms, in which cancer cells use preexisting blood vessels, rather than inducing the growth of new ones. The process of vessel co-option, for it is less dependent on pro-angiogenic signaling, is associated with resistance to anti-angiogenic therapy in patients with colorectal liver metastasis (178, 293). Whether ECs of co-opted vessels change their metabolic phenotype to maintain functionality when associated with cancer cells will be an exciting avenue to pursue.

B. Pulmonary Arterial Hypertension

Pulmonary arterial hypertension (PAH) is a progressive and life-threatening pathology characterized by lung EC dysfunction and adverse vascular remodeling. Excessive angiogenesis in the form of excessive EC proliferation and suppressed apoptosis (141, 355), resulting in aberrant vascular remodeling and lumen-obliterative pulmonary vascular lesions (311), are now considered to underlie the pathophysiology of PAH, rather than only vasoconstriction (another PAH characteristic) as thought previously (521). Such aberrant lung endothelial phenotypes lead to increased pulmonary arterial pressure ($>$25 mmHg) at rest and heightened pulmonary vascular resistance (162, 364, 537), ultimately leading to right ventricular cardiac failure and premature death.

PAH is no longer considered a disease exclusively affecting pulmonary arteries, but involves several other (extrapulmonary) organs as well (reviewed in Refs. 426, 521). PAEC dysfunction, typified by excessive proliferation, decreased apoptosis, and an obliterative vascular repair program, is considered as an early event in the pathogenesis of PAH; however, at later stages of the disease many more vascular cell types, such as SMCs, pericytes, and (myo)fibroblasts, become involved as well (457, 521). One key feature of PAH-associated vascular cells studied is mitochondrial remodeling resulting in suppressed glucose oxidation and secondary upregulation of glycolysis (described further below) (426, 521). The latter is in line with the increased proliferative phenotype, considering that under physiological conditions, glycolysis stimulates angiogenesis (114). Finally, the infiltration and presence of activated inflammatory and immune circulating cells indicate that inflammation may also play a significant role in PAH's pathogenesis (537). It becomes clear that the pathogenesis of PAH involves multiple pathways rather than one single mechanism. Despite

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

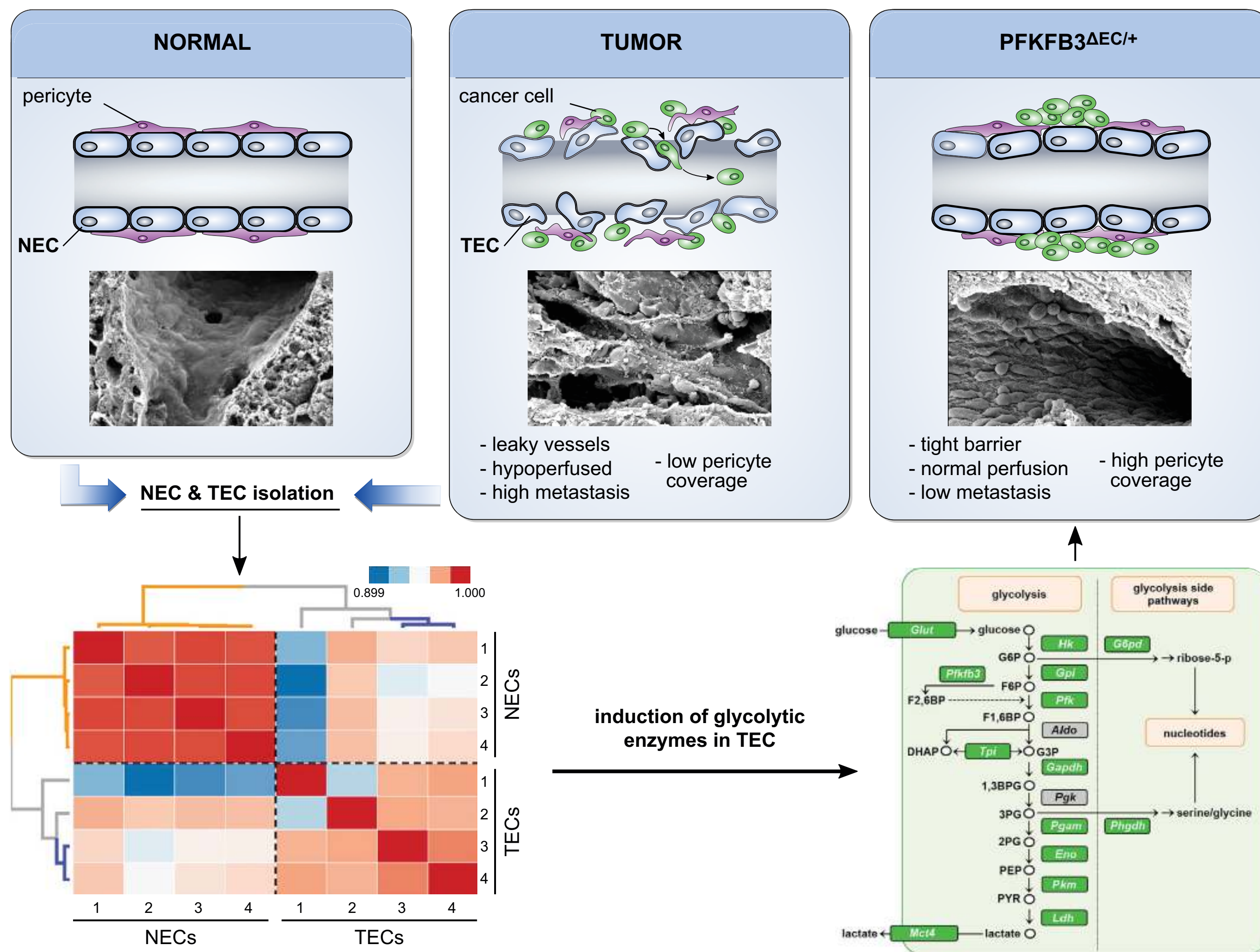


FIGURE 13. Reduction of hyperglycolysis in tumor ECs normalizes tumor vessels. Normal, mature blood vessels (*top left*) have a highly organized endothelial lining to ensure optimal blood flow and barrier function and are stabilized through pericyte coverage. In contrast, tumor vessels (*top middle*) are highly abnormal and tortuous and have a severely disorganized endothelium and reduced pericyte coverage. As a result, blood flow through these vessels is reduced, leading to a hypoperfused and consequently more aggressive, metastasis-prone tumor. In addition, tumor vessels are leaky which grants cancer cells easy access to the systemic circulation to establish distant metastases. Transcriptomic analysis of ECs isolated from normal vessels (normal ECs or NECs) versus ECs from tumor vessels (tumor ECs or TECs) and subsequent correlation heatmap analysis and hierarchical clustering of 1,255 metabolic genes (*bottom left*, color coding: high degree of correlation in red; lower degree of correlation in blue; numbers at the bottom and on the right indicate individual samples) shows differential metabolic wiring between NECs and TECs. Further analysis revealed that specifically glycolysis is induced in TECs and has the highest percentage of upregulated enzymes of all central metabolism pathways analyzed (*bottom right*, green coding indicates increased expression; gray coding indicates unaltered expression levels). Reverting hyperglycolysis in TECs in endothelial PFKFB3 haplodeficient mice leads to tumor vessel normalization featuring restored endothelial barrier function, increased pericyte coverage, and normalized blood flow which sustains tumor perfusion and renders the tumor more benign and less metastasis-prone (*top right*). [Correlation heatmap (*bottom left*) and pathway analysis (*bottom right*) as well as scanning electron microscopy images in the *top panels* are adapted from Cantelmo et al. (66), with permission from Elsevier.]

the role of PAECs early on in the pathogenesis of PAH, SMCs have been most extensively investigated, leaving questions on how metabolic aberrations specifically affect PAECs in PAH largely unanswered.

eNOS is the key regulator of basal vessel tone in the lungs (74, 159). As mentioned above in the sections on diabetes and atherosclerosis, aberrant production of eNOS-derived NO is a hallmark of EC dysfunction present in several vasculopathies and results in overall oxidative stress and vasoconstriction. Oxidative stress also plays a central role in vascular remodeling in PAH (53, 354, 578). Total body and pulmonary bioavailability of NO is reduced in PAH patients (87, 163, 200, 337, 606), while levels of the potent

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

vasoconstrictor ET-1 are increased (197, 444, 463). Previously, contradictory reports on eNOS expression levels in lungs of PAH patients made it unclear as to how eNOS-derived NO concentrations were lower in PAH patients than healthy controls (196, 353, 606). However, it was recently reported that this reduction in NO is due to increased threonine (T495) phosphorylation of eNOS via PKC with inhibitory functional consequences for eNOS, rather than decreased eNOS expression levels (194, 520). ET-1 is responsible for eNOS uncoupling by PKC-mediated phosphorylation at T495 and translocation of eNOS from the plasma membrane to mitochondria, where the eNOS-derived superoxide and $ONOO^-$ reduce respiratory capacity and as such disrupt mitochondrial bioenergetics in PAECs. Furthermore, the mitochondrial ROS induces HIF-1 activity and the subsequent switch to glycolysis (520). β-Blockers, pharmacologically inhibiting PKC activity, have been shown to restore NO bioavailability in PAH-associated pulmonary ECs (194). Interestingly, administration of eNOS-transduced endothelial progenitor cells to rats with established PAH resulted in regeneration of pulmonary endothelium and markedly improved their survival (640).

Decreased levels of arginine and BH_4 may be another cause of diminished eNOS-derived NO bioavailability in this disease. While there are sufficient levels of the eNOS cofactor BH_4 (194), others previously reported higher arg II expression and lower NO production in pulmonary ECs derived from PAH patients, indicating aberrant arginine metabolism (606). Similar results were reported in vitro in pulmonary ECs under hypoxic conditions (as a model for PAH), implicating hypoxia as well in the pathophysiology of PAH and vascular remodeling (69, 290). Even though arg II inhibitors are commercially available, their use should be addressed with caution as PAECs have elevated protein levels of arg II upon its pharmacological inhibition, partially due to increased protein half-life (289).

Increased EC mitophagy is a critical initiating event in PAH pathogenesis and results in EC loss due to inadequate biosynthesis of new mitochondria (225). This is in line with reports on early PAH-associated EC apoptosis and subsequent selection for ECs with excessive proliferative and apoptosis-resistant phenotypes (474). A decrease in EC mitochondrial biogenesis [also characterized by decreased PGC1α expression (225)] and subsequent reduction in the number of fully functional endothelial mitochondria may provide an explanation for the PAH-associated metabolic phenotype, i.e., a shift from oxidative phosphorylation (glucose oxidation) to glycolysis. Furthermore, PAH-associated ECs display increased expression and activity of HIF-1α, caused by oxidative stress (via decreased mitochondrial MnSOD expression) and lower levels of NO, ultimately downregulating the number of mitochondria (170, 607). The increased HIF-1α levels, even under normoxic conditions, induce transcription of hypoxia-responsive genes (46, 344) and thereby contribute to the metabolic shift from oxidative to glycolytic metabolism. Transcriptomic and metabolomic analysis of PMVECs from PAH patients confirmed increased gene expression of key glycolytic enzymes and GLUT1 and global activation of the glycolytic pathway (169).

However, increased glycolysis alone seems insufficient to sustain the total metabolic needs of a hyperproliferative phenotype. Indeed, the hyperproliferative phenotype of PAH-associated ECs has also been attributed to upregulation of the PPP, nucleotide salvage pathway, and the mitogenic polyamine biosynthesis pathway (169, 381). Moreover, stiffening of the pulmonary vascular extracellular matrix in PAH induces upregulation of glutaminase 1 (GLS1), pyruvate carboxylase (PC), and lactate dehydrogenase A (LDH-A) metabolic enzymatic activity, in part to promote glutamine metabolism in addition to glycolysis to meet the metabolic demands of increased proliferation and vascular remodeling (39). Finally, PAH-associated pulmonary ECs also display decreases in carnitine and FAO pathways, further highlighting their reduced oxidative metabolic nature (169). Noteworthy, PAH has recently been characterized further by intracellular lipid accumulation (upregulation of FA transporter molecule CD36) and reduced FA consumption in myocardium of PAH patients, due to reduced FAO, resulting in right ventricular lipotoxicity and ultimately right cardiac failure and death (55, 525, 526). Whether similar lipotoxicity also occurs in ECs specifically has not been reported yet.

C. Ocular Neovascularization

Ocular neovascularization is a feature of many eye diseases with diverse etiologies and is characterized by excess angiogenesis in one of the ocular tissues (retina, cornea, optic disk, and iris). Much like tumor vessels (see above in this section), the neovessels are structurally abnormal and leaky with blinding hemorrhages and fibrosis as a final outcome (307). In proliferative diabetic retinopathy (PDR) for example, excess angiogenesis occurs in response to hypoxic lesions that result from EC dysfunction and lack of efficient blood supply (449, 561). The resulting retinal neovessels are nonfunctional, lack pericyte coverage, and display increased vessel leakiness and as such aggravate the vascular undersupply of the retina and affect vision due to frequent vitreous hemorrhaging (451). The metabolic maladaptations in diabetic dysfunctional ECs have been discussed above and should further be explored as therapeutic targets, given that PDR accounts for ~5% of blindness cases worldwide.

Retinopathy of prematurity (ROP) is a blinding disorder that affects premature infants and involves excess oxygen-induced retinal neovascularization. In the mouse ROP

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

model, mice are exposed to increased oxygen tension, for a limited amount of time during which the retinal vasculature is still developing (P7–P12), causing retinal vaso-obliteration. Upon exposure to room air, the retina of the mice then becomes relatively hypoxic, leading to activation of angiogenesis and pathological vascular tuft formation (485). PFKFB3 blockade by 3PO in ROP mice at P12–17 attenuates vascular tuft formation (483). Likewise, treatment of ROP pups with etomoxir to inhibit FAO reduces vascular tuft formation (481) **(FIGURE 14)**.

The novel concept of targeting EC metabolism has also been applied in other preclinical models for pathological ocular neovascularization. Mouse choroidal neovascularization (CNV) is a preclinical model of wet age-related macular degeneration (AMD) and can be induced in mice by laser injury of the Bruch's membrane. CNV in wet AMD is initiated by hypoxia and local inflammation and can be inhibited by targeting the angiogenic growth factors VEGF and PlGF (554). Experimental evidence shows that glycolytic flux is a determining factor in CNV in mice, since treatment of mice with 3PO reduces CNV lesions (483) and even amplifies the efficiency of VEGFR2 inhibition (483). Together, these findings illustrate the importance of EC metabolism in different aspects of ocular diseases characterized by excessive neovessel formation.

IX. METABOLIC MEMORY OF ENDOTHELIAL CELLS: ENDOTHELIAL CELL METABOLISM AND EPIGENETICS

A peculiar trait of EC metabolism is the presumed existence of a so-called "metabolic memory." In the context of diabetes, both biological and clinical data suggest that hyperglycemia-induced oxidative stress exerts long-lasting detrimental effects on vascular function, even after restoration of glycemic levels. In addition to the role of additional diabetes-related factors, a metabolic/hyperglycemic memory [with hyperglycemia driving stable molecular modifications, via (aberrant) epigenetic mechanisms, and perpetuating EC dysfunction despite normalization of glucose levels] may explain the poor reversibility of hyperglycemia-induced metabolic modifications (75, 98, 99, 470). Accumulating evidence supports such hypothesis that hyperglycemic stress (constant or transient), driven by a self-sustaining cycle of oxidative stress, has persistent effects on the vasculature (reviewed in Refs. 245, 246, 271, 419).

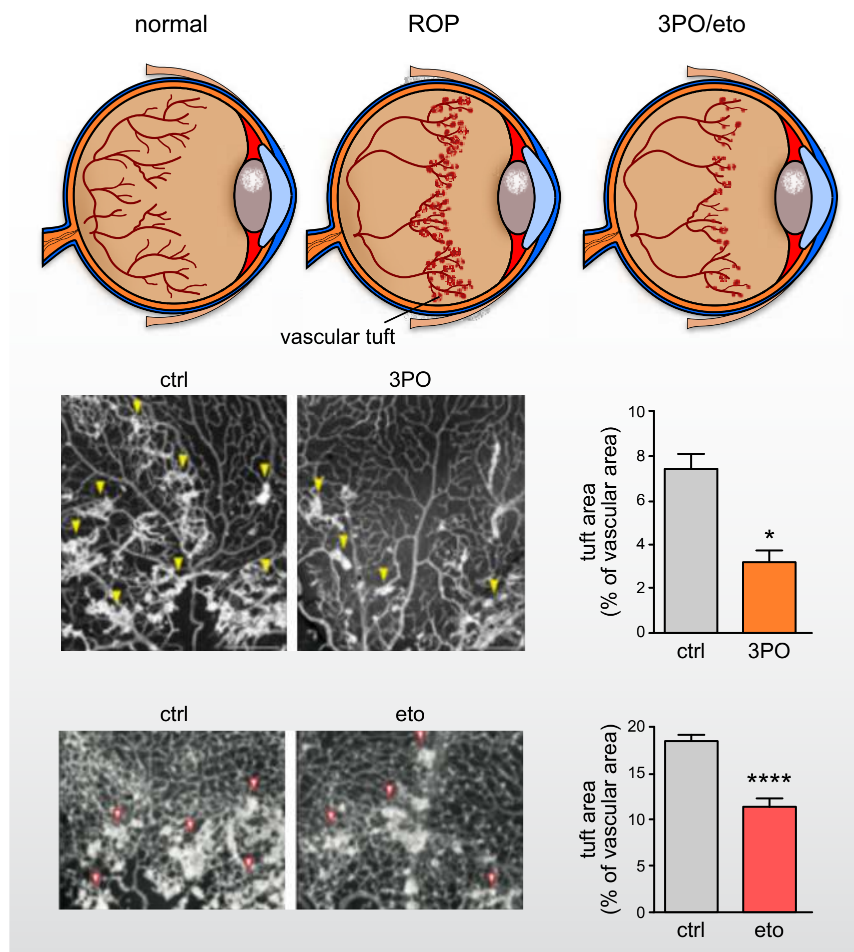


FIGURE 14. Blocking glycolysis or FAO mitigates pathological ocular neovascularization. In a mouse model for retinopathy of prematurity (ROP), treatment of the mice with 70 mg/kg of the PFKFB3 blocker 3PO significantly reduces the formation of pathological vascular tufts (yellow arrowheads). In the same model, blocking FAO with the CPT1a blocker etomoxir also attenuates tuft formation. The retinal microvasculature is stained with isolectin B4. Pictures and corresponding quantifications of vascular tuft area are from Schoors et al. (481), with permission from Macmillan Publishers Ltd., and Schoors et al. (483), with permission from Elsevier. The drawings at the top of this figure provide schematic overviews to recapitulate these findings. 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one; eto, etomoxir.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

Mitochondrial DNA damage, for example, persists despite restoration of normoglycemia (338). Moreover, hyperglycemia-induced oxidative stress promotes a pro-inflammatory memory in ECs, as elevated levels of PKC-activated NOX subunit p47phox persist upon lowering glucose levels (246). The mitochondrial adaptor protein $p66^{Shc}$, a key redox enzyme, plays a pivotal role in this oxidative stress-dependent metabolic and vascular memory. Its hyperglycemia-induced upregulation persists after glucose normalization and is associated with mitochondrial oxidative stress, reduced NO availability, and reduced ROS scavenging via MnSOD, NFκB-induced inflammation, and cellular death as well as insulin resistance in ECs (64, 78, 96, 415, 416, 418). The persistent upregulation of $p66^{Shc}$ in ECs also sustains increased methylglyoxal levels and eNOS inhibition, thereby promoting endothelial dysfunction (100, 418, 643) **(FIGURE 15)**. In line with this, deficiency of $p66^{shc}$ in mice (though not EC-specific) protects against hyperglycemia-induced endothelial dysfunction and high-fat diet-induced atherosclerosis (65, 637).

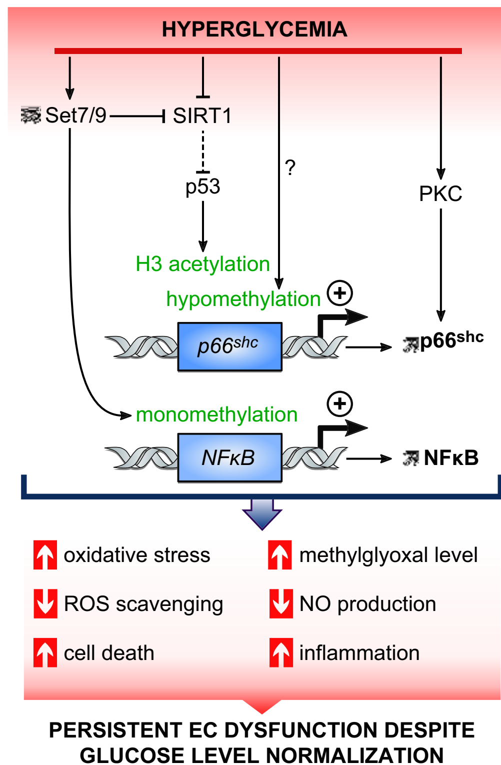


FIGURE 15. Molecular mechanisms underlying the hyperglycemic memory in ECs. Schematic and simplified scheme showing different (interconnected) molecular mechanisms and their contribution to a hyperglycemic memory in ECs. Hyperglycemic stimuli, even when followed by normal glucose restoration, act on different key mediators such as the methyltransferase Set7/9, the deacetylase SIRT1 and PKC which (epigenetically) change the expression levels/activity of NFκB and the mitochondrial adaptor $p66^{Shc}$ to cause a persistent pro-inflammatory and increased oxidative stress state. More details on the intricate signaling leading to this hyperglycemic memory are provided in the main text. SIRT1, sirtuin 1; H3, histone 3; PKC, protein kinase C; NFκB, nuclear factor-kappa B; NO, nitric oxide; ROS, reactive oxygen species.

Hyperglycemic memory is closely linked to persistent epigenetic modification. As such, the sustained overexpression of $p66^{Shc}$ upon glycemic normalization in ECs is dependent on promoter CpG hypomethylation and histone 3 acetylation (418). Increased acetylation of histone 3 in ECs exposed to high glucose can be relieved by overexpression of deacetylase SIRT1, thereby inhibiting $p66^{Shc}$ expression and protecting against hyperglycemia-induced EC dysfunction in vitro and in vivo (419, 643). Overexpression of endothelial SIRT1 also restores hyperglycemia-perturbed activity and expression levels of AMPK and MnSOD, respectively (592, 642). In this context, it is also interesting to note that mice overexpressing SIRT1 in the endothelium show improved endothelial vasodilation and less atherosclerotic lesions on a high-fat diet (637, 643). Additional evidence reports that p53 may be a critical intermediate between SIRT1 and $p66^{Shc}$ (272, 328). p53 gets acetylated and transcriptionally active upon reduction of SIRT1 deacetylase activity and has $p66^{Shc}$ as one of its transcriptional targets (reviewed in Ref. 419). Overall, the SIRT1-p53-$p66^{Shc}$ pathway may be responsible for the sustained EC dysfunction after glucose normalization. Interestingly, the methyltransferase Set7/9 is activated by hyperglycemic signals and inhibits SIRT1 activity. Set7/9 further leads to histone methylation of over 8,000 gene promoters, including the NFκB promoter leading to its activation and therefore contributing to endothelial dysfunction (271, 417). Taken together, epigenetic modification events seem to be critical for sustained EC dysfunction after glucose normalization and influence long-term complications of metabolic disease **(FIGURE 15)**.

A body of evidence suggests that oxidative stress in the diabetic and atherosclerotic vasculature influences epigenetic modifications (150, 400, 413, 433, 524, 573), impacting expression of key pro-inflammatory and pro-atherosclerotic targets (VCAM-1, E-selectin, ICAM-1), and resulting in the self-maintenance of a harmful metabolic memory and EC dysfunction (172, 455). Furthermore, hyperglycemia and the diabetic state trigger a metabolic memory via epigenetic modulations in SMCs and macrophages (reviewed in Ref. 271), key cell types involved in the pathogenesis of atherosclerosis. Further investigating the mechanisms underlying this metabolic memory, including mapping the epigenome of diabetic ECs, and looking for means to revert this to the normal state, will be key in minimizing long-term diabetic vascular complications.

Of note, the anti-diabetic drug metformin was recently put forward as a promising therapeutic drug against the development of a metabolic memory, independent of its glucose-lowering activity via the following mechanism. Indeed, short-term high glucose treatment causes persistent EC se-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

nescence (metabolic memory) by reducing SIRT1 deacetylase activity and inducing p300 acetyltransferase activity. Similarly to the findings described above, this causes p53 to become hyperacetylated and active, thereby driving p53/p21-mediated senescence. As a potent SIRT1 activator, metformin reduces the occurrence of this memory phenotype (634).

X. METABOLIC CROSSTALK

Intra- and intercellular communications provide important cues to ensure adequate tissue biogenesis and orchestrate physiological processes. Upon external stimuli (e.g., inflammation, hypoxia, tumorigenesis), these interactions may derail and become implicated in the etiology and evolution of several pathologies. One of the most fundamental ways of metabolite-based communication between ECs and other cell types in the body is by controlling endothelial uptake and transport of nutrients such as glucose and FAs. Additionally, surrounding mural or immune cells produce a wide variety of external stimuli (i.e., Dll4/Notch, VEGF/VEGFR2, FGF/FGFR; see sects. II and III) that can alter EC metabolism. Subsequent alterations in the levels of endothelial lactate production and excretion, for example, then impact the surrounding cells creating a metabolic crosstalk loop. Whether other metabolic intermediates of glycolysis, the TCA cycle, or FA metabolism participate in an intercellular crosstalk remains largely to be elucidated.

With glycolysis being the primary metabolic pathway for energy production in ECs (114), and lactate being the major end product of glycolysis, ECs excrete large amounts of lactate (423). Physiological lactate concentrations range from 1.5 to 3 mM in blood and tissues at baseline conditions (508) and can rise to >10 mM in disease settings such as atherosclerosis, rheumatoid arthritis, and cancer (90, 214, 235). Endothelial production of lactate and extracellular export can act as a vasoactive signal for pericytes. This interaction appears to be dynamically regulated based on the cellular energy status: when energy supplies are low, lactate causes vasodilation, whereas when energy supplies are abundant, lactate acts as a vasoconstrictor (616). In addition, lactate has diverse functions in a variety of cell types, including direct regulation of global gene transcription in skeletal muscle and neurons (224, 622) and polarization of immune cells (90, 214). Lactate has further been shown to inhibit glycolytic activity in T-cells, possibly through downregulation of HK1 or direct inhibition of PFK, and to inhibit cytotoxic T-cell responses and cause chronic inflammation through enhanced IL-17A secretion, which activates Th17 cells (31). In cancer cells, lactate has various functions (235), including stimulating cell migration and progression (29, 47), promoting glutamine uptake and metabolism (428), and direct binding to n-MYC downstream-regulated gene 3 protein (NDRG3), a PHD2-binding protein, resulting in the stabilization and activation of Raf-ERK1/2 signaling, thereby promoting hypoxia responses in cancer cells and angiogenesis (305). The wide variety of responses to lactate in other cells types warrants further investigation and consideration; strategies targeting EC glycolysis may yield secondary benefit through limiting lactate production **(FIGURE 16)**.

Conversely, lactate produced by other cell types has been shown to have numerous effects on ECs. For example, lactate promotes an "angiogenic switch" in ECs, through the upregulation of VEGF and VEGFR2 (297), and promotes EC migration (32). Furthermore, lactate activates PI3K/Akt signaling in ECs via ligand-mediated activation of the tyrosine kinases Axl, Tie2, and VEGFR2, promoting angiogenesis (471) **(FIGURE 16)**. In the tumor context (see above in this section), lactate influx in ECs through MCT1 leads to stimulation of the NFκB/IL8 pathway in a ROS- and IκBα-dependent manner, promoting tumor angiogenesis (557). Targeting MCT1 in ECs rescues lactate-mediated HIF1α activation and reduces tumor angiogenesis (506). Further investigation on the efficacy of targeting EC lactate transporters in other disease settings will be of future interest.

Over the last decade, the study of extracellular vesicles (EVs) has received growing interest. This less conventional mode of communication involves a broad variety of cells and is particularly modulated by extracellular signals. EVs can contain a number of factors including metabolites, nucleic acids (mRNA, DNA, miRNA), and proteins (soluble and transmembrane), all of which can directly stimulate target cells (92). Cardiomyocytes and ECs, the two main cell types in the heart, directly influence each other, particularly upon glycemic changes. Indeed, under in vitro glucose starvation conditions, the EV content of cardiomyocytes is remodeled and stimulates angiogenesis and proliferation in recipient ECs (187). Starved cardiomyocyte-derived EVs enhance glucose transporter expression (GLUT1 and 4) along with the glycolytic activity in ECs (186). Interestingly, in response to these vesicles, ECs secrete pyruvate in their microenvironment, presumably to sustain cardiomyocyte function (187). Finally, given that the contents of EC-derived EVs may reflect cellular stress or disease state, it merits further attention whether these EVs could act as diagnostic markers in vascular disorders **(FIGURE 16)**.

XI. THERAPEUTIC OPPORTUNITIES AND FUTURE PERSPECTIVES

As evidenced above, ECs rewire their central metabolism when becoming dysfunctional and during pathological vessel overgrowth. This obvious connection between metabolic maladaptation in ECs and illness calls for the exploration of novel metabolism-centric treatment strategies. Growing interest in and deeper understanding of the most detailed features of EC metabolism will advance translation into therapeutics. As such, by targeting ECs in their very core, metabolism-centric therapies might offer novel thera-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

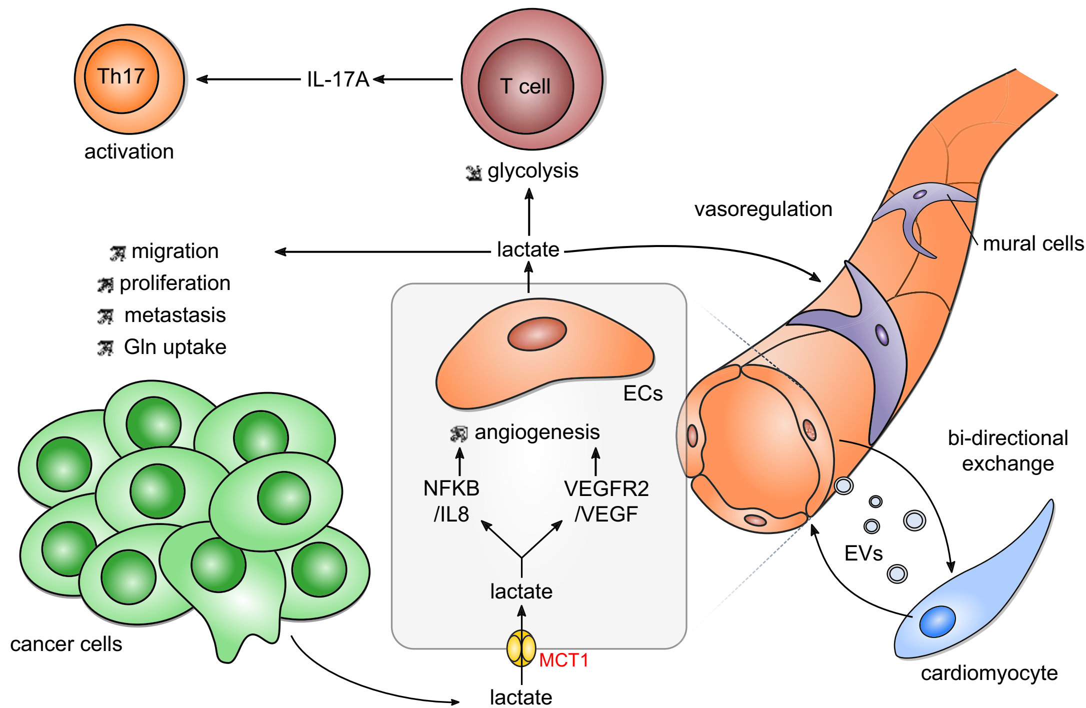


FIGURE 16. Two-way metabolic crosstalk between ECs and other cell types. Schematic and simplified view on possible metabolic crosstalk between ECs and other cell types. ECs use lactate, the end product of glycolysis, to signal to other cell types and thereby influence the immune compartment, cancer cell growth but also vasoconstriction/vasodilation (for details see main text). Conversely, lactate derived from other cell types, for example, cancer cells, can be taken up by ECs through the MCT1 transporter and drive (tumor-)angiogenesis by inducing VEGF/VEGFR2 or NFκB/IL8 signaling. Another way of crosstalk is through the exchange of EVs (containing metabolites, nucleic acids, and proteins) as exemplified here by the bidirectional exchange of EVs between ECs and cardiomyocytes (for more details, see main text). Gln, glutamine; EV, extracellular vesicles; IL8, interleukin 8; IL17A, interleukin 17A; MCT1, monocarboxylate transporter 1; NFκB, nuclear factor-kappa B; Th17, T helper 17 cell; VEGFR2, VEGF receptor 2.

peutic opportunities, as compared with current growth factor-centric anti-angiogenic approaches that suffer from acquired resistance and escape mechanisms (reviewed in Ref. 583). Also, unlike current anti-angiogenic therapies, which block a single target (such "targeted" strategies regularly fail in the clinic because of the induction of compensatory mechanisms), targeting EC metabolism is a more global approach, as angiogenic factors converge onto EC metabolism and ECs need energy, biomass, and redox control to form new vessel sprouts, and, as explained below in this section, it is possible to target key metabolic players without inciting compensatory feedback mechanisms.

Of utmost importance for future research is the quest for additional specific, perhaps even unique, metabolic features that distinguish ECs from other cell types. From a therapeutic point of view, such unique targets will be key to successful metabolism-centric anti-angiogenic strategies. As mentioned above (see sect. III), ECs are highly glycolytic and use FAO for a rather unique purpose (114, 481). Whether glutamine (or other amino acids) is used to serve a metabolic purpose that is unique for ECs, remains to be determined. Apart from unique traits, the search for synthetic lethality will most probably be in the limelight of future EC metabolism research. Inhibition of one particular metabolic target might cause ECs to re-route their metabolism and to overcome the initial blockade. Identification and subsequent targeting of the compensatory metabolic pathway creates opportunities for more efficient therapeutic strategies. Forward and unbiased metabolomics approaches (see further below in this section) should facilitate identification of such synthetically lethal metabolic rewiring.

Initial proof-of-principle for metabolism-centric treatment of pathological angiogenesis is provided by (pharmacological) inhibition of PFKFB3 (482, 483, 608). Revoking the dogmatic view that successful antimetabolic therapies require complete and permanent glycolysis inhibition proved crucial in this matter. Indeed, the PFKFB3 inhibitor tool compound 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) causes EC glycolysis only to drop transiently and by no more than 40%. In vivo, this matches the surplus in glycolysis needed for quiescent ECs to start forming new sprouts and apparently suffices to mitigate pathological angiogenesis (482, 483). ECs, especially tumor ECs, are so sensitive to partial glycolysis inhibition (because they are so glycolysis addicted and critically rely on this metabolic pathway, more than other cell types), which thereby also offers greater selectivity to paralyze ECs without neces-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

sarily inducing toxic effects in other cell types. Complete glycolysis inhibition on the other hand affects healthy quiescent vessels as well as other cell types, relying on glycolysis too. In contrast to ECs, which cannot adapt to (deep) glycolysis blockade, other cell types can switch to other (oxidative) metabolic pathways to survive (482). More complete and sustained inhibition of glycolysis with 2DG indeed causes toxicity in ECs in vitro (483) and cancer patients under 2DG treatment present adverse effects and the disease still progresses (206, 442). The advantages 3PO brings over 2DG relate to dosing and mode of action and have been discussed previously (206, 483). PFK158, a novel, more potent derivative of 3PO (535), successfully made it through a phase 1 multicenter clinical trial in patients with solid malignancies (http://www.advancedcancertherapeutics.com/clinical-trials.html). Whether it can be used as a novel anti-angiogenic drug will need further investigation. Additional screens for novel PFKFB3 inhibitors have been undertaken (54), yet await further testing in (pre)clinical settings.

Other novel strategies can come from unexpected sources. Propranolol, a nonselective β-adrenergic receptor-blocking agent, is successfully being used to treat infantile hemangiomas (IH, most common vascular tumor in children) (304). A possible mechanism by which propranolol stops the growth and induces involution of IHs is the upregulation of PTEN and subsequent inhibition of AKT activity, as shown in the hemangioma-derived endothelial cell line XPTS-1 (316). Given that Akt activity affects glycolysis (and other metabolic pathways) (462), the potential impact of propranolol on EC metabolism could be exploited for therapeutic options in vascular disorders (with a hyperglycolysis component).

As discussed above, tumor vessels are abnormal and their ECs display a hyperglycolytic metabolism, a reduction of which through partial PFKFB3 inactivation normalized the affected vasculature, reduced metastasis, and improved chemotherapy, even of a standard dose (66). Overall, precisely because ECs are so glycolysis addicted and tumor ECs even more so, they also become more sensitive to glycolysis inhibition, even by very low amounts. In fact, available preclinical evidence suggests that the "less is more" principle might well be a key paradigm-shifting objective in treating pathological angiogenesis with perturbed vascular cell metabolism. In a way, the hyperglycolysis in TECs bears resemblance to an overheated car engine; it suffices to cool the overheated engine down to normal levels to prevent activated ECs to form excess vessels and restore normalized vessel structure and function. In addition, precisely because 3PO blocks an "activator of an activator" of glycolysis and lowers glycolysis by no more than 35% in vivo, this treatment regimen does not evoke metabolic compensatory mechanisms (114, 482, 483), although further clinical evaluation will be necessary. Since vascular pericytes are also highly glycolytic, like ECs, a low dose of 3PO rendered these mural cells also more quiescent and increased their adhesiveness to ECs (by upregulating N-CAM adhesion molecule), thereby promoting pericyte coverage and tumor vessel normalization (66). Lastly, targeting endothelial PFKFB3 with 3PO may possibly be a valuable strategy in treating atherosclerosis too, as the resulting dampened glycolytic flux could maintain EC quiescence even under turbulent shear stress conditions, similar to atheroprotective KLF2-induced repression of PFKFB3 under uniform laminar shear stress conditions (126).

In vascular disorders with an endothelial hyperglycolysis component, metabolic inhibitors might offer an additional advantage in terms of routes of administration. Wet age-related macular degeneration (AMD), a leading cause of blindness in the elderly, is treated with anti-VEGF therapy administered by intraocular injection. This leaves the patient with local side effects and personal discomfort originating from the (repeated) needle injections in the eye, in addition to resistance in a formidable fraction of treated patients. In a preclinical mouse model for AMD, systemic delivery of the glycolytic inhibitor 3PO reduces choroidal neovascularization without any need for intraocular injections (483). Along the same lines, systemic treatment of mice with the FAO inhibitor etomoxir reduced pathological vascular tuft formation in an ROP model (see sect. VIII) and inhibited lymphatic neovessels from vascularizing the cornea after corneal cauterization (591), an established model for blinding corneal neovascularization (591). In line with the above discussed newly uncovered role for FAO-derived acetyl-CoA in histone acetylation in LECs and how this drives lymphangiogenesis, supplying the mice with acetate to restore acetyl-CoA fully abolished the inhibitory effects of etomoxir on lymphatic neovascularization (591). These in vivo findings may offer novel therapeutic opportunities towards blocking lymphangiogenesis in a cancer setting or conversely towards restoring lymphangiogenesis in lymphedema, for which currently no efficient therapies are available.

We have illustrated that metabolic adaptations resulting in EC dysfunction in both diabetes and atherosclerosis are mainly caused by oxidative stress. Despite the promising findings from studies targeting metabolic regulators [i.e., AMPK (72), PARP1 antagonists (133)] to decrease this oxidative stress, outcomes of clinical trials using antioxidant therapies have been rather disappointing (309). Also, dietary BH_4 and arginine supplementation to avert eNOS uncoupling showed no benefits in patients (106, 587). As such, a "prophylaxis rather than cure" approach or combination therapy may be required. A promising metabolic target is aldose reductase (enzyme converting excess glucose into sorbitol in the polyol pathway), which has several inhibitors currently undergoing clinical trials for diabetic vascular complications (208).

Even though specific targeting of the endothelium for therapeutic purposes remains challenging, some new strategies are arising. For instance, polymeric nanoparticles consisting of

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

low-molecular-weight polyamines and lipids deliver siRNAs to lung ECs without reducing target gene expression in hepatocytes or pulmonary/peritoneal immune cells (109). Taking advantage of diseased EC-specific gene expression to guide drug carriers towards the desired site of delivery might possibly become a promising approach, which, on top, would require lower drug concentrations. As such, nanoparticles equipped to recognize and bind EC-expressed inflammation markers might be a tool for delivering metabolic drugs specifically to the atherosclerotic region (reviewed in Ref. 329). Another example involves the higher expression of the apelin receptor APJ in tumor versus healthy ECs; apelin-conjugated liposomes were efficiently taken up by tumor ECs in tumor-bearing mice (269). Likewise, integrin $\alpha\nu\beta3$-targeted nanoparticles carrying cytotoxic doxorubicin accumulated at and induced apoptosis at integrin $\alpha\nu\beta3$ expressing tumor vessels (386). In case these approaches become better validated and withstand scrutiny of clinical trials, loading the nanocarriers with metabolic inhibitors could become a means to specifically target derailed EC metabolism in disease.

Finally, the near future will bring a logic yet necessary change to the EC metabolism field in the way new metabolic targets will be identified. Indeed, genotype-to-phenotype studies, characterizing the angiogenic role of a preselected metabolic enzyme (reverse approach), have provided seminal insights into EC metabolism. Given that the metabolome represents the final product of the (epi)genome, and complements transcriptomics and proteomics (18, 140), increasingly more studies engage in unbiased and untargeted omics approaches (forward approach), using ECs from healthy and diseased tissues, also of patients (18, 205, 570, 648). The integration of such omics data, for example through in silico genome-scale metabolic modeling (GEM), enables a forward, data-driven, and all-encompassing omics approach to identify new metabolic targets as possible disease-drivers or -modifiers (49, 274, 629). In contrast to the cancer metabolism field, the EC metabolism field is in its early infancy and has yet to apply such forward approaches (189, 366). With the current advances in isolation and culturing techniques of ECs from different tissues and/or disease states (e.g., TEC vs. NEC, diabetes, atherosclerosis, and arterial hypertension; see sects. VII and VIII), the stage is all set to explore and exploit forward omics approaches (66). The advent and general applicability of techniques to study in vivo EC metabolism, for example by in vivo metabolic tracing and metabolic imaging (112, 226, 388), will greatly deepen our current understandings, especially given the substantial influence of the microenvironment on cellular metabolism (67, 112).

XII. CONCLUDING SUMMARY

Here, we comprehensively reviewed the existing literature on EC metabolism, both the more basic aspects by listing and describing the different metabolic pathways and its determinative role in developmental and pathological angiogenesis. Indeed, EC metabolism stretches far beyond the dogmatic view of simply fueling cell growth and activity. Several metabolic pathways act in parallel with genetic/growth factor signaling to sustain appropriate EC behavior. This concept substantially gains importance when derailed metabolism renders ECs dysfunctional and causes some of the most life-threatening disorders known to humans.

With seminal findings on endothelial glycolysis and FAO being translated towards possible targets in these disorders, other metabolic pathways still require further insight into their exact roles in angiogenesis. Does glutamine metabolism hold the key to further unlock EC metabolism-centric anti-angiogenic strategies? It is undoubtedly a highly attractive target both because of already gathered knowledge on its role in ECs (see sect. III) and the recent development of inhibitors with high specificity for rate-limiting enzymes in glutamine metabolism (361). What is the role of other AAs (like serine, asparagine, aspartate,...), the metabolism of which is hardly being studied in ECs, let alone in vessel sprouting?

Emerging evidence suggests that diseases affect EC subtypes in a vascular bed-specific pattern. For instance, diabetes causes EC dysfunction in skin, peripheral nerves, heart, and kidney but stimulates EC growth in the retina (see sects. VII and VIII). Yet, current anti-angiogenic therapies do not discriminate between different EC types in different vascular beds. This then raises the question if future anti-angiogenic therapy should be "tailored" to EC subtype and vascular bed. The observations that EC heterogeneity is indeed paralleled by differences in metabolism (see sect. VI) create both the opportunity and the formidable challenge to accomplish such "tailored" strategies.

Developing further knowledge on the most intricate aspects of EC metabolism such as metabolic compartmentalization, metabolic cross talk with other cell types, and EC subtype-specific metabolic traits is another future challenge in this young field and a prerequisite to the development of novel therapeutic strategies.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: P. Carmeliet, Laboratory of Angiogenesis & Vascular Metabolism, Center for Cancer Biology, Dept. of Oncology, KU Leuven, Campus Gasthuisberg O&N4, Herestraat 49–912, B-3000 Leuven, Belgium (e-mail: peter.carmeliet@kuleuven.vib.be).

GRANTS

P. Carmeliet is funded by The Research Foundation-Flanders (FWO), Belgian Science Policy Grant IUAP P7/03, long-term structural Methusalem funding from the Flemish Government, European Research Council Advanced Research Grant EU-ERC269073, and The AXA Research Fund. P. de Zeeuw and U. Harjes are funded by FWO. L.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

Treps is supported by the Bettencourt Schueller Foundation and a Marie Curie Postdoctoral Fellowship.

DISCLOSURES

P. Carmeliet declares to be named as inventor on patent applications claiming subject matter related to findings reviewed in this manuscript. The other authors report no conflict of interest financial or otherwise.

REFERENCES

1. Action to Control Cardiovascular Risk in Diabetes Study Group; Gerstein HC, Miller ME, Byington RP, Goff DC, Jr, Bigger JT, Buse JB, Cushman WC, Genuth S, Ismail-Beigi F, Grimm RH, Jr, Probstfield JL, Simons-Morton DG, Friedewald WT. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med* 358: 2545–2559, 2008. doi:10.1056/NEJMoa0802743.

2. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol* 8: 464–478, 2007. doi:10.1038/nrm2183.

3. Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C. Liver glucose metabolism in humans. *Biosci Rep* 36: e00416, 2016. doi:10.1042/BSR20160385.

4. ADVANCE Collaborative Group; Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, Marre M, Cooper M, Glasziou P, Grobbee D, Hamet P, Harrap S, Heller S, Liu L, Mancia G, Mogensen CE, Pan C, Poulter N, Rodgers A, Williams B, Bompoint S, de Galan BE, Joshi R, Travert F. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 358: 2560–2572, 2008. doi:10.1056/NEJMoa0802987.

5. Agathocleous M, Harris WA. Metabolism in physiological cell proliferation and differentiation. *Trends Cell Biol* 23: 484–492, 2013. doi:10.1016/j.tcb.2013.05.004.

6. Akino T, Hida K, Hida Y, Tsuchiya K, Freedman D, Muraki C, Ohga N, Matsuda K, Akiyama K, Harabayashi T, Shinohara N, Nonomura K, Klagsbrun M, Shindoh M. Cytogenetic abnormalities of tumor-associated endothelial cells in human malignant tumors. *Am J Pathol* 175: 2657–2667, 2009. doi:10.2353/ajpath.2009.090202.

7. Akiyama K, Maishi N, Ohga N, Hida Y, Ohba Y, Alam MT, Kawamoto T, Ohmura H, Yamada K, Torii C, Shindoh M, Hida K. Inhibition of multidrug transporter in tumor endothelial cells enhances antiangiogenic effects of low-dose metronomic paclitaxel. *Am J Pathol* 185: 572–580, 2015. doi:10.1016/j.ajpath.2014.10.017.

9. Akiyama K, Ohga N, Hida Y, Kawamoto T, Sadamoto Y, Ishikawa S, Maishi N, Akino T, Kondoh M, Matsuda A, Inoue N, Shindoh M, Hida K. Tumor endothelial cells acquire drug resistance by MDR1 up-regulation via VEGF signaling in tumor microenvironment. *Am J Pathol* 180: 1283–1293, 2012. doi:10.1016/j.ajpath.2011.11.029.

10. Al-Delaimy WK, Merchant AT, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Effect of type 2 diabetes and its duration on the risk of peripheral arterial disease among men. *Am J Med* 116: 236–240, 2004. doi:10.1016/j.amjmed.2003.09.038.

11. Al-Habori M. Microcompartmentation, metabolic channelling and carbohydrate metabolism. *Int J Biochem Cell Biol* 27: 123–132, 1995. doi:10.1016/1357-2725(94)00079-Q.

12. Alitalo K. The lymphatic vasculature in disease. *Nat Med* 17: 1371–1380, 2011. doi:10.1038/nm.2545.

13. Altaany Z, Ju Y, Yang G, Wang R. The coordination of *S*-sulfhydration, *S*-nitrosylation, and phosphorylation of endothelial nitric oxide synthase by hydrogen sulfide. *Sci Signal* 7: ra87, 2014. doi:10.1126/scisignal.2005478.

14. Amin DN, Hida K, Bielenberg DR, Klagsbrun M. Tumor endothelial cells express epidermal growth factor receptor (EGFR) but not ErbB3 and are responsive to EGF and to EGFR kinase inhibitors. *Cancer Res* 66: 2173–2180, 2006. doi:10.1158/0008-5472.CAN-05-3387.

15. Aranguren XL, Agirre X, Beerens M, Coppiello G, Uriz M, Vandersmissen I, Benkheil M, Panadero J, Aguado N, Pascual-Montano A, Segura V, Prósper F, Luttun A. Unraveling a novel transcription factor code determining the human arterial-specific endothelial cell signature. *Blood* 122: 3982–3992, 2013. doi:10.1182/blood-2013-02-483255.

16. Arcaro G, Cretti A, Balzano S, Lechi A, Muggeo M, Bonora E, Bonadonna RC. Insulin causes endothelial dysfunction in humans: sites and mechanisms. *Circulation* 105: 576–582, 2002. doi:10.1161/hc0502.103333.

17. Arima S, Nishiyama K, Ko T, Arima Y, Hakozaki Y, Sugihara K, Koseki H, Uchijima Y, Kurihara Y, Kurihara H. Angiogenic morphogenesis driven by dynamic and heterogeneous collective endothelial cell movement. *Development* 138: 4763–4776, 2011. doi:10.1242/dev.068023.

18. Armitage EG, Barbas C. Metabolomics in cancer biomarker discovery: current trends and future perspectives. *J Pharm Biomed Anal* 87: 1–11, 2014. doi:10.1016/j.jpba.2013.08.041.

19. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev Cell* 21: 193–215, 2011. doi:10.1016/j.devcel.2011.07.001.

20. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964–967, 1997. doi:10.1126/science.275.5302.964.

21. Astanina K, Koch M, Jüngst C, Zumbusch A, Kiemer AK. Lipid droplets as a novel cargo of tunnelling nanotubes in endothelial cells. *Sci Rep* 5: 11453, 2015. doi:10.1038/srep11453.

22. Averna M, Stifanese R, De Tullio R, Passalacqua M, Salamino F, Pontremoli S, Melloni E. Functional role of HSP90 complexes with endothelial nitric-oxide synthase (eNOS) and calpain on nitric oxide generation in endothelial cells. *J Biol Chem* 283: 29069–29076, 2008. doi:10.1074/jbc.M803638200.

23. Babaei-Jadidi R, Karachalias N, Ahmed N, Battah S, Thornalley PJ. Prevention of incipient diabetic nephropathy by high-dose thiamine and benfotiamine. *Diabetes* 52: 2110–2120, 2003. doi:10.2337/diabetes.52.8.2110.

24. Ball LE, Berkaw MN, Buse MG. Identification of the major site of *O*-linked beta-*N*-acetylglucosamine modification in the C terminus of insulin receptor substrate-1. *Mol Cell Proteomics* 5: 313–323, 2006. doi:10.1074/mcp.M500314-MCP200.

25. Balligand JL, Feron O, Dessy C. eNOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. *Physiol Rev* 89: 481–534, 2009. doi:10.1152/physrev.00042.2007.

26. Banerjee PS, Lagerlöf O, Hart GW. Roles of *O*-GlcNAc in chronic diseases of aging. *Mol Aspects Med* 51: 1–15, 2016. doi:10.1016/j.mam.2016.05.005.

27. Battelli MG, Polito L, Bolognesi A. Xanthine oxidoreductase in atherosclerosis pathogenesis: not only oxidative stress. *Atherosclerosis* 237: 562–567, 2014. doi:10.1016/j.atherosclerosis.2014.10.006.

28. Baum C, Johannsen SS, Zeller T, Atzler D, Ojeda FM, Wild PS, Sinning CR, Lackner KJ, Gori T, Schwedhelm E, Böger RH, Blankenberg S, Münzel T, Schnabel RB; Gutenberg Health Study Investigators. ADMA and arginine derivatives in relation to non-invasive vascular function in the general population. *Atherosclerosis* 244: 149–156, 2016. doi:10.1016/j.atherosclerosis.2015.10.101.

29. Baumann F, Leukel P, Doerfelt A, Beier CP, Dettmer K, Oefner PJ, Kastenberger M, Kreutz M, Nickl-Jockschat T, Bogdahn U, Bosserhoff AK, Hau P. Lactate promotes glioma migration by TGF-beta2-dependent regulation of matrix metalloproteinase-2. *Neuro-oncol* 11: 368–380, 2009. doi:10.1215/15228517-2008-106.

30. Becker BF, Jacob M, Leipert S, Salmon AH, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. *Br J Clin Pharmacol* 80: 389–402, 2015. doi:10.1111/bcp.12629.

31. Becker JC, Andersen MH, Schrama D, Thor Straten P. Immune-suppressive properties of the tumor microenvironment. *Cancer Immunol Immunother* 62: 1137–1148, 2013. doi:10.1007/s00262-013-1434-6.

32. Beckert S, Hierlemann H, Müschenborn N, Witte M, Ranke M, Coerper S. Experimental ischemic wounds: correlation of cell proliferation and insulin-like growth factor I expression and its modification by different local IGF-I release systems. *Wound Repair Regen* 13: 278–283, 2005. doi:10.1111/j.1067-1927.2005.130310.x.

33. Beckman JA, Creager MA. Vascular Complications of Diabetes. *Circ Res* 118: 1771–1785, 2016. doi:10.1161/CIRCRESAHA.115.306884.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

34. Beckman JA, Paneni F, Cosentino F, Creager MA. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part II. *Eur Heart J* 34: 2444–2452, 2013. doi:10.1093/eurheartj/eht142.

35. Belaoussoff M, Farrington SM, Baron MH. Hematopoietic induction and respecification of A-P identity by visceral endoderm signaling in the mouse embryo. *Development* 125: 5009–5018, 1998.

36. Bendall JK, Douglas G, McNeill E, Channon KM, Crabtree MJ. Tetrahydrobiopterin in cardiovascular health and disease. *Antioxid Redox Signal* 20: 3040–3077, 2014. doi:10.1089/ars.2013.5566.

37. Benedito R, Roca C, Sörensen I, Adams S, Gossler A, Fruttiger M, Adams RH. The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis. *Cell* 137: 1124–1135, 2009. doi:10.1016/j.cell.2009.03.025.

38. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. *Nat Rev Cancer* 8: 592–603, 2008. doi:10.1038/nrc2442.

39. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, Zhao J, Tai Y, Tang Y, Zhang YY, Rehman S, Sugahara M, Qi Z, Gorcsan J III, Vargas SO, Saggar R, Saggar R, Wallace WD, Ross DJ, Haley KJ, Waxman AB, Parikh VN, De Marco T, Hsue PY, Morris A, Simon MA, Norris KA, Gaggioli C, Loscalzo J, Fessel J, Chan SY. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *J Clin Invest* 126: 3313–3335, 2016. doi:10.1172/JCI86387.

40. Betz B, Möller-Ehrlich K, Kress T, Kniepert J, Schwedhelm E, Böger RH, Wanner C, Sauvant C, Schneider R. Increased symmetrical dimethylarginine in ischemic acute kidney injury as a causative factor of renal L-arginine deficiency. *Transl Res* 162: 67–76, 2013. doi:10.1016/j.trsl.2013.04.005.

41. Betz C, Lenard A, Belting HG, Affolter M. Cell behaviors and dynamics during angiogenesis. *Development* 143: 2249–2260, 2016. doi:10.1242/dev.135616.

42. Bian F, Yang X, Zhou F, Wu PH, Xing S, Xu G, Li W, Chi J, Ouyang C, Zhang Y, Xiong B, Li Y, Zheng T, Wu D, Chen X, Jin S. C-reactive protein promotes atherosclerosis by increasing LDL transcytosis across endothelial cells. *Br J Pharmacol* 171: 2671–2684, 2014. doi:10.1111/bph.12616.

43. Boas SE, Merks RM. Tip cell overtaking occurs as a side effect of sprouting in computational models of angiogenesis. *BMC Syst Biol* 9: 86, 2015. doi:10.1186/s12918-015-0230-7.

44. Böger RH. Asymmetric dimethylarginine: understanding the physiology, genetics, and clinical relevance of this novel biomarker. Proceedings of the 4th International Symposium on ADMA. *Pharmacol Res* 60: 447, 2009. doi:10.1016/j.phrs.2009.10.001.

45. Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Böger SM. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of *S*-adenosylmethionine-dependent methyltransferases. *Circ Res* 87: 99–105, 2000. doi:10.1161/01.RES.87.2.99.

46. Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Thébaud B, Bonnet S, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir EK, Archer SL. An abnormal mitochondrial-hypoxia inducible factor-1alpha-Kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: similarities to human pulmonary arterial hypertension. *Circulation* 113: 2630–2641, 2006. doi:10.1161/CIRCULATIONAHA.105.609008.

47. Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, Frank PG, Flomenberg N, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Ketones and lactate "fuel" tumor growth and metastasis: evidence that epithelial cancer cells use oxidative mitochondrial metabolism. *Cell Cycle* 9: 3506–3514, 2010. doi:10.4161/cc.9.17.12731.

48. Boon RA, Leyen TA, Fontijn RD, Fledderus JO, Baggen JM, Volger OL, van Nieuw Amerongen GP, Horrevoets AJ. KLF2-induced actin shear fibers control both alignment to flow and JNK signaling in vascular endothelium. *Blood* 115: 2533–2542, 2010. doi:10.1182/blood-2009-06-228726.

49. Bordbar A, Palsson BO. Using the reconstructed genome-scale human metabolic network to study physiology and pathology. *J Intern Med* 271: 131–141, 2012. doi:10.1111/j.1365-2796.2011.02494.x.

50. Borniquel S, Valle I, Cadenas S, Lamas S, Monsalve M. Nitric oxide regulates mitochondrial oxidative stress protection via the transcriptional coactivator PGC-1alpha. *FASEB J* 20: 1889–1891, 2006. doi:10.1096/fj.05-5189fje.

51. Boucher JL, Custot J, Vadon S, Delaforge M, Lepoivre M, Tenu JP, Yapo A, Mansuy D. *N*- omega-hydroxyl-L-arginine, an intermediate in the L-arginine to nitric oxide pathway, is a strong inhibitor of liver and macrophage arginase. *Biochem Biophys Res Commun* 203: 1614–1621, 1994. doi:10.1006/bbrc.1994.2371.

52. Bourajjaj M, Stehouwer CD, van Hinsbergh VW, Schalkwijk CG. Role of methylglyoxal adducts in the development of vascular complications in diabetes mellitus. *Biochem Soc Trans* 31: 1400–1402, 2003. doi:10.1042/bst0311400.

53. Bowers R, Cool C, Murphy RC, Tuder RM, Hopken MW, Flores SC, Voelkel NF. Oxidative stress in severe pulmonary hypertension. *Am J Respir Crit Care Med* 169: 764–769, 2004. doi:10.1164/rccm.200301-147OC.

54. Boyd S, Brookfield JL, Critchlow SE, Cumming IA, Curtis NJ, Debreczeni J, Degorce SL, Donald C, Evans NJ, Groombridge S, Hopcroft P, Jones NP, Kettle JG, Lamont S, Lewis HJ, MacFaull P, McLoughlin SB, Rigoreau LJ, Smith JM, St-Gallay S, Stock JK, Turnbull AP, Wheatley ER, Winter J, Wingfield J. Structure-Based Design of Potent and Selective Inhibitors of the Metabolic Kinase PFKFB3. *J Med Chem* 58: 3611–3625, 2015. doi:10.1021/acs.jmedchem.5b00352.

55. Brittain EL, Talati M, Fessel JP, Zhu H, Penner N, Calcutt MW, West JD, Funke M, Lewis GD, Gerszten RE, Hamid R, Pugh ME, Austin ED, Newman JH, Hemnes AR. Fatty Acid Metabolic Defects and Right Ventricular Lipotoxicity in Human Pulmonary Arterial Hypertension. *Circulation* 133: 1936–1944, 2016. doi:10.1161/CIRCULATIONAHA.115.019351.

56. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett* 356: 295–298, 1994. doi:10.1016/0014-5793(94)01290-3.

57. Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303: 2011–2015, 2004. doi:10.1126/science.1094637.

58. Buchwald P. A local glucose- and oxygen concentration-based insulin secretion model for pancreatic islets. *Theor Biol Med Model* 8: 20, 2011. doi:10.1186/1742-4682-8-20.

59. Bukeirat M, Sarkar SN, Hu H, Quintana DD, Simpkins JW, Ren X. MiR-34a regulates blood-brain barrier permeability and mitochondrial function by targeting cytochrome c. *J Cereb Blood Flow Metab* 36: 387–392, 2016. doi:10.1177/0271678X15606147.

60. Bussolati B, Deambrosis I, Russo S, Deregibus MC, Camussi G. Altered angiogenesis and survival in human tumor-derived endothelial cells. *FASEB J* 17: 1159–1161, 2003.

61. Cade WT. Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Phys Ther* 88: 1322–1335, 2008. doi:10.2522/ptj.20080008.

62. Cahill CM, Rogers JT. Interleukin (IL) 1beta induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/IkappaB kinase alpha pathway targeting activator protein-1. *J Biol Chem* 283: 25900–25912, 2008. doi:10.1074/jbc.M707692200.

63. Cai S, Khoo J, Mussa S, Alp NJ, Channon KM. Endothelial nitric oxide synthase dysfunction in diabetic mice: importance of tetrahydrobiopterin in eNOS dimerisation. *Diabetologia* 48: 1933–1940, 2005. doi:10.1007/s00125-005-1857-5.

64. Cai W, He JC, Zhu L, Chen X, Striker GE, Vlassara H. AGE-receptor-1 counteracts cellular oxidant stress induced by AGEs via negative regulation of p66shc-dependent FKHRL1 phosphorylation. *Am J Physiol Cell Physiol* 294: C145–C152, 2008. doi:10.1152/ajpcell.00350.2007.

65. Camici GG, Schiavoni M, Francia P, Bachschmid M, Martin-Padura I, Hersberger M, Tanner FC, Pelicci P, Volpe M, Anversa P, Lüscher TF, Cosentino F. Genetic deletion of p66(Shc) adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. *Proc Natl Acad Sci USA* 104: 5217–5222, 2007. doi:10.1073/pnas.0609656104.

66. Cantelmo AR, Conradi LC, Brajic A, Goveia J, Kalucka J, Pircher A, Chaturvedi P, Hol J, Thienpont B, Teuwen LA, Schoors S, Boeckx B, Vriens J, Kuchnio A, Veys K, Cruys B, Finotto L, Treps L, Stav-Noraas TE, Bifari F, Stapor P, Decimo I, Kampen K, De Bock K, Haraldsen G, Schoonjans L, Rabelink T, Eelen G, Ghesquière B, Rehman J, Lambrechts D, Malik AB, Dewerchin M, Carmeliet P. Inhibition of the Glycolytic Activator PFKFB3 in Endothelium Induces Tumor Vessel Normalization, Impairs Metastasis, and Improves Chemotherapy. *Cancer Cell* 30: 968–985, 2016. doi:10.1016/j.ccell.2016.10.006.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

67. Cantor JR, Abu-Remaileh M, Kanarek N, Freinkman E, Gao X, Louissaint A, Jr, Lewis CA, Sabatini DM. Physiologic Medium Rewires Cellular Metabolism and Reveals Uric Acid as an Endogenous Inhibitor of UMP Synthase. *Cell* 169: 258–272.e17, 2017. doi:10.1016/j.cell.2017.03.023.

68. Carbone V, Ishikura S, Hara A, El-Kabbani O. Structure-based discovery of human L-xylulose reductase inhibitors from database screening and molecular docking. *Bioorg Med Chem* 13: 301–312, 2005. doi:10.1016/j.bmc.2004.10.030.

69. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 407: 249–257, 2000. doi:10.1038/35025220.

70. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. *Nature* 473: 298–307, 2011. doi:10.1038/nature10144.

71. Castellana M, Wilson MZ, Xu Y, Joshi P, Cristea IM, Rabinowitz JD, Gitai Z, Wingreen NS. Enzyme clustering accelerates processing of intermediates through metabolic channeling. *Nat Biotechnol* 32: 1011–1018, 2014. doi:10.1038/nbt.3018.

72. Ceolotto G, Gallo A, Papparella I, Franco L, Murphy E, Iori E, Pagnin E, Fadini GP, Albiero M, Semplicini A, Avogaro A. Rosiglitazone reduces glucose-induced oxidative stress mediated by NAD(P)H oxidase via AMPK-dependent mechanism. *Arterioscler Thromb Vasc Biol* 27: 2627–2633, 2007. doi:10.1161/ATVBAHA.107.155762.

73. Ceriello A, Ihnat MA, Thorpe JE. Clinical review 2: The "metabolic memory": is more than just tight glucose control necessary to prevent diabetic complications? *J Clin Endocrinol Metab* 94: 410–415, 2009. doi:10.1210/jc.2008-1824.

74. Champion HC, Bivalacqua TJ, Greenberg SS, Giles TD, Hyman AL, Kadowitz PJ. Adenoviral gene transfer of endothelial nitric-oxide synthase (eNOS) partially restores normal pulmonary arterial pressure in eNOS-deficient mice. *Proc Natl Acad Sci USA* 99: 13248–13253, 2002. doi:10.1073/pnas.182225899.

75. Chan PS, Kanwar M, Kowluru RA. Resistance of retinal inflammatory mediators to suppress after reinstitution of good glycemic control: novel mechanism for metabolic memory. *J Diabetes Complications* 24: 55–63, 2010. doi:10.1016/j.jdiacomp.2008.10.002.

76. Chaveroux C, Sarcinelli C, Barbet V, Belfeki S, Barthelaix A, Ferraro-Peyret C, Lebecque S, Renno T, Bruhat A, Fafournoux P, Manié SN. Nutrient shortage triggers the hexosamine biosynthetic pathway via the GCN2-ATF4 signalling pathway. *Sci Rep* 6: 27278, 2016. doi:10.1038/srep27278.

77. Chen F, Lucas R, Fulton D. The subcellular compartmentalization of arginine metabolizing enzymes and their role in endothelial dysfunction. *Front Immunol* 4: 184, 2013. doi:10.3389/fimmu.2013.00184.

78. Chen HZ, Wan YZ, Liu DP. Cross-talk between SIRT1 and p66Shc in vascular diseases. *Trends Cardiovasc Med* 23: 237–241, 2013. doi:10.1016/j.tcm.2013.01.001.

79. Chen L, Zhao J, Tang Q, Li H, Zhang C, Yu R, Zhao Y, Huo Y, Wu C. PFKFB3 Control of Cancer Growth by Responding to Circadian Clock Outputs. *Sci Rep* 6: 24324, 2016. doi:10.1038/srep24324.

80. Chen Y, Zhao S, Wang Y, Li Y, Bai L, Liu R, Fan J, Liu E. Homocysteine reduces protein *S*-nitrosylation in endothelium. *Int J Mol Med* 34: 1277–1285, 2014. doi:10.3892/ijmm.2014.1920.

81. Chen Z, Wen L, Martin M, Hsu CY, Fang L, Lin FM, Lin TY, Geary MJ, Geary GG, Zhao Y, Johnson DA, Chen JW, Lin SJ, Chien S, Huang HD, Miller YI, Huang PH, Shyy JY. Oxidative stress activates endothelial innate immunity via sterol regulatory element binding protein 2 (SREBP2) transactivation of microRNA-92a. *Circulation* 131: 805–814, 2015. doi:10.1161/CIRCULATIONAHA.114.013675.

82. Cheung AK, Fung MK, Lo AC, Lam TT, So KF, Chung SS, Chung SK. Aldose reductase deficiency prevents diabetes-induced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of *db/db* mice. *Diabetes* 54: 3119–3125, 2005. doi:10.2337/diabetes.54.11.3119.

83. Chew GT, Watts GF. Coenzyme Q10 and diabetic endotheliopathy: oxidative stress and the 'recoupling hypothesis'. *QJM* 97: 537–548, 2004. doi:10.1093/qjmed/hch089.

84. Chon J, Stover PJ, Field MS. Targeting nuclear thymidylate biosynthesis. *Mol Aspects Med* 53: 48–56, 2017. doi:10.1016/j.mam.2016.11.005.

85. Cirillo P, Pellegrino G, Conte S, Maresca F, Pacifico F, Leonardi A, Trimarco B. Fructose induces prothrombotic phenotype in human endothelial cells: a new role for "added sugar" in cardio-metabolic risk. *J Thromb Thrombolysis* 40: 444–451, 2015. doi:10.1007/s11239-015-1243-1.

86. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of *S*-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci USA* 95: 7631–7636, 1998. doi:10.1073/pnas.95.13.7631.

87. Coggins MP, Bloch KD. Nitric oxide in the pulmonary vasculature. *Arterioscler Thromb Vasc Biol* 27: 1877–1885, 2007. doi:10.1161/ATVBAHA.107.142943.

88. Cohen P, Levy JD, Zhang Y, Frontini A, Kolodin DP, Svensson KJ, Lo JC, Zeng X, Ye L, Khandekar MJ, Wu J, Gunawardana SC, Banks AS, Camporez JP, Jurczak MJ, Kajimura S, Piston DW, Mathis D, Cinti S, Shulman GI, Seale P, Spiegelman BM. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* 156: 304–316, 2014. doi:10.1016/j.cell.2013.12.021.

89. Colavitti R, Pani G, Bedogni B, Anzevino R, Borrello S, Waltenberger J, Galeotti T. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. *J Biol Chem* 277: 3101–3108, 2002. doi:10.1074/jbc.M107711200.

90. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513: 559–563, 2014. doi:10.1038/nature13490.

91. Coletta C, Papapetropoulos A, Erdelyi K, Olah G, Módis K, Panopoulos P, Asimakopoulou A, Gerö D, Sharina I, Martin E, Szabo C. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc Natl Acad Sci USA* 109: 9161–9166, 2012. doi:10.1073/pnas.1202916109.

92. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 30: 255–289, 2014. doi:10.1146/annurev-cellbio-101512-122326.

93. Coppiello G, Collantes M, Sirerol-Piquer MS, Vandenwijngaert S, Schoors S, Swinnen M, Vandersmissen I, Herijgers P, Topal B, van Loon J, Goffin J, Prósper F, Carmeliet P, García-Verdugo JM, Janssens S, Peñuelas I, Aranguren XL, Luttun A. Meox2/Tcf15 heterodimers program the heart capillary endothelium for cardiac fatty acid uptake. *Circulation* 131: 815–826, 2015. doi:10.1161/CIRCULATIONAHA.114.013721.

94. Corada M, Nyqvist D, Orsenigo F, Caprini A, Giampietro C, Taketo MM, Iruela-Arispe ML, Adams RH, Dejana E. The Wnt/beta-catenin pathway modulates vascular remodeling and specification by upregulating Dll4/Notch signaling. *Dev Cell* 18: 938–949, 2010. doi:10.1016/j.devcel.2010.05.006.

95. Cory JG, Cory AH. Critical roles of glutamine as nitrogen donors in purine and pyrimidine nucleotide synthesis: asparaginase treatment in childhood acute lymphoblastic leukemia. *In Vivo* 20: 587–589, 2006.

96. Cosentino F, Francia P, Camici GG, Pelicci PG, Lüscher TF, Volpe M. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. *Arterioscler Thromb Vasc Biol* 28: 622–628, 2008. doi:10.1161/ATVBAHA.107.156059.

97. Cossette É, Cloutier I, Tardif K, DonPierre G, Tanguay JF. Estradiol inhibits vascular endothelial cells pro-inflammatory activation induced by C-reactive protein. *Mol Cell Biochem* 373: 137–147, 2013. doi:10.1007/s11010-012-1482-9.

98. Costantino S, Paneni F, Cosentino F. Hyperglycemia: a bad signature on the vascular system. *Cardiovasc Diagn Ther* 5: 403–406, 2015.

99. Costantino S, Paneni F, Lüscher TF, Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. *Eur Heart J* 37: 572–576, 2016. doi:10.1093/eurheartj/ehv599.

100. Costantino S, Paneni F, Lüscher TF, Cosentino F. Pin1 inhibitor Juglone prevents diabetic vascular dysfunction. *Int J Cardiol* 203: 702–707, 2016. doi:10.1016/j.ijcard.2015.10.221.

101. Coutelle O, Hornig-Do HT, Witt A, Andree M, Schiffmann LM, Piekarek M, Brinkmann K, Seeger JM, Liwschitz M, Miwa S, Hallek M, Krönke M, Trifunovic A, Eming SA, Wiesner RJ, Hacker UT, Kashkar H. Embelin inhibits endothelial mitochondrial respiration and impairs neoangiogenesis during tumor growth and wound healing. *EMBO Mol Med* 6: 624–639, 2014.

102. Cruys B, Wong BW, Kuchnio A, Verdegem D, Cantelmo AR, Conradi LC, Vandekeere S, Bouché A, Cornelissen I, Vinckier S, Merks RM, Dejana E, Gerhardt H, Dewerchin M, Bentley K, Carmeliet P. Glycolytic regulation of cell rearrangement in angiogenesis. *Nat Commun* 7: 12240, 2016. doi:10.1038/ncomms12240.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

103. Cubbon RM, Murgatroyd SR, Ferguson C, Bowen TS, Rakobowchuk M, Baliga V, Cannon D, Rajwani A, Abbas A, Kahn M, Birch KM, Porter KE, Wheatcroft SB, Rossiter HB, Kearney MT. Human exercise-induced circulating progenitor cell mobilization is nitric oxide-dependent and is blunted in South Asian men. *Arterioscler Thromb Vasc Biol* 30: 878–884, 2010. doi:10.1161/ATVBAHA.109.201012.

104. Cucullo L, Hossain M, Puvenna V, Marchi N, Janigro D. The role of shear stress in blood-brain barrier endothelial physiology. *BMC Neurosci* 12: 40, 2011. doi:10.1186/1471-2202-12-40.

105. Culic O, Gruwel ML, Schrader J. Energy turnover of vascular endothelial cells. *Am J Physiol Cell Physiol* 273: C205–C213, 1997.

106. Cunnington C, Van Assche T, Shirodaria C, Kylintireas I, Lindsay AC, Lee JM, Antoniades C, Margaritis M, Lee R, Cerrato R, Crabtree MJ, Francis JM, Sayeed R, Ratnatunga C, Pillai R, Choudhury RP, Neubauer S, Channon KM. Systemic and vascular oxidation limits the efficacy of oral tetrahydrobiopterin treatment in patients with coronary artery disease. *Circulation* 125: 1356–1366, 2012. doi:10.1161/CIRCULATIONAHA.111.038919.

107. Dagher Z, Ruderman N, Tornheim K, Ido Y. Acute regulation of fatty acid oxidation and AMP-activated protein kinase in human umbilical vein endothelial cells. *Circ Res* 88: 1276–1282, 2001. doi:10.1161/hh1201.092998.

108. Daghigh F, Fukuto JM, Ash DE. Inhibition of rat liver arginase by an intermediate in NO biosynthesis, N^G-hydroxy-L-arginine: implications for the regulation of nitric oxide biosynthesis by arginase. *Biochem Biophys Res Commun* 202: 174–180, 1994. doi:10.1006/bbrc.1994.1909.

109. Dahlman JE, Barnes C, Khan O, Thiriot A, Jhunjunwala S, Shaw TE, Xing Y, Sager HB, Sahay G, Speciner L, Bader A, Bogorad RL, Yin H, Racie T, Dong Y, Jiang S, Seedorf D, Dave A, Sandu KS, Webber MJ, Novobrantseva T, Ruda VM, Lytton-Jean AKR, Levins CG, Kalish B, Mudge DK, Perez M, Abezgauz L, Dutta P, Smith L, Charisse K, Kieran MW, Fitzgerald K, Nahrendorf M, Danino D, Tuder RM, von Andrian UH, Akinc A, Schroeder A, Panigrahy D, Kotelianski V, Langer R, Anderson DG. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. *Nat Nanotechnol* 9: 648–655, 2014. doi:10.1038/nnano.2014.84.

110. Dane MJ, van den Berg BM, Lee DH, Boels MG, Tiemeier GL, Avramut MC, van Zonneveld AJ, van der Vlag J, Vink H, Rabelink TJ. A microscopic view on the renal endothelial glycocalyx. *Am J Physiol Renal Physiol* 308: F956–F966, 2015. doi:10.1152/ajprenal.00532.2014.

111. Das Evcimen N, King GL. The role of protein kinase C activation and the vascular complications of diabetes. *Pharmacol Res* 55: 498–510, 2007. doi:10.1016/j.phrs.2007.04.016.

112. Davidson SM, Papagiannakopoulos T, Olenchock BA, Heyman JE, Keibler MA, Luengo A, Bauer MR, Jha AK, O'Brien JP, Pierce KA, Gui DY, Sullivan LB, Wasylenko TM, Subbaraj L, Chin CR, Stephanopolous G, Mott BT, Jacks T, Clish CB, Vander Heiden MG. Environment Impacts the Metabolic Dependencies of Ras-Driven Non-Small Cell Lung Cancer. *Cell Metab* 23: 517–528, 2016. doi:10.1016/j.cmet.2016.01.007.

113. Davies BS, Beigneux AP, Barnes RH II, Tu Y, Gin P, Weinstein MM, Nobumori C, Nyrén R, Goldberg I, Olivecrona G, Bensadoun A, Young SG, Fong LG. GPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. *Cell Metab* 12: 42–52, 2010. doi:10.1016/j.cmet.2010.04.016.

114. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, Quaegebeur A, Ghesquière B, Cauwenberghs S, Eelen G, Phng LK, Betz I, Tembuyser B, Brepoels K, Welti J, Geudens I, Segura I, Cruys B, Bifari F, Decimo I, Blanco R, Wyns S, Vangindertael J, Rocha S, Collins RT, Munck S, Daelemans D, Imamura H, Devlieger R, Rider M, Van Veldhoven PP, Schuit F, Bartrons R, Hofkens J, Fraisl P, Telang S, Deberardinis RJ, Schoonjans L, Vinckier S, Chesney J, Gerhardt H, Dewerchin M, Carmeliet P. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* 154: 651–663, 2013. doi:10.1016/j.cell.2013.06.037.

115. De Bruin RG, van der Veer EP, Prins J, Lee DH, Dane MJ, Zhang H, Roeten MK, Bijkerk R, de Boer HC, Rabelink TJ, van Zonneveld AJ, van Gils JM. The RNA-binding protein quaking maintains endothelial barrier function and affects VE-cadherin and β-catenin protein expression. *Sci Rep* 6: 21643, 2016. doi:10.1038/srep21643.

116. De Saedeleer CJ, Copetti T, Porporato PE, Verrax J, Feron O, Sonveaux P. Lactate activates HIF-1 in oxidative but not in Warburg-phenotype human tumor cells. *PLoS One* 7: e46571, 2012. doi:10.1371/journal.pone.0046571.

117. Dehennaut V, Leprince D, Lefebvre T. *O*-GlcNAcylation, an Epigenetic Mark. Focus on the Histone Code, TET Family Proteins, and Polycomb Group Proteins. *Front Endocrinol (Lausanne)* 5: 155, 2014.

118. Desco MC, Asensi M, Márquez R, Martínez-Valls J, Vento M, Pallardó FV, Sastre J, Viña J. Xanthine oxidase is involved in free radical production in type 1 diabetes: protection by allopurinol. *Diabetes* 51: 1118–1124, 2002. doi:10.2337/diabetes.51.4.1118.

119. Devalaraja-Narashimha K, Padanilam BJ. PARP-1 inhibits glycolysis in ischemic kidneys. *J Am Soc Nephrol* 20: 95–103, 2009. doi:10.1681/ASN.2008030325.

120. Dhar-Chowdhury P, Malester B, Rajacic P, Coetzee WA. The regulation of ion channels and transporters by glycolytically derived ATP. *Cell Mol Life Sci* 64: 3069–3083, 2007. doi:10.1007/s00018-007-7332-3.

121. Di Pietro N, Formoso G, Pandolfi A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. *Vascul Pharmacol* 84: 1–7, 2016. doi:10.1016/j.vph.2016.05.013.

122. Dicker KT, Gurski LA, Pradhan-Bhatt S, Witt RL, Farach-Carson MC, Jia X. Hyaluronan: a simple polysaccharide with diverse biological functions. *Acta Biomater* 10: 1558–1570, 2014. doi:10.1016/j.actbio.2013.12.019.

123. Diebold I, Petry A, Sabrane K, Djordjevic T, Hess J, Görlach A. The HIF1 target gene NOX2 promotes angiogenesis through urotensin-II. *J Cell Sci* 125: 956–964, 2012. doi:10.1242/jcs.094060.

124. Diebold LP, Chandel NS. HSC Fate Is Tethered to Mitochondria. *Cell Stem Cell* 18: 303–304, 2016. doi:10.1016/j.stem.2016.02.007.

125. Dinkova-Kostova AT, Abramov AY. The emerging role of Nrf2 in mitochondrial function. *Free Radic Biol Med* 88, Pt B: 179–188, 2015. doi:10.1016/j.freeradbiomed.2015.04.036.

126. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, Chen W, Zeiher AM, Potente M, Dimmeler S, Boon RA. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler Thromb Vasc Biol* 35: 137–145, 2015. doi:10.1161/ATVBAHA.114.304277.

127. Doll DN, Hu H, Sun J, Lewis SE, Simpkins JW, Ren X. Mitochondrial crisis in cerebrovascular endothelial cells opens the blood-brain barrier. *Stroke* 46: 1681–1689, 2015. doi:10.1161/STROKEAHA.115.009099.

128. Domigan CK, Warren CM, Antanesian V, Happel K, Ziyad S, Lee S, Krall A, Duan L, Torres-Collado AX, Castellani LW, Elashoff D, Christofk HR, van der Bliek AM, Potente M, Iruela-Arispe ML. Autocrine VEGF maintains endothelial survival through regulation of metabolism and autophagy. *J Cell Sci* 128: 2236–2248, 2015. doi:10.1242/jcs.163774.

129. Dranka BP, Hill BG, Darley-Usmar VM. Mitochondrial reserve capacity in endothelial cells: the impact of nitric oxide and reactive oxygen species. *Free Radic Biol Med* 48: 905–914, 2010. doi:10.1016/j.freeradbiomed.2010.01.015.

130. Drummond GR, Sobey CG. Endothelial NADPH oxidases: which NOX to target in vascular disease? *Trends Endocrinol Metab* 25: 452–463, 2014. doi:10.1016/j.tem.2014.06.012.

131. Du J, Fan LM, Mai A, Li JM. Crucial roles of Nox2-derived oxidative stress in deteriorating the function of insulin receptors and endothelium in dietary obesity of middle-aged mice. *Br J Pharmacol* 170: 1064–1077, 2013. doi:10.1111/bph.12336.

132. Du X, Edelstein D, Obici S, Higham N, Zou MH, Brownlee M. Insulin resistance reduces arterial prostacyclin synthase and eNOS activities by increasing endothelial fatty acid oxidation. *J Clin Invest* 116: 1071–1080, 2006. doi:10.1172/JCI23354.

133. Du X, Matsumura T, Edelstein D, Rossetti L, Zsengellér Z, Szabó C, Brownlee M. Inhibition of GAPDH activity by poly(ADP-ribose) polymerase activates three major pathways of hyperglycemic damage in endothelial cells. *J Clin Invest* 112: 1049–1057, 2003. doi:10.1172/JCI18127.

134. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest* 108: 1341–1348, 2001. doi:10.1172/JCI11235.

135. Du XL, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, Wu J, Brownlee M. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci USA* 97: 12222–12226, 2000. doi:10.1073/pnas.97.22.12222.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

136. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD; VADT Investigators. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 360: 129–139, 2009. doi:10.1056/NEJMoa0808431.

137. Dudley AC. Tumor endothelial cells. *Cold Spring Harb Perspect Med* 2: a006536, 2012. doi:10.1101/cshperspect.a006536.

138. Dueber JE, Wu GC, Malmirchegini GR, Moon TS, Petzold CJ, Ullal AV, Prather KL, Keasling JD. Synthetic protein scaffolds provide modular control over metabolic flux. *Nat Biotechnol* 27: 753–759, 2009. doi:10.1038/nbt.1557.

139. Duncan ER, Crossey PA, Walker S, Anilkumar N, Poston L, Douglas G, Ezzat VA, Wheatcroft SB, Shah AM, Kearney MT. Effect of endothelium-specific insulin resistance on endothelial function in vivo. *Diabetes* 57: 3307–3314, 2008. doi:10.2337/db07-1111.

140. Dunn WB. Current trends and future requirements for the mass spectrometric investigation of microbial, mammalian and plant metabolomes. *Phys Biol* 5: 011001, 2008. doi:10.1088/1478-3975/5/1/011001.

141. Duong HT, Comhair SA, Aldred MA, Mavrakis L, Savasky BM, Erzurum SC, Asosingh K. Pulmonary artery endothelium resident endothelial colony-forming cells in pulmonary arterial hypertension. *Pulm Circ* 1: 475–486, 2011. doi:10.4103/2045-8932.93547.

142. Dzau VJ, Gnecchi M, Pachori AS, Morello F, Melo LG. Therapeutic potential of endothelial progenitor cells in cardiovascular diseases. *Hypertension* 46: 7–18, 2005. doi:10.1161/01.HYP.0000168923.92885.f7.

143. Ebong EE, Lopez-Quintero SV, Rizzo V, Spray DC, Tarbell JM. Shear-induced endothelial NOS activation and remodeling via heparan sulfate, glypican-1, and syndecan-1. *Integr Biol* 6: 338–347, 2014. doi:10.1039/C3IB40199E.

144. Eelen G, Cruys B, Welti J, De Bock K, Carmeliet P. Control of vessel sprouting by genetic and metabolic determinants. *Trends Endocrinol Metab* 24: 589–596, 2013. doi:10.1016/j.tem.2013.08.006.

145. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. *Circ Res* 116: 1231–1244, 2015. doi:10.1161/CIRCRESAHA.116.302855.

146. Egan CG, Caporali F, Garcia-Gonzalez E, Galeazzi M, Sorrentino V. Endothelial progenitor cells and colony-forming units in rheumatoid arthritis: association with clinical characteristics. *Rheumatology (Oxford)* 47: 1484–1488, 2008. doi:10.1093/rheumatology/ken299.

147. Ehnfors J, Kost-Alimova M, Persson NL, Bergsmedh A, Castro J, Levchenko-Tegnebratt T, Yang L, Panaretakis T, Holmgren L. Horizontal transfer of tumor DNA to endothelial cells in vivo. *Cell Death Differ* 16: 749–757, 2009. doi:10.1038/cdd.2009.7.

148. Eichmann A, Simons M. Need glucose to sprout: local metabolic control of angiogenesis. *EMBO Mol Med* 5: 1459–1461, 2013. doi:10.1002/emmm.201303174.

149. Eklund L, Saharinen P. Angiopoietin signaling in the vasculature. *Exp Cell Res* 319: 1271–1280, 2013. doi:10.1016/j.yexcr.2013.03.011.

150. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG, Cooper ME, Brownlee M. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med* 205: 2409–2417, 2008. doi:10.1084/jem.20081188.

151. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. *Nat Rev Cancer* 8: 579–591, 2008. doi:10.1038/nrc2403.

152. Elmasri H, Ghelfi E, Yu CW, Traphagen S, Cernadas M, Cao H, Shi GP, Plutzky J, Sahin M, Hotamisligil G, Cataltepe S. Endothelial cell-fatty acid binding protein 4 promotes angiogenesis: role of stem cell factor/c-kit pathway. *Angiogenesis* 15: 457–468, 2012. doi:10.1007/s10456-012-9274-0.

153. Elmasri H, Karaaslan C, Teper Y, Ghelfi E, Weng M, Ince TA, Kozakewich H, Bischoff J, Cataltepe S. Fatty acid binding protein 4 is a target of VEGF and a regulator of cell proliferation in endothelial cells. *FASEB J* 23: 3865–3873, 2009. doi:10.1096/fj.09-134882.

154. Emerging Risk Factors Collaboration' Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, Walker M, Thompson A, Sarwar N, Caslake M, Butterworth AS, Amouyel P, Assmann G, Bakker SJ, Barr EL, Barrett-Connor E, Benjamin EJ, Björkelund C, Brenner H, Brunner E, Clarke R, Cooper JA, Cremer P, Cushman M, Dagenais GR, D'Agostino RB Sr, Dankner R, Davey-Smith G, Deeg D, Dekker JM, Engström G, Folsom AR, Fowkes FG, Gallacher J, Gaziano JM, Giampaoli S, Gillum RF, Hofman A, Howard BV, Ingelsson E, Iso H, Jørgensen T, Kiechl S, Kitamura A, Kiyohara Y, Koenig W, Kromhout D, Kuller LH, Lawlor DA, Meade TW, Nissinen A, Nordestgaard BG, Onat A, Panagiotakos DB, Psaty BM, Rodriguez B, Rosengren A, Salomaa V, Kauhanen J, Salonen JT, Shaffer JA, Shea S, Ford I, Stehouwer CD, Strandberg TE, Tipping RW, Tosetto A, Wassertheil-Smoller S, Wennberg P, Westendorp RG, Whincup PH, Wilhelmsen L, Woodward M, Lowe GD, Wareham NJ, Khaw KT, Sattar N, Packard CJ, Gudnason V, Ridker PM, Pepys MB, Thompson SG, Danesh J. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med* 367: 1310–1320, 2012. doi:10.1056/NEJMoa1107477.

155. Epstein T, Xu L, Gillies RJ, Gatenby RA. Separation of metabolic supply and demand: aerobic glycolysis as a normal physiological response to fluctuating energetic demands in the membrane. *Cancer Metab* 2: 7, 2014. doi:10.1186/2049-3002-2-7.

156. Erez A, Nagamani SC, Shchelochkov OA, Premkumar MH, Campeau PM, Chen Y, Garg HK, Li L, Mian A, Bertin TK, Black JO, Zeng H, Tang Y, Reddy AK, Summar M, O'Brien WE, Harrison DG, Mitch WE, Marini JC, Aschner JL, Bryan NS, Lee B. Requirement of argininosuccinate lyase for systemic nitric oxide production. *Nat Med* 17: 1619–1626, 2011. doi:10.1038/nm.2544.

157. Facucho-Oliveira JM, St John JC. The relationship between pluripotency and mitochondrial DNA proliferation during early embryo development and embryonic stem cell differentiation. *Stem Cell Rev* 5: 140–158, 2009. doi:10.1007/s12015-009-9058-0.

158. Fadini GP, Sartore S, Schiavon M, Albiero M, Baesso I, Cabrelle A, Agostini C, Avogaro A. Diabetes impairs progenitor cell mobilisation after hindlimb ischaemia-reperfusion injury in rats. *Diabetologia* 49: 3075–3084, 2006. doi:10.1007/s00125-006-0401-6.

159. Fagan KA, McMurtry I, Rodman DM. Nitric oxide synthase in pulmonary hypertension: lessons from knockout mice. *Physiol Res* 49: 539–548, 2000.

160. Fang L, Choi SH, Baek JS, Liu C, Almazan F, Ulrich F, Wiesner P, Taleb A, Deer E, Pattison J, Torres-Vázquez J, Li AC, Miller YI. Control of angiogenesis by AIBP-mediated cholesterol efflux. *Nature* 498: 118–122, 2013. doi:10.1038/nature12166.

161. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* 116: 829–840, 2010. doi:10.1182/blood-2009-12-257832.

162. Farber HW, Loscalzo J. Pulmonary arterial hypertension. *N Engl J Med* 351: 1655–1665, 2004. doi:10.1056/NEJMra035488.

163. Farha S, Sharp J, Asosingh K, Park M, Comhair SA, Tang WH, Thomas J, Farver C, Hsieh F, Loyd JE, Erzurum SC. Mast cell number, phenotype, and function in human pulmonary arterial hypertension. *Pulm Circ* 2: 220–228, 2012. doi:10.4103/2045-8932.97609.

165. Federici M, Menghini R, Mauriello A, Hribal ML, Ferrelli F, Lauro D, Sbraccia P, Spagnoli LG, Sesti G, Lauro R. Insulin-dependent activation of endothelial nitric oxide synthase is impaired by O-linked glycosylation modification of signaling proteins in human coronary endothelial cells. *Circulation* 106: 466–472, 2002. doi:10.1161/01.CIR.0000023043.02648.51.

166. Feliers D, Lee DY, Gorin Y, Kasinath BS. Symmetric dimethylarginine alters endothelial nitric oxide activity in glomerular endothelial cells. *Cell Signal* 27: 1–5, 2015. doi:10.1016/j.cellsig.2014.09.024.

167. Ferguson JE III, Kelley RW, Patterson C. Mechanisms of endothelial differentiation in embryonic vasculogenesis. *Arterioscler Thromb Vasc Biol* 25: 2246–2254, 2005. doi:10.1161/01.ATV.0000183609.55154.44.

168. Ferri C, Pittoni V, Piccoli A, Laurenti O, Cassone MR, Bellini C, Properzi G, Valesini G, De Mattia G, Santucci A. Insulin stimulates endothelin-1 secretion from human endothelial cells and modulates its circulating levels in vivo. *J Clin Endocrinol Metab* 80: 829–835, 1995.

169. Fessel JP, Hamid R, Wittmann BM, Robinson LJ, Blackwell T, Tada Y, Tanabe N, Tatsumi K, Hemnes AR, West JD. Metabolomic analysis of bone morphogenetic protein receptor type 2 mutations in human pulmonary endothelium reveals widespread metabolic reprogramming. *Pulm Circ* 2: 201–213, 2012. doi:10.4103/2045-8932.97606.

170. Fijalkowska I, Xu W, Comhair SA, Janocha AJ, Mavrakis LA, Krishnamachary B, Zhen L, Mao T, Richter A, Erzurum SC, Tuder RM. Hypoxia inducible-factor1alpha regu-

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

lates the metabolic shift of pulmonary hypertensive endothelial cells. *Am J Pathol* 176: 1130–1138, 2010. doi:10.2353/ajpath.2010.090832.

171. Fleming T, Cuny J, Nawroth G, Djuric Z, Humpert PM, Zeier M, Bierhaus A, Nawroth PP. Is diabetes an acquired disorder of reactive glucose metabolites and their intermediates? *Diabetologia* 55: 1151–1155, 2012. doi:10.1007/s00125-012-2452-1.

172. Forbes JM, Coughlan MT, Cooper ME. Oxidative stress as a major culprit in kidney disease in diabetes. *Diabetes* 57: 1446–1454, 2008. doi:10.2337/db08-0057.

173. Fordjour PA, Wang Y, Shi Y, Agyemang K, Akinyi M, Zhang Q, Fan G. Possible mechanisms of C-reactive protein mediated acute myocardial infarction. *Eur J Pharmacol* 760: 72–80, 2015. doi:10.1016/j.ejphar.2015.04.010.

174. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* 33: 829–837, 2012. doi:10.1093/eurheartj/ehr304.

175. Fraisl P. Crosstalk between oxygen- and nitric oxide-dependent signaling pathways in angiogenesis. *Exp Cell Res* 319: 1331–1339, 2013. doi:10.1016/j.yexcr.2013.02.010.

176. Franco M, Roswall P, Cortez E, Hanahan D, Pietras K. Pericytes promote endothelial cell survival through induction of autocrine VEGF-A signaling and Bcl-w expression. *Blood* 118: 2906–2917, 2011. doi:10.1182/blood-2011-01-331694.

177. François M, Caprini A, Hosking B, Orsenigo F, Wilhelm D, Browne C, Paavonen K, Karnezis T, Shayan R, Downes M, Davidson T, Tutt D, Cheah KS, Stacker SA, Muscat GE, Achen MG, Dejana E, Koopman P. Sox18 induces development of the lymphatic vasculature in mice. *Nature* 456: 643–647, 2008. doi:10.1038/nature07391.

178. Frentzas S, Simoneau E, Bridgeman VL, Vermeulen PB, Foo S, Kostaras E, Nathan M, Wotherspoon A, Gao ZH, Shi Y, Van den Eynden G, Daley F, Peckitt C, Tan X, Salman A, Lazaris A, Gazinska P, Berg TJ, Eltahir Z, Ritsma L, Van Rheenen J, Khashper A, Brown G, Nystrom H, Sund M, Van Laere S, Loyer E, Dirix L, Cunningham D, Metrakos P, Reynolds AR. Vessel co-option mediates resistance to anti-angiogenic therapy in liver metastases. *Nat Med* 22: 1294–1302, 2016. doi:10.1038/nm.4197.

179. Funahashi Y, Shawber CJ, Vorontchikhina M, Sharma A, Outtz HH, Kitajewski J. Notch regulates the angiogenic response via induction of VEGFR-1. *J Angiogenes Res* 2: 3, 2010. doi:10.1186/2040-2384-2-3.

180. Gaengel K, Genové G, Armulik A, Betsholtz C. Endothelial-mural cell signaling in vascular development and angiogenesis. *Arterioscler Thromb Vasc Biol* 29: 630–638, 2009. doi:10.1161/ATVBAHA.107.161521.

181. Gage MC, Yuldasheva NY, Viswambharan H, Sukumar P, Cubbon RM, Galloway S, Imrie H, Skromna A, Smith J, Jackson CL, Kearney MT, Wheatcroft SB. Endothelium-specific insulin resistance leads to accelerated atherosclerosis in areas with disturbed flow patterns: a role for reactive oxygen species. *Atherosclerosis* 230: 131–139, 2013. doi:10.1016/j.atherosclerosis.2013.06.017.

182. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 27: 165–197, 2009. doi:10.1146/annurev.immunol.021908.132620.

183. Gao HX, Regier EE, Close KL. European Society of Cardiology Congress 2015 (ESC 2015). *J Diabetes* 8: 7–8, 2016. doi:10.1111/1753-0407.12350.

184. Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 458: 762–765, 2009. doi:10.1038/nature07823.

185. Gao XH, Krokowski D, Guan BJ, Bederman I, Majumder M, Parisien M, Diatchenko L, Kabil O, Willard B, Banerjee R, Wang B, Bebek G, Evans CR, Fox PL, Gerson SL, Hoppel CL, Liu M, Arvan P, Hatzoglou M. Quantitative H2S-mediated protein sulfhydration reveals metabolic reprogramming during the integrated stress response. *eLife* 4: e10067, 2015. doi:10.7554/eLife.10067.

186. Garcia NA, Moncayo-Arlandi J, Sepulveda P, Diez-Juan A. Cardiomyocyte exosomes regulate glycolytic flux in endothelium by direct transfer of GLUT transporters and glycolytic enzymes. *Cardiovasc Res* 109: 397–408, 2016. doi:10.1093/cvr/cvv260.

187. Garcia NA, Ontoria-Oviedo I, González-King H, Diez-Juan A, Sepúlveda P. Glucose Starvation in Cardiomyocytes Enhances Exosome Secretion and Promotes Angiogenesis in Endothelial Cells. *PLoS One* 10: e0138849, 2015. doi:10.1371/journal.pone.0138849.

188. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* 4: 891–899, 2004. doi:10.1038/nrc1478.

189. Gaul DA, Mezencev R, Long TQ, Jones CM, Benigno BB, Gray A, Fernández FM, McDonald JF. Highly-accurate metabolomic detection of early-stage ovarian cancer. *Sci Rep* 5: 16351, 2015. doi:10.1038/srep16351.

190. Gebala V, Collins R, Geudens I, Phng LK, Gerhardt H. Blood flow drives lumen formation by inverse membrane blebbing during angiogenesis in vivo. *Nat Cell Biol* 18: 443–450, 2016. doi:10.1038/ncb3320.

191. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, Aiello LP, Kern TS, King GL. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nat Med* 15: 1298–1306, 2009. doi:10.1038/nm.2052.

192. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 161: 1163–1177, 2003. doi:10.1083/jcb.200302047.

193. Ghosh C, Hossain M, Puvenna V, Martinez-Gonzalez J, Alexopolous A, Janigro D, Marchi N. Expression and functional relevance of UGT1A4 in a cohort of human drug-resistant epileptic brains. *Epilepsia* 54: 1562–1570, 2013. doi:10.1111/epi.12318.

194. Ghosh S, Gupta M, Xu W, Mavrakis DA, Janocha AJ, Comhair SA, Haque MM, Stuehr DJ, Yu J, Polgar P, Naga Prasad SV, Erzurum SC. Phosphorylation inactivation of endothelial nitric oxide synthesis in pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 310: L1199–L1205, 2016. doi:10.1152/ajplung.00092.2016.

195. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res* 107: 1058–1070, 2010. doi:10.1161/CIRCRESAHA.110.223545.

196. Giaid A, Saleh D. Reduced expression of endothelial nitric oxide synthase in the lungs of patients with pulmonary hypertension. *N Engl J Med* 333: 214–221, 1995. doi:10.1056/NEJM199507273330403.

197. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 328: 1732–1739, 1993. doi:10.1056/NEJM199306173282402.

198. Gimbrone MA, Jr, García-Cardeña G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. *Circ Res* 118: 620–636, 2016. doi:10.1161/CIRCRESAHA.115.306301.

199. Gimond C, Marchetti S, Pagès G. Differentiation of mouse embryonic stem cells into endothelial cells: genetic selection and potential use in vivo. *Methods Mol Biol* 330: 303–329, 2006.

200. Girgis RE, Champion HC, Diette GB, Johns RA, Permutt S, Sylvester JT. Decreased exhaled nitric oxide in pulmonary arterial hypertension: response to bosentan therapy. *Am J Respir Crit Care Med* 172: 352–357, 2005. doi:10.1164/rccm.200412-1684OC.

201. Glatz JF, Luiken JJ, Bonen A. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. *Physiol Rev* 90: 367–417, 2010. doi:10.1152/physrev.00003.2009.

202. Głowińska-Olszewska B, Moniuszko M, Hryniewicz A, Jeznach M, Rusak M, Dąbrowska M, Łuczyński W, Bodzenta-Łukaszyk A, Bossowski A. Relationship between circulating endothelial progenitor cells and endothelial dysfunction in children with type 1 diabetes: a novel paradigm of early atherosclerosis in high-risk young patients. *Eur J Endocrinol* 168: 153–161, 2013. doi:10.1530/EJE-12-0857.

203. González M, Gallardo V, Rodríguez N, Salomón C, Westermeier F, Guzmán-Gutiérrez E, Abarzúa F, Leiva A, Casanello P, Sobrevia L. Insulin-stimulated L-arginine transport requires SLC7A1 gene expression and is associated with human umbilical vein relaxation. *J Cell Physiol* 226: 2916–2924, 2011. doi:10.1002/jcp.22635.

204. Görlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res* 87: 26–32, 2000. doi:10.1161/01.RES.87.1.26.

205. Goveia J, Pircher A, Conradi LC, Kalucka J, Lagani V, Dewerchin M, Eelen G, DeBerardinis RJ, Wilson ID, Carmeliet P. Meta-analysis of clinical metabolic profiling studies in cancer: challenges and opportunities. *EMBO Mol Med* 8: 1134–1142, 2016. doi:10.15252/emmm.201606798.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

206. Granchi C, Minutolo F. Anticancer agents that counteract tumor glycolysis. *ChemMedChem* 7: 1318–1350, 2012. doi:10.1002/cmdc.201200176.

207. Gray SP, Di Marco E, Kennedy K, Chew P, Okabe J, El-Osta A, Calkin AC, Biessen EA, Touyz RM, Cooper ME, Schmidt HH, Jandeleit-Dahm KA. Reactive Oxygen Species Can Provide Atheroprotection via NOX4-Dependent Inhibition of Inflammation and Vascular Remodeling. *Arterioscler Thromb Vasc Biol* 36: 295–307, 2016. doi:10.1161/ATVBAHA.115.307012.

208. Grewal AS, Bhardwaj S, Pandita D, Lather V, Sekhon BS. Updates on Aldose Reductase Inhibitors for Management of Diabetic Complications and Non-diabetic Diseases. *Mini Rev Med Chem* 16: 120–162, 2016. doi:10.2174/1389557515666150909143737.

209. Groschner LN, Waldeck-Weiermair M, Malli R, Graier WF. Endothelial mitochondria–less respiration, more integration. *Pflugers Arch* 464: 63–76, 2012. doi:10.1007/s00424-012-1085-z.

211. Gurda D, Handschuh L, Kotkowiak W, Jakubowski H. Homocysteine thiolactone and *N*-homocysteinylated protein induce pro-atherogenic changes in gene expression in human vascular endothelial cells. *Amino Acids* 47: 1319–1339, 2015. doi:10.1007/s00726-015-1956-7.

212. Guzik TJ, Harrison DG. Vascular NADPH oxidases as drug targets for novel antioxidant strategies. *Drug Discov Today* 11: 524–533, 2006. doi:10.1016/j.drudis.2006.04.003.

213. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, Channon KM. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation* 105: 1656–1662, 2002. doi:10.1161/01.CIR.0000012748.58444.08.

214. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, Bland EJ, Bombardieri M, Pitzalis C, Perretti M, Marelli-Berg FM, Mauro C. Lactate Regulates Metabolic and Pro-inflammatory Circuits in Control of T Cell Migration and Effector Functions. *PLoS Biol* 13: e1002202, 2015. doi:10.1371/journal.pbio.1002202.

215. Hagberg C, Mehlem A, Falkevall A, Muhl L, Eriksson U. Endothelial fatty acid transport: role of vascular endothelial growth factor B. *Physiology (Bethesda)* 28: 125–134, 2013. doi:10.1152/physiol.00042.2012.

216. Hagberg CE, Falkevall A, Wang X, Larsson E, Huusko J, Nilsson I, van Meeteren LA, Samen E, Lu L, Vanwildemeersch M, Klar J, Genove G, Pietras K, Stone-Elander S, Claesson-Welsh L, Ylä-Herttuala S, Lindahl P, Eriksson U. Vascular endothelial growth factor B controls endothelial fatty acid uptake. *Nature* 464: 917–921, 2010. doi:10.1038/nature08945.

217. Haines RJ, Corbin KD, Pendleton LC, Meininger CJ, Eichler DC. Insulin transcriptionally regulates argininosuccinate synthase to maintain vascular endothelial function. *Biochem Biophys Res Commun* 421: 9–14, 2012. doi:10.1016/j.bbrc.2012.03.074.

218. Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, Lin J, Bierhaus A, Nawroth P, Hannak D, Neumaier M, Bergfeld R, Giardino I, Brownlee M. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. *Nat Med* 9: 294–299, 2003. doi:10.1038/nm834.

219. Harjes U, Bridges E, Gharpure KM, Roxanis I, Sheldon H, Miranda F, Mangala LS, Pradeep S, Lopez-Berestein G, Ahmed A, Fielding B, Sood AK, Harris AL. Antiangiogenic and tumour inhibitory effects of downregulating tumour endothelial FABP4. *Oncogene* 36: 912–921, 2017. doi:10.1038/onc.2016.256.

220. Harjes U, Bridges E, McIntyre A, Fielding BA, Harris AL. Fatty acid-binding protein 4, a point of convergence for angiogenic and metabolic signaling pathways in endothelial cells. *J Biol Chem* 289: 23168–23176, 2014. doi:10.1074/jbc.M114.576512.

221. Harjes U, Kalucka J, Carmeliet P. Targeting fatty acid metabolism in cancer and endothelial cells. *Crit Rev Oncol Hematol* 97: 15–21, 2016. doi:10.1016/j.critrevonc.2015.10.011.

222. Harris AL. Hypoxia–a key regulatory factor in tumour growth. *Nat Rev Cancer* 2: 38–47, 2002. doi:10.1038/nrc704.

223. Hasegawa Y, Saito T, Ogihara T, Ishigaki Y, Yamada T, Imai J, Uno K, Gao J, Kaneko K, Shimosawa T, Asano T, Fujita T, Oka Y, Katagiri H. Blockade of the nuclear factor-κB pathway in the endothelium prevents insulin resistance and prolongs life spans. *Circulation* 125: 1122–1133, 2012. doi:10.1161/CIRCULATIONAHA.111.054346.

224. Hashimoto T, Hussien R, Oommen S, Gohil K, Brooks GA. Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *FASEB J* 21: 2602–2612, 2007. doi:10.1096/fj.07-8174com.

225. Haslip M, Dostanic I, Huang Y, Zhang Y, Russell KS, Jurczak MJ, Mannam P, Giordano F, Erzurum SC, Lee PJ. Endothelial uncoupling protein 2 regulates mitophagy and pulmonary hypertension during intermittent hypoxia. *Arterioscler Thromb Vasc Biol* 35: 1166–1178, 2015. doi:10.1161/ATVBAHA.114.304865.

226. Hensley CT, Faubert B, Yuan Q, Lev-Cohain N, Jin E, Kim J, Jiang L, Ko B, Skelton R, Loudat L, Wodzak M, Klimko C, McMillan E, Butt Y, Ni M, Oliver D, Torrealba J, Malloy CR, Kernstine K, Lenkinski RE, DeBerardinis RJ. Metabolic Heterogeneity in Human Lung Tumors. *Cell* 164: 681–694, 2016. doi:10.1016/j.cell.2015.12.034.

227. Herranz D, Ambesi-Impiombato A, Sudderth J, Sánchez-Martín M, Belver L, Tosello V, Xu L, Wendorff AA, Castillo M, Haydu JE, Márquez J, Matés JM, Kung AL, Rayport S, Cordon-Cardo C, DeBerardinis RJ, Ferrando AA. Metabolic reprogramming induces resistance to anti-NOTCH1 therapies in T cell acute lymphoblastic leukemia. *Nat Med* 21: 1182–1189, 2015. doi:10.1038/nm.3955.

228. Hida K, Akiyama K, Ohga N, Maishi N, Hida Y. Tumour endothelial cells acquire drug resistance in a tumour microenvironment. *J Biochem* 153: 243–249, 2013. doi:10.1093/jb/mvs152.

229. Hida K, Hida Y, Amin DN, Flint AF, Panigrahy D, Morton CC, Klagsbrun M. Tumor-associated endothelial cells with cytogenetic abnormalities. *Cancer Res* 64: 8249–8255, 2004. doi:10.1158/0008-5472.CAN-04-1567.

230. Hida K, Klagsbrun M. A new perspective on tumor endothelial cells: unexpected chromosome and centrosome abnormalities. *Cancer Res* 65: 2507–2510, 2005. doi:10.1158/0008-5472.CAN-05-0002.

231. Hida K, Maishi N, Sakurai Y, Hida Y, Harashima H. Heterogeneity of tumor endothelial cells and drug delivery. *Adv Drug Deliv Rev* 99, Pt B: 140–147, 2016. doi:10.1016/j.addr.2015.11.008.

232. Hida K, Ohga N, Akiyama K, Maishi N, Hida Y. Heterogeneity of tumor endothelial cells. *Cancer Sci* 104: 1391–1395, 2013. doi:10.1111/cas.12251.

233. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 348: 593–600, 2003. doi:10.1056/NEJMoa022287.

234. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, Onozuka H, Kinoshita T, Saito N, Ochiai A, Tomita M, Esumi H, Soga T. Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res* 69: 4918–4925, 2009. doi:10.1158/0008-5472.CAN-08-4806.

235. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. *Cancer Res* 71: 6921–6925, 2011. doi:10.1158/0008-5472.CAN-11-1457.

236. Ho PY, Zhong WB, Ho YS, Lee WS. Terbinafine inhibits endothelial cell migration through suppression of the Rho-mediated pathway. *Mol Cancer Ther* 5: 3130–3138, 2006. doi:10.1158/1535-7163.MCT-06-0457.

237. Hodge RG, Ridley AJ. Regulating Rho GTPases and their regulators. *Nat Rev Mol Cell Biol* 17: 496–510, 2016. doi:10.1038/nrm.2016.67.

238. Hong YK, Harvey N, Noh YH, Schacht V, Hirakawa S, Detmar M, Oliver G. Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate. *Dev Dyn* 225: 351–357, 2002. doi:10.1002/dvdy.10163.

239. Hosios AM, Hecht VC, Danai LV, Johnson MO, Rathmell JC, Steinhauser ML, Manalis SR, Vander Heiden MG. Amino Acids Rather than Glucose Account for the Majority of Cell Mass in Proliferating Mammalian Cells. *Dev Cell* 36: 540–549, 2016. doi:10.1016/j.devcel.2016.02.012.

240. Hu H, Juvekar A, Lyssiotis CA, Lien EC, Albeck JG, Oh D, Varma G, Hung YP, Ullas S, Lauring J, Seth P, Lundquist MR, Tolan DR, Grant AK, Needleman DJ, Asara JM, Cantley LC, Wulf GM. Phosphoinositide 3-Kinase Regulates Glycolysis through Mobilization of Aldolase from the Actin Cytoskeleton. *Cell* 164: 433–446, 2016. doi:10.1016/j.cell.2015.12.042.

241. Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z. Glutaminase 2, a novel p53 target gene regulating energy metabolism and antioxidant function. *Proc Natl Acad Sci USA* 107: 7455–7460, 2010. doi:10.1073/pnas.1001006107.

242. Huang H, Vandekeere S, Kalucka J, Bierhansl L, Zecchin A, Brüning U, Visnagri A, Yuldasheva N, Goveia J, Cruys B, Brepoels K, Wyns S, Rayport S, Ghesquière B, Vinckier S, Schoonjans L, Cubbon R, Dewerchin M, Eelen G, Carmeliet P. Role of glutamine and interlinked asparagine metabolism in vessel formation. *EMBO J* 36: 2334–2352, 2017. doi:10.15252/embj.201695518.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

243. Hunt TK, Aslam RS, Beckert S, Wagner S, Ghani QP, Hussain MZ, Roy S, Sen CK. Aerobically derived lactate stimulates revascularization and tissue repair via redox mechanisms. *Antioxid Redox Signal* 9: 1115–1124, 2007. doi:10.1089/ars.2007.1674.

244. Hwang J, Ing MH, Salazar A, Lassègue B, Griendling K, Navab M, Sevanian A, Hsiai TK. Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation. *Circ Res* 93: 1225–1232, 2003. doi:10.1161/01.RES.0000104087.29395.66.

245. Ihnat MA, Thorpe JE, Ceriello A. Hypothesis: the 'metabolic memory', the new challenge of diabetes. *Diabet Med* 24: 582–586, 2007. doi:10.1111/j.1464-5491.2007.02138.x.

246. Ihnat MA, Thorpe JE, Kamat CD, Szabó C, Green DE, Warnke LA, Lacza Z, Cselenyák A, Ross K, Shakir S, Piconi L, Kaltreider RC, Ceriello A. Reactive oxygen species mediate a cellular 'memory' of high glucose stress signalling. *Diabetologia* 50: 1523–1531, 2007. doi:10.1007/s00125-007-0684-2.

247. Ii M, Takenaka H, Asai J, Ibusuki K, Mizukami Y, Maruyama K, Yoon YS, Wecker A, Luedemann C, Eaton E, Silver M, Thorne T, Losordo DW. Endothelial progenitor thrombospondin-1 mediates diabetes-induced delay in reendothelialization following arterial injury. *Circ Res* 98: 697–704, 2006. doi:10.1161/01.RES.0000209948.50943.ea.

248. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H, Nawata H. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes* 49: 1939–1945, 2000. doi:10.2337/diabetes.49.11.1939.

249. Inoguchi T, Tsubouchi H, Etoh T, Kakimoto M, Sonta T, Utsumi H, Sumimoto H, Yu HY, Sonoda N, Inuo M, Sato N, Sekiguchi N, Kobayashi K, Nawata H. A possible target of antioxidative therapy for diabetic vascular complications-vascular NAD(P)H oxidase. *Curr Med Chem* 10: 1759–1764, 2003. doi:10.2174/0929867033457133.

250. Inoguchi T, Xia P, Kunisaki M, Higashi S, Feener EP, King GL. Insulin's effect on protein kinase C and diacylglycerol induced by diabetes and glucose in vascular tissues. *Am J Physiol Endocrinol Metab* 267: E369–E379, 1994.

250a. International Diabetes Federation. *IDF Diabetes Atlas*. Brussels, Belgium: International Diabetes Federation, 2015.

251. Ishii M, Shimizu S, Nagai T, Shiota K, Kiuchi Y, Yamamoto T. Stimulation of tetrahydrobiopterin synthesis induced by insulin: possible involvement of phosphatidylinositol 3-kinase. *Int J Biochem Cell Biol* 33: 65–73, 2001. doi:10.1016/S1357-2725(00)00070-4.

252. Iso T, Maeda K, Hanaoka H, Suga T, Goto K, Syamsunarno MR, Hishiki T, Nagahata Y, Matsui H, Arai M, Yamaguchi A, Abumrad NA, Sano M, Suematsu M, Endo K, Hotamisligil GS, Kurabayashi M. 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: 2549–2557, 2013. doi:10.1161/ATVBAHA.113.301588.

253. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 99: 3092–3095, 1999. doi:10.1161/01.CIR.99.24.3092.

254. Jae SY, Kurl S, Laukkanen JA, Lee CD, Choi YH, Fernhall B, Franklin BA. Relation of C-reactive protein, fibrinogen, and cardiorespiratory fitness to risk of systemic hypertension in men. *Am J Cardiol* 115: 1714–1719, 2015. doi:10.1016/j.amjcard.2015.03.016.

255. Jakobsson L, Franco CA, Bentley K, Collins RT, Ponsioen B, Aspalter IM, Rosewell I, Busse M, Thurston G, Medvinsky A, Schulte-Merker S, Gerhardt H. Endothelial cells dynamically compete for the tip cell position during angiogenic sprouting. *Nat Cell Biol* 12: 943–953, 2010. doi:10.1038/ncb2103.

256. Jandeleit-Dahm KA, Schmidt HH. Comment on: Sukumar et al. Nox2 NADPH oxidase has a critical role in insulin resistance-related endothelial cell dysfunction. Diabetes 2013;62:2130-2134. *Diabetes* 62: e30, 2013. doi:10.2337/db13-1286.

257. Jang C, Oh SF, Wada S, Rowe GC, Liu L, Chan MC, Rhee J, Hoshino A, Kim B, Ibrahim A, Baca LG, Kim E, Ghosh CC, Parikh SM, Jiang A, Chu Q, Forman DE, Lecker SH, Krishnaiah S, Rabinowitz JD, Weljie AM, Baur JA, Kasper DL, Arany Z. A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance. *Nat Med* 22: 421–426, 2016. doi:10.1038/nm.4057.

258. Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginases. *Comp Biochem Physiol B Biochem Mol Biol* 114: 107–132, 1996. doi:10.1016/0305-0491(95)02138-8.

259. Jeremy JY, Rowe D, Emsley AM, Newby AC. Nitric oxide and the proliferation of vascular smooth muscle cells. *Cardiovasc Res* 43: 580–594, 1999. doi:10.1016/S0008-6363(99)00171-6.

260. Jia G, Aroor AR, Whaley-Connell AT, Sowers JR. Fructose and uric acid: is there a role in endothelial function? *Curr Hypertens Rep* 16: 434, 2014. doi:10.1007/s11906-014-0434-z.

261. Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (*fa/fa*) rats. *J Clin Invest* 104: 447–457, 1999. doi:10.1172/JCI5971.

262. Jo-Watanabe A, Ohse T, Nishimatsu H, Takahashi M, Ikeda Y, Wada T, Shirakawa J, Nagai R, Miyata T, Nagano T, Hirata Y, Inagi R, Nangaku M. Glyoxalase I reduces glycative and oxidative stress and prevents age-related endothelial dysfunction through modulation of endothelial nitric oxide synthase phosphorylation. *Aging Cell* 13: 519–528, 2014. doi:10.1111/acel.12204.

263. Jude EB, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. *Diabetes Care* 24: 1433–1437, 2001. doi:10.2337/diacare.24.8.1433.

264. Judkins CP, Diep H, Broughton BR, Mast AE, Hooker EU, Miller AA, Selemidis S, Dusting GJ, Sobey CG, Drummond GR. Direct evidence of a role for Nox2 in superoxide production, reduced nitric oxide bioavailability, and early atherosclerotic plaque formation in ApoE-/- mice. *Am J Physiol Heart Circ Physiol* 298: H24–H32, 2010. doi:10.1152/ajpheart.00799.2009.

265. Kadlec AO, Beyer AM, Ait-Aissa K, Gutterman DD. Mitochondrial signaling in the vascular endothelium: beyond reactive oxygen species. *Basic Res Cardiol* 111: 26, 2016. doi:10.1007/s00395-016-0546-5.

266. Kaiser N, Sasson S, Feener EP, Boukobza-Vardi N, Higashi S, Moller DE, Davidheiser S, Przybylski RJ, King GL. Differential regulation of glucose transport and transporters by glucose in vascular endothelial and smooth muscle cells. *Diabetes* 42: 80–89, 1993. doi:10.2337/diab.42.1.80.

267. Kakoki M, Kim HS, Edgell CJ, Maeda N, Smithies O, Mattson DL. Amino acids as modulators of endothelium-derived nitric oxide. *Am J Physiol Renal Physiol* 291: F297–F304, 2006. doi:10.1152/ajprenal.00417.2005.

268. Kalucka J, Missiaen R, Georgiadou M, Schoors S, Lange C, De Bock K, Dewerchin M, Carmeliet P. Metabolic control of the cell cycle. *Cell Cycle* 14: 3379–3388, 2015. doi:10.1080/15384101.2015.1090068.

269. Kawahara H, Naito H, Takara K, Wakabayashi T, Kidoya H, Takakura N. Tumor endothelial cell-specific drug delivery system using apelin-conjugated liposomes. *PLoS One* 8: e65499, 2013. doi:10.1371/journal.pone.0065499.

270. Kearney MT. Changing the way we think about endothelial cell insulin sensitivity, nitric oxide, and the pathophysiology of type 2 diabetes: the FoxO is loose. *Diabetes* 62: 1386–1388, 2013. doi:10.2337/db13-0183.

271. Keating ST, Plutzky J, El-Osta A. Epigenetic Changes in Diabetes and Cardiovascular Risk. *Circ Res* 118: 1706–1722, 2016. doi:10.1161/CIRCRESAHA.116.306819.

272. Kim CS, Jung SB, Naqvi A, Hoffman TA, DeRicco J, Yamamori T, Cole MP, Jeon BH, Irani K. p53 impairs endothelium-dependent vasomotor function through transcriptional upregulation of p66shc. *Circ Res* 103: 1441–1450, 2008. doi:10.1161/CIRCRESAHA.108.181644.

273. Kim F, Tysseling KA, Rice J, Pham M, Haji L, Gallis BM, Baas AS, Paramsothy P, Giachelli CM, Corson MA, Raines EW. Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKKbeta. *Arterioscler Thromb Vasc Biol* 25: 989–994, 2005. doi:10.1161/01.ATV.0000160549.60980.a8.

274. Kim MK, Lun DS. Methods for integration of transcriptomic data in genome-scale metabolic models. *Comput Struct Biotechnol J* 11: 59–65, 2014. doi:10.1016/j.csbj.2014.08.009.

275. Kim YW, Byzova TV. Oxidative stress in angiogenesis and vascular disease. *Blood* 123: 625–631, 2014. doi:10.1182/blood-2013-09-512749.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

276. Kivelä R, Bry M, Robciuc MR, Räsänen M, Taavitsainen M, Silvola JM, Saraste A, Hulmi JJ, Anisimov A, Mäyränpää MI, Lindeman JH, Eklund L, Hellberg S, Hlushchuk R, Zhuang ZW, Simons M, Djonov V, Knuuti J, Mervaala E, Alitalo K. VEGF-B-induced vascular growth leads to metabolic reprogramming and ischemia resistance in the heart. *EMBO Mol Med* 6: 307–321, 2014.

277. Kleemann R, Gervois PP, Verschuren L, Staels B, Princen HM, Kooistra T. Fibrates down-regulate IL-1-stimulated C-reactive protein gene expression in hepatocytes by reducing nuclear p50-NFkappa B-C/EBP-beta complex formation. *Blood* 101: 545–551, 2003. doi:10.1182/blood-2002-06-1762.

278. Klotz L, Norman S, Vieira JM, Masters M, Rohling M, Dubé KN, Bollini S, Matsuzaki F, Carr CA, Riley PR. Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature* 522: 62–67, 2015. doi:10.1038/nature14483.

279. Kofler NM, Simons M. Angiogenesis versus arteriogenesis: neuropilin 1 modulation of VEGF signaling. *F1000Prime Rep* 7: 26, 2015. doi:10.12703/P7-26.

280. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. *Mediators Inflamm* 2014: 694312, 2014. doi:10.1155/2014/694312.

281. Kolderup A, Svihus B. Fructose Metabolism and Relation to Atherosclerosis, Type 2 Diabetes, and Obesity. *J Nutr Metab* 2015: 823081, 2015. doi:10.1155/2015/823081.

282. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. *Int J Vasc Med* 2012: 918267, 2012.

283. Kondo T, Vicent D, Suzuma K, Yanagisawa M, King GL, Holzenberger M, Kahn CR. Knockout of insulin and IGF-1 receptors on vascular endothelial cells protects against retinal neovascularization. *J Clin Invest* 111: 1835–1842, 2003. doi:10.1172/JCI200317455.

284. Kops GJ, Dansen TB, Polderman PE, Saarloos I, Wirtz KW, Coffer PJ, Huang TT, Bos JL, Medema RH, Burgering BM. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. *Nature* 419: 316–321, 2002. doi:10.1038/nature01036.

285. Kota SK, Meher LK, Jammula S, Kota SK, Krishna SV, Modi KD. Aberrant angiogenesis: the gateway to diabetic complications. *Indian J Endocrinol Metab* 16: 918–930, 2012. doi:10.4103/2230-8210.102992.

286. Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. *Cancer Res* 66: 632–637, 2006. doi:10.1158/0008-5472.CAN-05-3260.

287. Kovács K, Decatur C, Toro M, Pham DG, Liu H, Jing Y, Murray TG, Lampidis TJ, Merchan JR. 2-Deoxy-Glucose Downregulates Endothelial AKT and ERK via Interference With *N*-Linked Glycosylation, Induction of Endoplasmic Reticulum Stress, and GSK3β Activation. *Mol Cancer Ther* 15: 264–275, 2016. doi:10.1158/1535-7163.MCT-14-0315.

288. Koziel A, Woyda-Ploszczyca A, Kicinska A, Jarmuszkiewicz W. The influence of high glucose on the aerobic metabolism of endothelial EA.hy926 cells. *Pflugers Arch* 464: 657–669, 2012. doi:10.1007/s00424-012-1156-1.

289. Krotova K, Patel JM, Block ER, Zharikov S. Endothelial arginase II responds to pharmacological inhibition by elevation in protein level. *Mol Cell Biochem* 343: 211–216, 2010. doi:10.1007/s11010-010-0515-5.

290. Krotova K, Patel JM, Block ER, Zharikov S. Hypoxic upregulation of arginase II in human lung endothelial cells. *Am J Physiol Cell Physiol* 299: C1541–C1548, 2010. doi:10.1152/ajpcell.00068.2010.

291. Krützfeldt A, Spahr R, Mertens S, Siegmund B, Piper HM. Metabolism of exogenous substrates by coronary endothelial cells in culture. *J Mol Cell Cardiol* 22: 1393–1404, 1990. doi:10.1016/0022-2828(90)90984-A.

292. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, Kumagai K, Kawai T, Hashimoto S, Kobayashi T, Sato M, Tokuyama K, Nishimura S, Tsunoda M, Ide T, Murakami K, Yamazaki T, Ezaki O, Kawamura K, Masuda H, Moroi M, Sugi K, Oike Y, Shimokawa H, Yanagihara N, Tsutsui M, Terauchi Y, Tobe K, Nagai R, Kamata K, Inoue K, Kodama T, Ueki K, Kadowaki T. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 13: 294–307, 2011. doi:10.1016/j.cmet.2011.01.018.

293. Kuczynski EA, Yin M, Bar-Zion A, Lee CR, Butz H, Man S, Daley F, Vermeulen PB, Yousef GM, Foster FS, Reynolds AR, Kerbel RS. Co-option of Liver Vessels and Not Sprouting Angiogenesis Drives Acquired Sorafenib Resistance in Hepatocellular Carcinoma. *J Natl Cancer Inst* 108: djw030, 2016. doi:10.1093/jnci/djw030.

294. Kukidome D, Nishikawa T, Sonoda K, Imoto K, Fujisawa K, Yano M, Motoshima H, Taguchi T, Matsumura T, Araki E. 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: 120–127, 2006. doi:10.2337/diabetes.55.01.06.db05-0943.

295. Kumagai R, Lu X, Kassab GS. Role of glycocalyx in flow-induced production of nitric oxide and reactive oxygen species. *Free Radic Biol Med* 47: 600–607, 2009. doi:10.1016/j.freeradbiomed.2009.05.034.

296. Kumar A, Kumar S, Vikram A, Hoffman TA, Naqvi A, Lewarchik CM, Kim YR, Irani K. Histone and DNA methylation-mediated epigenetic downregulation of endothelial Kruppel-like factor 2 by low-density lipoprotein cholesterol. *Arterioscler Thromb Vasc Biol* 33: 1936–1942, 2013. doi:10.1161/ATVBAHA.113.301765.

297. Kumar VB, Viji RI, Kiran MS, Sudhakaran PR. Endothelial cell response to lactate: implication of PAR modification of VEGF. *J Cell Physiol* 211: 477–485, 2007. doi:10.1002/jcp.20955.

298. Kurosu T, Ohga N, Hida Y, Maishi N, Akiyama K, Kakuguchi W, Kuroshima T, Kondo M, Akino T, Totsuka Y, Shindoh M, Higashino F, Hida K. HuR keeps an angiogenic switch on by stabilising mRNA of VEGF and COX-2 in tumour endothelium. *Br J Cancer* 104: 819–829, 2011. doi:10.1038/bjc.2011.20.

299. Kurtoglu M, Maher JC, Lampidis TJ. Differential toxic mechanisms of 2-deoxy-D-glucose versus 2-fluorodeoxy-D-glucose in hypoxic and normoxic tumor cells. *Antioxid Redox Signal* 9: 1383–1390, 2007. doi:10.1089/ars.2007.1714.

300. Langbein H, Brunssen C, Hofmann A, Cimalla P, Brux M, Bornstein SR, Deussen A, Koch E, Morawietz H. NADPH oxidase 4 protects against development of endothelial dysfunction and atherosclerosis in LDL receptor deficient mice. *Eur Heart J* 37: 1753–1761, 2016. doi:10.1093/eurheartj/ehv564.

301. Laufs U, La Fata V, Plutzky J, Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97: 1129–1135, 1998. doi:10.1161/01.CIR.97.12.1129.

302. Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, Tarpey M, Fukai T, Harrison DG. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 103: 1282–1288, 2001. doi:10.1161/01.CIR.103.9.1282.

303. Le Clainche C, Carlier MF. Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol Rev* 88: 489–513, 2008. doi:10.1152/physrev.00021.2007.

304. Léauté-Labrèze C, Hoeger P, Mazereeuw-Hautier J, Guibaud L, Baselga E, Posiunas G, Phillips RJ, Caceres H, Lopez Gutierrez JC, Ballona R, Friedlander SF, Powell J, Perek D, Metz B, Barbarot S, Maruani A, Szalai ZZ, Krol A, Boccara O, Foelster-Holst R, Febrer Bosch MI, Su J, Buckova H, Torrelo A, Cambazard F, Grantzow R, Wargon O, Wyrzykowski D, Roessler J, Bernabeu-Wittel J, Valencia AM, Przewratil P, Glick S, Pope E, Birchall N, Benjamin L, Mancini AJ, Vabres P, Souteyrand P, Frieden IJ, Berul CI, Mehta CR, Prey S, Boralevi F, Morgan CC, Heritier S, Delarue A, Voisard JJ. A randomized, controlled trial of oral propranolol in infantile hemangioma. *N Engl J Med* 372: 735–746, 2015. doi:10.1056/NEJMoa1404710.

305. Lee DC, Sohn HA, Park ZY, Oh S, Kang YK, Lee KM, Kang M, Jang YJ, Yang SJ, Hong YK, Noh H, Kim JA, Kim DJ, Bae KH, Kim DM, Chung SJ, Yoo HS, Yu DY, Park KC, Yeom YI. A lactate-induced response to hypoxia. *Cell* 161: 595–609, 2015. doi:10.1016/j.cell.2015.03.011.

306. Lee JV, Carrer A, Shah S, Snyder NW, Wei S, Venneti S, Worth AJ, Yuan ZF, Lim HW, Liu S, Jackson E, Aiello NM, Haas NB, Rebbeck TR, Judkins A, Won KJ, Chodosh LA, Garcia BA, Stanger BZ, Feldman MD, Blair IA, Wellen KE. Akt-dependent metabolic reprogramming regulates tumor cell histone acetylation. *Cell Metab* 20: 306–319, 2014. doi:10.1016/j.cmet.2014.06.004.

307. Lee P, Wang CC, Adamis AP. Ocular neovascularization: an epidemiologic review. *Surv Ophthalmol* 43: 245–269, 1998. doi:10.1016/S0039-6257(98)00035-6.

308. Leighton B, Curi R, Hussein A, Newsholme EA. Maximum activities of some key enzymes of glycolysis, glutaminolysis, Krebs cycle and fatty acid utilization in bovine pulmonary endothelial cells. *FEBS Lett* 225: 93–96, 1987. doi:10.1016/0014-5793(87)81137-7.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

309. Leopold JA. Antioxidants and coronary artery disease: from pathophysiology to preventive therapy. *Coron Artery Dis* 26: 176–183, 2015. doi:10.1097/MCA.0000000000000187.

310. Leopold JA, Cap A, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase deficiency promotes endothelial oxidant stress and decreases endothelial nitric oxide bioavailability. *FASEB J* 15: 1771–1773, 2001.

311. Leopold JA, Maron BA. Molecular Mechanisms of Pulmonary Vascular Remodeling in Pulmonary Arterial Hypertension. *Int J Mol Sci* 17: E761, 2016. doi:10.3390/ijms17050761.

312. Leopold JA, Walker J, Scribner AW, Voetsch B, Zhang YY, Loscalzo AJ, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase modulates vascular endothelial growth factor-mediated angiogenesis. *J Biol Chem* 278: 32100–32106, 2003. doi:10.1074/jbc.M301293200.

313. Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. *Arterioscler Thromb Vasc Biol* 23: 411–417, 2003. doi:10.1161/01.ATV.0000056744.26901.BA.

314. Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R. Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 99: 4391–4396, 2002. doi:10.1073/pnas.032074999.

315. Lewis CA, Parker SJ, Fiske BP, McCloskey D, Gui DY, Green CR, Vokes NI, Feist AM, Vander Heiden MG, Metallo CM. Tracing compartmentalized NADPH metabolism in the cytosol and mitochondria of mammalian cells. *Mol Cell* 55: 253–263, 2014. doi:10.1016/j.molcel.2014.05.008.

316. Li D, Li P, Guo Z, Wang H, Pan W. Downregulation of miR-382 by propranolol inhibits the progression of infantile hemangioma via the PTEN-mediated AKT/mTOR pathway. *Int J Mol Med* 39: 757–763, 2017. doi:10.3892/ijmm.2017.2863.

317. Li FY, Lam KS, Tse HF, Chen C, Wang Y, Vanhoutte PM, Xu A. 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: 1267–1277, 2012. doi:10.1161/CIRCULATIONAHA.112.108159.

318. Li H, Horke S, Förstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis* 237: 208–219, 2014. doi:10.1016/j.atherosclerosis.2014.09.001.

319. Li M, Li C, Allen A, Stanley CA, Smith TJ. Glutamate dehydrogenase: structure, allosteric regulation, and role in insulin homeostasis. *Neurochem Res* 39: 433–445, 2014. doi:10.1007/s11064-013-1173-2.

320. Li Q, Park K, Li C, Rask-Madsen C, Mima A, Qi W, Mizutani K, Huang P, King GL. Induction of vascular insulin resistance and endothelin-1 expression and acceleration of atherosclerosis by the overexpression of protein kinase C-β isoform in the endothelium. *Circ Res* 113: 418–427, 2013. doi:10.1161/CIRCRESAHA.113.301074.

321. Li XF, O'Donoghue JA. Hypoxia in microscopic tumors. *Cancer Lett* 264: 172–180, 2008. doi:10.1016/j.canlet.2008.02.037.

322. Libby P, Bornfeldt KE, Tall AR. Atherosclerosis: Successes, Surprises, and Future Challenges. *Circ Res* 118: 531–534, 2016. doi:10.1161/CIRCRESAHA.116.308334.

323. Liersch R, Nay F, Lu L, Detmar M. Induction of lymphatic endothelial cell differentiation in embryoid bodies. *Blood* 107: 1214–1216, 2006. doi:10.1182/blood-2005-08-3400.

324. Lilly B. We have contact: endothelial cell-smooth muscle cell interactions. *Physiology (Bethesda)* 29: 234–241, 2014.

325. Lira VA, Brown DL, Lira AK, Kavazis AN, Soltow QA, Zeanah EH, Criswell DS. Nitric oxide and AMPK cooperatively regulate PGC-1 in skeletal muscle cells. *J Physiol* 588: 3551–3566, 2010. doi:10.1113/jphysiol.2010.194035.

326. Liu J, Wang C, Liu F, Lu Y, Cheng J. Metabonomics revealed xanthine oxidase-induced oxidative stress and inflammation in the pathogenesis of diabetic nephropathy. *Anal Bioanal Chem* 407: 2569–2579, 2015. doi:10.1007/s00216-015-8481-0.

327. Liu W, Wang T, He X, Liu X, Wang B, Liu Y, Li Z, Tan R, Ding C, Wang H, Zeng H. CYP2J2 Overexpression Increases EETs and Protects Against HFD-Induced Atherosclerosis in ApoE-/- Mice. *J Cardiovasc Pharmacol* 67: 491–502, 2016. doi:10.1097/FJC.0000000000000371.

328. Liu X, Wang D, Zhao Y, Tu B, Zheng Z, Wang L, Wang H, Gu W, Roeder RG, Zhu WG. Methyltransferase Set7/9 regulates p53 activity by interacting with Sirtuin 1 (SIRT1). *Proc Natl Acad Sci USA* 108: 1925–1930, 2011. doi:10.1073/pnas.1019619108.

329. Lobatto ME, Fuster V, Fayad ZA, Mulder WJ. Perspectives and opportunities for nanomedicine in the management of atherosclerosis. *Nat Rev Drug Discov* 10: 835–852, 2011. doi:10.1038/nrd3578.

330. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. *Diabetes* 53: 195–199, 2004. doi:10.2337/diabetes.53.1.195.

331. Lorenzi M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Exp Diabetes Res* 2007: 61038, 2007. doi:10.1155/2007/61038.

332. Lorin J, Zeller M, Guilland JC, Cottin Y, Vergely C, Rochette L. Arginine and nitric oxide synthase: regulatory mechanisms and cardiovascular aspects. *Mol Nutr Food Res* 58: 101–116, 2014. doi:10.1002/mnfr.201300033.

333. Loyer X, Potteaux S, Vion AC, Guérin CL, Boulkroun S, Rautou PE, Ramkhelawon B, Esposito B, Dalloz M, Paul JL, Julia P, Maccario J, Boulanger CM, Mallat Z, Tedgui A. Inhibition of microRNA-92a prevents endothelial dysfunction and atherosclerosis in mice. *Circ Res* 114: 434–443, 2014. doi:10.1161/CIRCRESAHA.114.302213.

334. Luchsinger LL, de Almeida MJ, Corrigan DJ, Mumau M, Snoeck HW. Mitofusin 2 maintains haematopoietic stem cells with extensive lymphoid potential. *Nature* 529: 528–531, 2016. doi:10.1038/nature16500.

335. Luo B, Soesanto Y, McClain DA. Protein modification by *O*-linked GlcNAc reduces angiogenesis by inhibiting Akt activity in endothelial cells. *Arterioscler Thromb Vasc Biol* 28: 651–657, 2008. doi:10.1161/ATVBAHA.107.159533.

336. Luo W, Hu Q, Wang D, Deeb KK, Ma Y, Morrison CD, Liu S, Johnson CS, Trump DL. Isolation and genome-wide expression and methylation characterization of CD31+ cells from normal and malignant human prostate tissue. *Oncotarget* 4: 1472–1483, 2013. doi:10.18632/oncotarget.1269.

337. Machado RF, Londhe Nerkar MV, Dweik RA, Hammel J, Janocha A, Pyle J, Laskowski D, Jennings C, Arroliga AC, Erzurum SC. Nitric oxide and pulmonary arterial pressures in pulmonary hypertension. *Free Radic Biol Med* 37: 1010–1017, 2004. doi:10.1016/j.freeradbiomed.2004.06.039.

338. Madsen-Bouterse SA, Mohammad G, Kanwar M, Kowluru RA. Role of mitochondrial DNA damage in the development of diabetic retinopathy, and the metabolic memory phenomenon associated with its progression. *Antioxid Redox Signal* 13: 797–805, 2010. doi:10.1089/ars.2009.2932.

339. Maishi N, Ohba Y, Akiyama K, Ohga N, Hamada J, Nagao-Kitamoto H, Alam MT, Yamamoto K, Kawamoto T, Inoue N, Taketomi A, Shindoh M, Hida Y, Hida K. Tumour endothelial cells in high metastatic tumours promote metastasis via epigenetic dysregulation of biglycan. *Sci Rep* 6: 28039, 2016. doi:10.1038/srep28039.

340. Majzner K, Chlopicki S, Baranska M. Lipid droplets formation in human endothelial cells in response to polyunsaturated fatty acids and 1-methyl-nicotinamide (MNA); confocal Raman imaging and fluorescence microscopy studies. *J Biophotonics* 9: 396–405, 2016. doi:10.1002/jbio.201500134.

341. Makanya AN, Hlushchuk R, Djonov VG. Intussusceptive angiogenesis and its role in vascular morphogenesis, patterning, and remodeling. *Angiogenesis* 12: 113–123, 2009. doi:10.1007/s10456-009-9129-5.

342. Mäkinen T, Veikkola T, Mustjoki S, Karpanen T, Catimel B, Nice EC, Wise L, Mercer A, Kowalski H, Kerjaschki D, Stacker SA, Achen MG, Alitalo K. Isolated lymphatic endothelial cells transduce growth, survival and migratory signals via the VEGF-C/D receptor VEGFR-3. *EMBO J* 20: 4762–4773, 2001. doi:10.1093/emboj/20.17.4762.

343. Makino A, Dai A, Han Y, Youssef KD, Wang W, Donthamsetty R, Scott BT, Wang H, Dillmann WH. *O*-GlcNAcase overexpression reverses coronary endothelial cell dysfunction in type 1 diabetic mice. *Am J Physiol Cell Physiol* 309: C593–C599, 2015. doi:10.1152/ajpcell.00069.2015.

344. Manalo DJ, Rowan A, Lavoie T, Natarajan L, Kelly BD, Ye SQ, Garcia JG, Semenza GL. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. *Blood* 105: 659–669, 2005. doi:10.1182/blood-2004-07-2958.

345. Mani S, Untereiner A, Wu L, Wang R. Hydrogen sulfide and the pathogenesis of atherosclerosis. *Antioxid Redox Signal* 20: 805–817, 2014. doi:10.1089/ars.2013.5324.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

346. Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiol Rev* 83: 183–252, 2003. doi:10.1152/physrev.00022.2002.

347. Mapanga RF, Essop MF. Damaging effects of hyperglycemia on cardiovascular function: spotlight on glucose metabolic pathways. *Am J Physiol Heart Circ Physiol* 310: H153–H173, 2016. doi:10.1152/ajpheart.00206.2015.

348. Marcelo KL, Goldie LC, Hirschi KK. Regulation of endothelial cell differentiation and specification. *Circ Res* 112: 1272–1287, 2013. doi:10.1161/CIRCRESAHA.113.300506.

349. Marsh SA, Collins HE, Chatham JC. Protein *O*-GlcNAcylation and cardiovascular (patho)physiology. *J Biol Chem* 289: 34449–34456, 2014. doi:10.1074/jbc.R114.585984.

350. Martin A, Komada MR, Sane DC. Abnormal angiogenesis in diabetes mellitus. *Med Res Rev* 23: 117–145, 2003. doi:10.1002/med.10024.

351. Martinez-Corral I, Ulvmar MH, Stanczuk L, Tatin F, Kizhatil K, John SW, Alitalo K, Ortega S, Makinen T. Nonvenous origin of dermal lymphatic vasculature. *Circ Res* 116: 1649–1654, 2015. doi:10.1161/CIRCRESAHA.116.306170.

352. Mashek DG, Coleman RA. Cellular fatty acid uptake: the contribution of metabolism. *Curr Opin Lipidol* 17: 274–278, 2006. doi:10.1097/01.mol.0000226119.20307.2b.

353. Mason NA, Springall DR, Burke M, Pollock J, Mikhail G, Yacoub MH, Polak JM. High expression of endothelial nitric oxide synthase in plexiform lesions of pulmonary hypertension. *J Pathol* 185: 313–318, 1998. doi:10.1002/(SICI)1096-9896(199807)185:3<313::AID-PATH93>3.0.CO;2-8.

354. Masri FA, Comhair SA, Dostanic-Larson I, Kaneko FT, Dweik RA, Arroliga AC, Erzurum SC. Deficiency of lung antioxidants in idiopathic pulmonary arterial hypertension. *Clin Transl Sci* 1: 99–106, 2008. doi:10.1111/j.1752-8062.2008.00035.x.

355. Masri FA, Xu W, Comhair SA, Asosingh K, Koo M, Vasanji A, Drazba J, Anand-Apte B, Erzurum SC. Hyperproliferative apoptosis-resistant endothelial cells in idiopathic pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 293: L548–L554, 2007. doi:10.1152/ajplung.00428.2006.

356. Masters C. Interactions between glycolytic enzymes and components of the cytomatrix. *J Cell Biol* 99: 222s–225s, 1984. doi:10.1083/jcb.99.1.222s.

357. Matafome P, Sena C, Seiça R. Methylglyoxal, obesity, and diabetes. *Endocrine* 43: 472–484, 2013. doi:10.1007/s12020-012-9795-8.

358. Matsuda K, Ohga N, Hida Y, Muraki C, Tsuchiya K, Kurosu T, Akino T, Shih SC, Totsuka Y, Klagsbrun M, Shindoh M, Hida K. Isolated tumor endothelial cells maintain specific character during long-term culture. *Biochem Biophys Res Commun* 394: 947–954, 2010. doi:10.1016/j.bbrc.2010.03.089.

359. Matsumoto S, Koshiishi I, Inoguchi T, Nawata H, Utsumi H. Confirmation of superoxide generation via xanthine oxidase in streptozotocin-induced diabetic mice. *Free Radic Res* 37: 767–772, 2003. doi:10.1080/1071576031000107344.

360. Mazzone M, Dettori D, de Oliveira RL, Loges S, Schmidt T, Jonckx B, Tian YM, Lanahan AA, Pollard P, de Almodovar CR, De Smet F, Vinckier S, Aragonés J, Debackere K, Luttun A, Wyns S, Jordan B, Pisacane A, Gallez B, Lampugnani MG, Dejana E, Simons M, Ratcliffe P, Maxwell P, Carmeliet P. Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. *Cell* 136: 839–851, 2009. doi:10.1016/j.cell.2009.01.020.

361. McDermott LA, Iyer P, Vernetti L, Rimer S, Sun J, Boby M, Yang T, Fioravanti M, O'Neill J, Wang L, Drakes D, Katt W, Huang Q, Cerione R. Design and evaluation of novel glutaminase inhibitors. *Bioorg Med Chem* 24: 1819–1839, 2016. doi:10.1016/j.bmc.2016.03.009.

362. McDonnell E, Crown SB, Fox DB, Kitir B, Ilkayeva OR, Olsen CA, Grimsrud PA, Hirschey MD. Lipids Reprogram Metabolism to Become a Major Carbon Source for Histone Acetylation. *Cell Reports* 17: 1463–1472, 2016. doi:10.1016/j.celrep.2016.10.012.

363. McIntyre A, Harris AL. The Role of pH Regulation in Cancer Progression. *Recent Results Cancer Res* 207: 93–134, 2016. doi:10.1007/978-3-319-42118-6_5.

364. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. *Circulation* 114: 1417–1431, 2006. doi:10.1161/CIRCULATIONAHA.104.503540.

365. McLellan AC, Thornalley PJ, Benn J, Sonksen PH. Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci (Lond)* 87: 21–29, 1994. doi:10.1042/cs0870021.

366. Meller S, Meyer HA, Bethan B, Dietrich D, Maldonado SG, Lein M, Montani M, Reszka R, Schatz P, Peter E, Stephan C, Jung K, Kamlage B, Kristiansen G. Integration of tissue metabolomics, transcriptomics and immunohistochemistry reveals ERG- and gleason score-specific metabolomic alterations in prostate cancer. *Oncotarget* 7: 1421–1438, 2016. doi:10.18632/oncotarget.6370.

367. Menden H, Welak S, Cossette S, Ramchandran R, Sampath V. Lipopolysaccharide (LPS)-mediated angiopoietin-2-dependent autocrine angiogenesis is regulated by NADPH oxidase 2 (Nox2) in human pulmonary microvascular endothelial cells. *J Biol Chem* 290: 5449–5461, 2015. doi:10.1074/jbc.M114.600692.

368. Menghini R, Casagrande V, Cardellini M, Ballanti M, Davato F, Cardolini I, Stoehr R, Fabrizi M, Morelli M, Anemona L, Bernges I, Schwedhelm E, Ippoliti A, Mauriello A, Böger RH, Federici M. FoxO1 regulates asymmetric dimethylarginine via downregulation of dimethylaminohydrolase 1 in human endothelial cells and subjects with atherosclerosis. *Atherosclerosis* 242: 230–235, 2015. doi:10.1016/j.atherosclerosis.2015.07.026.

369. Merchan JR, Kovács K, Railsback JW, Kurtoglu M, Jing Y, Piña Y, Gao N, Murray TG, Lehrman MA, Lampidis TJ. Antiangiogenic activity of 2-deoxy-D-glucose. *PLoS One* 5: e13699, 2010. doi:10.1371/journal.pone.0013699.

370. Miele C, Riboulet A, Maitan MA, Oriente F, Romano C, Formisano P, Giudicelli J, Beguinot F, Van Obberghen E. Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. *J Biol Chem* 278: 47376–47387, 2003. doi:10.1074/jbc.M301088200.

371. Miranda CH, de Carvalho Borges M, Schmidt A, Marin-Neto JA, Pazin-Filho A. Evaluation of the endothelial glycocalyx damage in patients with acute coronary syndrome. *Atherosclerosis* 247: 184–188, 2016. doi:10.1016/j.atherosclerosis.2016.02.023.

372. Mitchell JA, Hecker M, Anggård EE, Vane JR. Cultured endothelial cells maintain their L-arginine level despite the continuous release of EDRF. *Eur J Pharmacol* 182: 573–576, 1990. doi:10.1016/0014-2999(90)90058-E.

373. Mo C, Wang L, Zhang J, Numazawa S, Tang H, Tang X, Han X, Li J, Yang M, Wang Z, Wei D, Xiao H. 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: 574–588, 2014. doi:10.1089/ars.2012.5116.

374. Molina-Sánchez P, Chèvre R, Rius C, Fuster JJ, Andrés V. Loss of p27 phosphorylation at Ser10 accelerates early atherogenesis by promoting leukocyte recruitment via RhoA/ROCK. *J Mol Cell Cardiol* 84: 84–94, 2015. doi:10.1016/j.yjmcc.2015.04.013.

375. Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca^{2+} but requires phosphorylation by Akt at Ser(1179). *J Biol Chem* 276: 30392–30398, 2001. doi:10.1074/jbc.M103702200.

376. Montagnani M, Golovchenko I, Kim I, Koh GY, Goalstone ML, Mundhekar AN, Johansen M, Kucik DF, Quon MJ, Draznin B. Inhibition of phosphatidylinositol 3-kinase enhances mitogenic actions of insulin in endothelial cells. *J Biol Chem* 277: 1794–1799, 2002. doi:10.1074/jbc.M103728200.

377. Morgan PE, Sheahan PJ, Davies MJ. Perturbation of human coronary artery endothelial cell redox state and NADPH generation by methylglyoxal. *PLoS One* 9: e86564, 2014. doi:10.1371/journal.pone.0086564.

378. Mori M. Regulation of nitric oxide synthesis and apoptosis by arginase and arginine recycling. *J Nutr* 137, Suppl 2: 1616S–1620S, 2007.

379. Morris SM Jr. Recent advances in arginine metabolism: roles and regulation of the arginases. *Br J Pharmacol* 157: 922–930, 2009. doi:10.1111/j.1476-5381.2009.00278.x.

380. Morris SM, Jr, Bhamidipati D, Kepka-Lenhart D. Human type II arginase: sequence analysis and tissue-specific expression. *Gene* 193: 157–161, 1997. doi:10.1016/S0378-1119(97)00099-1.

381. Morrison RF, Seidel ER. Vascular endothelial cell proliferation: regulation of cellular polyamines. *Cardiovasc Res* 29: 841–847, 1995. doi:10.1016/S0008-6363(96)88621-4.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

382. Moussaieff A, Rouleau M, Kitsberg D, Cohen M, Levy G, Barasch D, Nemirovski A, Shen-Orr S, Laevsky I, Amit M, Bomze D, Elena-Herrmann B, Scherf T, Nissim-Rafinia M, Kempa S, Itskovitz-Eldor J, Meshorer E, Aberdam D, Nahmias Y. Glycolysis-mediated changes in acetyl-CoA and histone acetylation control the early differentiation of embryonic stem cells. *Cell Metab* 21: 392–402, 2015. doi:10.1016/j.cmet.2015.02.002.

383. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER III, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB; Writing Group Members; American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. *Circulation* 133: e38–e360, 2016. doi:10.1161/CIR.0000000000000350.

384. Mugoni V, Postel R, Catanzaro V, De Luca E, Turco E, Digilio G, Silengo L, Murphy MP, Medana C, Stainier DY, Bakkers J, Santoro MM. Ubiad1 is an antioxidant enzyme that regulates eNOS activity by CoQ10 synthesis. *Cell* 152: 504–518, 2013. doi:10.1016/j.cell.2013.01.013.

385. Murphy C, Kanaganayagam GS, Jiang B, Chowienczyk PJ, Zbinden R, Saha M, Rahman S, Shah AM, Marber MS, Kearney MT. Vascular dysfunction and reduced circulating endothelial progenitor cells in young healthy UK South Asian men. *Arterioscler Thromb Vasc Biol* 27: 936–942, 2007. doi:10.1161/01.ATV.0000258788.11372.d0.

386. Murphy EA, Majeti BK, Barnes LA, Makale M, Weis SM, Lutu-Fuga K, Wrasidlo W, Cheresh DA. Nanoparticle-mediated drug delivery to tumor vasculature suppresses metastasis. *Proc Natl Acad Sci USA* 105: 9343–9348, 2008. doi:10.1073/pnas.0803728105.

387. Nakayama K, Nakayama M, Iwabuchi M, Terawaki H, Sato T, Kohno M, Ito S. Plasma alpha-oxoaldehyde levels in diabetic and nondiabetic chronic kidney disease patients. *Am J Nephrol* 28: 871–878, 2008. doi:10.1159/000139653.

388. Nardo G, Favaro E, Curtarello M, Moserle L, Zulato E, Persano L, Rossi E, Esposito G, Crescenzi M, Casanovas O, Sattler U, Mueller-Klieser W, Biesalski B, Thews O, Canese R, Iorio E, Zanovello P, Amadori A, Indraccolo S. Glycolytic phenotype and AMP kinase modify the pathologic response of tumor xenografts to VEGF neutralization. *Cancer Res* 71: 4214–4225, 2011. doi:10.1158/0008-5472.CAN-11-0242.

388a. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, Rand L, Siebert C; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329: 977–986, 1993. doi:10.1056/NEJM199309303291401.

389. Nemoto S, Finkel T. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. *Science* 295: 2450–2452, 2002. doi:10.1126/science.1069004.

390. Newsholme P, Procopio J, Lima MM, Pithon-Curi TC, Curi R. Glutamine and glutamate–their central role in cell metabolism and function. *Cell Biochem Funct* 21: 1–9, 2003. doi:10.1002/cbf.1003.

391. Nicenboim J, Malkinson G, Lupo T, Asaf L, Sela Y, Mayseless O, Gibbs-Bar L, Senderovich N, Hashimshony T, Shin M, Jerafi-Vider A, Avraham-Davidi I, Krupalnik V, Hofi R, Almog G, Astin JW, Golani O, Ben-Dor S, Crosier PS, Herzog W, Lawson ND, Hanna JH, Yanai I, Yaniv K. Lymphatic vessels arise from specialized angioblasts within a venous niche. *Nature* 522: 56–61, 2015. doi:10.1038/nature14425.

392. Nikiforov A, Dölle C, Niere M, Ziegler M. Pathways and subcellular compartmentation of NAD biosynthesis in human cells: from entry of extracellular precursors to mitochondrial NAD generation. *J Biol Chem* 286: 21767–21778, 2011. doi:10.1074/jbc.M110.213298.

393. Nolan DJ, Ginsberg M, Israely E, Palikuqi B, Poulos MG, James D, Ding BS, Schachterle W, Liu Y, Rosenwaks Z, Butler JM, Xiang J, Rafii A, Shido K, Rabbany SY, Elemento O, Rafii S. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell* 26: 204–219, 2013. doi:10.1016/j.devcel.2013.06.017.

394. Notsu Y, Yano S, Shibata H, Nagai A, Nabika T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. *Atherosclerosis* 239: 61–66, 2015. doi:10.1016/j.atherosclerosis.2014.12.030.

395. Novodvorsky P, Chico TJ. The role of the transcription factor KLF2 in vascular development and disease. *Prog Mol Biol Transl Sci* 124: 155–188, 2014. doi:10.1016/B978-0-12-386930-2.00007-0.

396. Obrosova IG, Minchenko AG, Vasupuram R, White L, Abatan OI, Kumagai AK, Frank RN, Stevens MJ. Aldose reductase inhibitor fidarestat prevents retinal oxidative stress and vascular endothelial growth factor overexpression in streptozotocin-diabetic rats. *Diabetes* 52: 864–871, 2003. doi:10.2337/diabetes.52.3.864.

397. Odani H, Shinzato T, Matsumoto Y, Usami J, Maeda K. Increase in three alpha,beta-dicarbonyl compound levels in human uremic plasma: specific in vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 256: 89–93, 1999. doi:10.1006/bbrc.1999.0221.

398. Ohga N, Ishikawa S, Maishi N, Akiyama K, Hida Y, Kawamoto T, Sadamoto Y, Osawa T, Yamamoto K, Kondoh M, Ohmura H, Shinohara N, Nonomura K, Shindoh M, Hida K. Heterogeneity of tumor endothelial cells: comparison between tumor endothelial cells isolated from high- and low-metastatic tumors. *Am J Pathol* 180: 1294–1307, 2012. doi:10.1016/j.ajpath.2011.11.035.

399. Ohkawara H, Ishibashi T, Saitoh S, Inoue N, Sugimoto K, Kamioka M, Uekita H, Kaneshiro T, Ando K, Takuwa Y, Maruyama Y, Takeishi Y. Preventive effects of pravastatin on thrombin-triggered vascular responses via Akt/eNOS and RhoA/Rac1 pathways in vivo. *Cardiovasc Res* 88: 492–501, 2010. doi:10.1093/cvr/cvq221.

400. Okabe J, Orlowski C, Balcerczyk A, Tikellis C, Thomas MC, Cooper ME, El-Osta A. Distinguishing hyperglycemic changes by Set7 in vascular endothelial cells. *Circ Res* 110: 1067–1076, 2012. doi:10.1161/CIRCRESAHA.112.266171.

401. Oldendorf WH, Cornford ME, Brown WJ. The large apparent work capability of the blood-brain barrier: a study of the mitochondrial content of capillary endothelial cells in brain and other tissues of the rat. *Ann Neurol* 1: 409–417, 1977. doi:10.1002/ana.410010502.

402. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling: in control of vascular function. *Nat Rev Mol Cell Biol* 7: 359–371, 2006. doi:10.1038/nrm1911.

403. Opitz CA, Heiland I. Dynamics of NAD-metabolism: everything but constant. *Biochem Soc Trans* 43: 1127–1132, 2015. doi:10.1042/BST20150133.

404. Osanai T, Nakamura M, Sasaki S, Tomita H, Saitoh M, Osawa H, Yamabe H, Murakami S, Magota K, Okumura K. Plasma concentration of coupling factor 6 and cardiovascular events in patients with end-stage renal disease. *Kidney Int* 64: 2291–2297, 2003. doi:10.1046/j.1523-1755.2003.00334.x.

405. Osanai T, Okada S, Sirato K, Nakano T, Saitoh M, Magota K, Okumura K. Mitochondrial coupling factor 6 is present on the surface of human vascular endothelial cells and is released by shear stress. *Circulation* 104: 3132–3136, 2001. doi:10.1161/hc5001.100832.

406. Osanai T, Sasaki S, Kamada T, Fujiwara N, Nakano T, Tomita H, Matsunaga T, Magota K, Okumura K. Circulating coupling factor 6 in human hypertension: role of reactive oxygen species. *J Hypertens* 21: 2323–2328, 2003. doi:10.1097/00004872-200312000-00021.

407. Osawa T, Ohga N, Akiyama K, Hida Y, Kitayama K, Kawamoto T, Yamamoto K, Maishi N, Kondoh M, Onodera Y, Fujie M, Shinohara N, Nonomura K, Shindoh M, Hida K. Lysyl oxidase secreted by tumour endothelial cells promotes angiogenesis and metastasis. *Br J Cancer* 109: 2237–2247, 2013. doi:10.1038/bjc.2013.535.

408. Ouzzine M, Gulberti S, Ramalanjaona N, Magdalou J, Fournel-Gigleux S. The UDP-glucuronosyltransferases of the blood-brain barrier: their role in drug metabolism and detoxication. *Front Cell Neurosci* 8: 349, 2014. doi:10.3389/fncel.2014.00349.

409. Pahwa R, Nallasamy P, Jialal I. Toll-like receptors 2 and 4 mediate hyperglycemia induced macrovascular aortic endothelial cell inflammation and perturbation of the endothelial glycocalyx. *J Diabetes Complications* 30: 563–572, 2016. doi:10.1016/j.jdiacomp.2016.01.014.

410. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MA, Sheedy FJ, Gleeson LE, van den Bosch MW, Quinn SR, Domingo-Fernandez R, Johnston DG, Jiang JK, Israelsen WJ, Keane J, Thomas C, Clish C, Vander Heiden M, Xavier RJ, O'Neill LA. Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the warburg effect in LPS-activated macrophages. *Cell Metab* 21: 65–80, 2015. doi:10.1016/j.cmet.2014.12.005.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

411. Pan S, World CJ, Kovacs CJ, Berk BC. Glucose 6-phosphate dehydrogenase is regulated through c-Src-mediated tyrosine phosphorylation in endothelial cells. *Arterioscler Thromb Vasc Biol* 29: 895–901, 2009. doi:10.1161/ATVBAHA.109.184812.

412. Panchatcharam M, Salous AK, Brandon J, Miriyala S, Wheeler J, Patil P, Sunkara M, Morris AJ, Escalante-Alcalde D, Smyth SS. Mice with targeted inactivation of ppap2b in endothelial and hematopoietic cells display enhanced vascular inflammation and permeability. *Arterioscler Thromb Vasc Biol* 34: 837–845, 2014. doi:10.1161/ATVBAHA.113.302335.

413. Pandey D, Hori D, Kim JH, Bergman Y, Berkowitz DE, Romer LH. NEDDylation promotes endothelial dysfunction: a role for HDAC2. *J Mol Cell Cardiol* 81: 18–22, 2015. doi:10.1016/j.yjmcc.2015.01.019.

414. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. *Eur Heart J* 34: 2436–2443, 2013. doi:10.1093/eurheartj/eht149.

415. Paneni F, Costantino S, Castello L, Battista R, Capretti G, Chiandotto S, D'Amario D, Scavone G, Villano A, Rustighi A, Crea F, Pitocco D, Lanza G, Volpe M, Del Sal G, Lüscher TF, Cosentino F. Targeting prolyl-isomerase Pin1 prevents mitochondrial oxidative stress and vascular dysfunction: insights in patients with diabetes. *Eur Heart J* 36: 817–828, 2015. doi:10.1093/eurheartj/ehu179.

416. Paneni F, Costantino S, Cosentino F. p66(Shc)-induced redox changes drive endothelial insulin resistance. *Atherosclerosis* 236: 426–429, 2014. doi:10.1016/j.atherosclerosis.2014.07.027.

417. Paneni F, Costantino S, Cosentino F. Role of oxidative stress in endothelial insulin resistance. *World J Diabetes* 6: 326–332, 2015. doi:10.4239/wjd.v6.i2.326.

418. Paneni F, Mocharla P, Akhmedov A, Costantino S, Osto E, Volpe M, Lüscher TF, Cosentino F. Gene silencing of the mitochondrial adaptor p66(Shc) suppresses vascular hyperglycemic memory in diabetes. *Circ Res* 111: 278–289, 2012. doi:10.1161/CIRCRESAHA.112.266593.

419. Paneni F, Volpe M, Lüscher TF, Cosentino F. SIRT1, p66(Shc), and Set7/9 in vascular hyperglycemic memory: bringing all the strands together. *Diabetes* 62: 1800–1807, 2013. doi:10.2337/db12-1648.

420. Pangare M, Makino A. Mitochondrial function in vascular endothelial cell in diabetes. *J Smooth Muscle Res* 48: 1–26, 2012. doi:10.1540/jsmr.48.1.

421. Park EJ, Grabińska KA, Guan Z, Sessa WC. NgBR is essential for endothelial cell glycosylation and vascular development. *EMBO Rep* 17: 167–177, 2016. doi:10.15252/embr.201540789.

422. Parmar KM, Larman HB, Dai G, Zhang Y, Wang ET, Moorthy SN, Kratz JR, Lin Z, Jain MK, Gimbrone MA, Jr, García-Cardeña G. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. *J Clin Invest* 116: 49–58, 2006. doi:10.1172/JCI24787.

423. Parra-Bonilla G, Alvarez DF, Al-Mehdi AB, Alexeyev M, Stevens T. Critical role for lactate dehydrogenase A in aerobic glycolysis that sustains pulmonary microvascular endothelial cell proliferation. *Am J Physiol Lung Cell Mol Physiol* 299: L513–L522, 2010. doi:10.1152/ajplung.00274.2009.

424. Patel-Hett S, D'Amore PA. Signal transduction in vasculogenesis and developmental angiogenesis. *Int J Dev Biol* 55: 353–363, 2011. doi:10.1387/ijdb.103213sp.

425. Patra KC, Hay N. The pentose phosphate pathway and cancer. *Trends Biochem Sci* 39: 347–354, 2014. doi:10.1016/j.tibs.2014.06.005.

426. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res* 115: 148–164, 2014. doi:10.1161/CIRCRESAHA.115.301130.

427. Pendyala S, Gorshkova IA, Usatyuk PV, He D, Pennathur A, Lambeth JD, Thannickal VJ, Natarajan V. Role of Nox4 and Nox2 in hyperoxia-induced reactive oxygen species generation and migration of human lung endothelial cells. *Antioxid Redox Signal* 11: 747–764, 2009. doi:10.1089/ars.2008.2203.

428. Pérez-Escuredo J, Dadhich RK, Dhup S, Cacace A, Van Hée VF, De Saedeleer CJ, Sboarina M, Rodriguez F, Fontenille MJ, Brisson L, Porporato PE, Sonveaux P. Lactate promotes glutamine uptake and metabolism in oxidative cancer cells. *Cell Cycle* 15: 72–83, 2016. doi:10.1080/15384101.2015.1120930.

429. Pérez-Escuredo J, Van Hée VF, Sboarina M, Falces J, Payen VL, Pellerin L, Sonveaux P. Monocarboxylate transporters in the brain and in cancer. *Biochim Biophys Acta* 1863: 2481–2497, 2016. doi:10.1016/j.bbamcr.2016.03.013.

430. Peters K, Kamp G, Berz A, Unger RE, Barth S, Salamon A, Rychly J, Kirkpatrick CJ. Changes in human endothelial cell energy metabolic capacities during in vitro cultivation. The role of "aerobic glycolysis" and proliferation. *Cell Physiol Biochem* 24: 483–492, 2009. doi:10.1159/000257490.

431. Petrova TV, Mäkinen T, Mäkelä TP, Saarela J, Virtanen I, Ferrell RE, Finegold DN, Kerjaschki D, Ylä-Herttuala S, Alitalo K. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J* 21: 4593–4599, 2002. doi:10.1093/emboj/cdf470.

432. Phng LK, Gerhardt H. Angiogenesis: a team effort coordinated by notch. *Dev Cell* 16: 196–208, 2009. doi:10.1016/j.devcel.2009.01.015.

433. Pirola L, Balcerczyk A, Tothill RW, Haviv I, Kaspi A, Lunke S, Ziemann M, Karagiannis T, Tonna S, Kowalczyk A, Beresford-Smith B, Macintyre G, Kelong M, Hongyu Z, Zhu J, El-Osta A. Genome-wide analysis distinguishes hyperglycemia regulated epigenetic signatures of primary vascular cells. *Genome Res* 21: 1601–1615, 2011. doi:10.1101/gr.116095.110.

434. Pollak N, Dölle C, Ziegler M. The power to reduce: pyridine nucleotides–small molecules with a multitude of functions. *Biochem J* 402: 205–218, 2007. doi:10.1042/BJ20061638.

435. Pope AJ, Druhan L, Guzman JE, Forbes SP, Murugesan V, Lu D, Xia Y, Chicoine LG, Parinandi NL, Cardounel AJ. Role of DDAH-1 in lipid peroxidation product-mediated inhibition of endothelial NO generation. *Am J Physiol Cell Physiol* 293: C1679–C1686, 2007. doi:10.1152/ajpcell.00224.2007.

436. Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. *Cell* 146: 873–887, 2011. doi:10.1016/j.cell.2011.08.039.

437. Pretsch W, Charles DJ, Merkle S. X-linked glucose-6-phosphate dehydrogenase deficiency in *Mus musculus*. *Biochem Genet* 26: 89–103, 1988. doi:10.1007/BF00555491.

438. Prigione A, Fauler B, Lurz R, Lehrach H, Adjaye J. The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem Cells* 28: 721–733, 2010. doi:10.1002/stem.404.

439. Pushpakumar S, Kundu S, Sen U. Endothelial dysfunction: the link between homocysteine and hydrogen sulfide. *Curr Med Chem* 21: 3662–3672, 2014. doi:10.2174/0929867321666140706142335.

440. Qiang L, Banks AS, Accili D. Uncoupling of acetylation from phosphorylation regulates FoxO1 function independent of its subcellular localization. *J Biol Chem* 285: 27396–27401, 2010. doi:10.1074/jbc.M110.140228.

441. Quehenberger P, Bierhaus A, Fasching P, Muellner C, Klevesath M, Hong M, Stier G, Sattler M, Schleicher E, Speiser W, Nawroth PP. Endothelin 1 transcription is controlled by nuclear factor-kappaB in AGE-stimulated cultured endothelial cells. *Diabetes* 49: 1561–1570, 2000. doi:10.2337/diabetes.49.9.1561.

442. Raez LE, Papadopoulos K, Ricart AD, Chiorean EG, Dipaola RS, Stein MN, Rocha Lima CM, Schlesselman JJ, Tolba K, Langmuir VK, Kroll S, Jung DT, Kurtoglu M, Rosenblatt J, Lampidis TJ. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 71: 523–530, 2013. doi:10.1007/s00280-012-2045-1.

443. Rafikov R, Fonseca FV, Kumar S, Pardo D, Darragh C, Elms S, Fulton D, Black SM. eNOS activation and NO function: structural motifs responsible for the posttranslational control of endothelial nitric oxide synthase activity. *J Endocrinol* 210: 271–284, 2011. doi:10.1530/JOE-11-0083.

444. Rafikova O, Rafikov R, Kumar S, Sharma S, Aggarwal S, Schneider F, Jonigk D, Black SM, Tofovic SP. Bosentan inhibits oxidative and nitrosative stress and rescues occlusive pulmonary hypertension. *Free Radic Biol Med* 56: 28–43, 2013. doi:10.1016/j.freeradbiomed.2012.09.013.

445. Raimondi C, Brash JT, Fantin A, Ruhrberg C. NRP1 function and targeting in neurovascular development and eye disease. *Prog Retin Eye Res* 52: 64–83, 2016. doi:10.1016/j.preteyeres.2016.02.003.

446. Rajapakse NW, Chong AL, Zhang WZ, Kaye DM. Insulin-mediated activation of the L-arginine nitric oxide pathway in man, and its impairment in diabetes. *PLoS One* 8: e61840, 2013. doi:10.1371/journal.pone.0061840.

447. Rajwani A, Cubbon RM, Wheatcroft SB. Cell-specific insulin resistance: implications for atherosclerosis. *Diabetes Metab Res Rev* 28: 627–634, 2012. doi:10.1002/dmrr.2336.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

448. Rak J, Yu JL, Klement G, Kerbel RS. Oncogenes and angiogenesis: signaling three-dimensional tumor growth. *J Investig Dermatol Symp Proc* 5: 24–33, 2000. doi:10.1046/j.1087-0024.2000.00012.x.

449. Ramsey DJ, Arden GB. Hypoxia and Dark Adaptation in Diabetic Retinopathy: Interactions, Consequences, and Therapy. *Curr Diab Rep* 15: 118, 2015. doi:10.1007/s11892-015-0686-2.

450. Rao KM. Molecular mechanisms regulating iNOS expression in various cell types. *J Toxicol Environ Health B Crit Rev* 3: 27–58, 2000. doi:10.1080/109374000281131.

451. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab* 17: 20–33, 2013. doi:10.1016/j.cmet.2012.11.012.

452. Rask-Madsen C, Li Q, Freund B, Feather D, Abramov R, Wu IH, Chen K, Yamamoto-Hiraoka J, Goldenbogen J, Sotiropoulos KB, Clermont A, Geraldes P, Dall'Osso C, Wagers AJ, Huang PL, Rekhter M, Scalia R, Kahn CR, King GL. Loss of insulin signaling in vascular endothelial cells accelerates atherosclerosis in apolipoprotein E null mice. *Cell Metab* 11: 379–389, 2010. doi:10.1016/j.cmet.2010.03.013.

453. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, Wang T, Gregg D, Ramaswami P, Pippen AM, Annex BH, Dong C, Taylor DA. Aging, progenitor cell exhaustion, and atherosclerosis. *Circulation* 108: 457–463, 2003. doi:10.1161/01.CIR.0000082924.75945.48.

454. Real-Hohn A, Zancan P, Da Silva D, Martins ER, Salgado LT, Mermelstein CS, Gomes AM, Sola-Penna M. Filamentous actin and its associated binding proteins are the stimulatory site for 6-phosphofructo-1-kinase association within the membrane of human erythrocytes. *Biochimie* 92: 538–544, 2010. doi:10.1016/j.biochi.2010.01.023.

455. Reddy MA, Natarajan R. Epigenetic mechanisms in diabetic vascular complications. *Cardiovasc Res* 90: 421–429, 2011. doi:10.1093/cvr/cvr024.

456. Reis M, Liebner S. Wnt signaling in the vasculature. *Exp Cell Res* 319: 1317–1323, 2013. doi:10.1016/j.yexcr.2012.12.023.

457. Ricard N, Tu L, Le Hiress M, Huertas A, Phan C, Thuillet R, Sattler C, Fadel E, Seferian A, Montani D, Dorfmüller P, Humbert M, Guignabert C. Increased pericyte coverage mediated by endothelial-derived fibroblast growth factor-2 and interleukin-6 is a source of smooth muscle-like cells in pulmonary hypertension. *Circulation* 129: 1586–1597, 2014. doi:10.1161/CIRCULATIONAHA.113.007469.

458. Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM, De Maria R. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* 468: 824–828, 2010. doi:10.1038/nature09557.

459. Richter T, Münch G, Lüth HJ, Arendt T, Kientsch-Engel R, Stahl P, Fengler D, Kuhla B. Immunochemical crossreactivity of antibodies specific for "advanced glycation end-products" with "advanced lipoxidation endproducts". *Neurobiol Aging* 26: 465–474, 2005. doi:10.1016/j.neurobiolaging.2004.04.009.

460. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ; JUPITER Study Group. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 359: 2195–2207, 2008. doi:10.1056/NEJMoa0807646.

461. Riganti C, Gazzano E, Polimeni M, Aldieri E, Ghigo D. The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. *Free Radic Biol Med* 53: 421–436, 2012. doi:10.1016/j.freeradbiomed.2012.05.006.

462. Robey RB, Hay N. Is Akt the "Warburg kinase"?-Akt-energy metabolism interactions and oncogenesis. *Semin Cancer Biol* 19: 25–31, 2009. doi:10.1016/j.semcancer.2008.11.010.

463. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. *Hypertens Res* 34: 431–440, 2011. doi:10.1038/hr.2010.264.

464. Roe ND, Ren J. Nitric oxide synthase uncoupling: a therapeutic target in cardiovascular diseases. *Vascul Pharmacol* 57: 168–172, 2012. doi:10.1016/j.vph.2012.02.004.

465. Romagnoli M, Gomez-Cabrera MC, Perrelli MG, Biasi F, Pallardó FV, Sastre J, Poli G, Viña J. Xanthine oxidase-induced oxidative stress causes activation of NF-kappaB and inflammation in the liver of type I diabetic rats. *Free Radic Biol Med* 49: 171–177, 2010. doi:10.1016/j.freeradbiomed.2010.03.024.

466. Rosso A, Balsamo A, Gambino R, Dentelli P, Falcioni R, Cassader M, Pegoraro L, Pagano G, Brizzi MF. p53 mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *J Biol Chem* 281: 4339–4347, 2006. doi:10.1074/jbc.M509293200.

467. Roukens MG, Alloul-Ramdhani M, Baan B, Kobayashi K, Peterson-Maduro J, van Dam H, Schulte-Merker S, Baker DA. Control of endothelial sprouting by a Tel-CtBP complex. *Nat Cell Biol* 12: 933–942, 2010. doi:10.1038/ncb2096.

468. Rowland A, Miners JO, Mackenzie PI. The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int J Biochem Cell Biol* 45: 1121–1132, 2013. doi:10.1016/j.biocel.2013.02.019.

469. Roy M, Reddy PH, Iijima M, Sesaki H. Mitochondrial division and fusion in metabolism. *Curr Opin Cell Biol* 33: 111–118, 2015. doi:10.1016/j.ceb.2015.02.001.

470. Roy S, Sala R, Cagliero E, Lorenzi M. Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. *Proc Natl Acad Sci USA* 87: 404–408, 1990. doi:10.1073/pnas.87.1.404.

471. Ruan GX, Kazlauskas A. Lactate engages receptor tyrosine kinases Axl, Tie2, and vascular endothelial growth factor receptor 2 to activate phosphoinositide 3-kinase/Akt and promote angiogenesis. *J Biol Chem* 288: 21161–21172, 2013. doi:10.1074/jbc.M113.474619.

472. Sahoo S, Meijles DN, Pagano PJ. NADPH oxidases: key modulators in aging and age-related cardiovascular diseases? *Clin Sci (Lond)* 130: 317–335, 2016. doi:10.1042/CS20150087.

473. Saiki R, Hayashi D, Ikuo Y, Nishimura K, Ishii I, Kobayashi K, Chiba K, Toida T, Kashiwagi K, Igarashi K. Acrolein stimulates the synthesis of IL-6 and C-reactive protein (CRP) in thrombosis model mice and cultured cells. *J Neurochem* 127: 652–659, 2013. doi:10.1111/jnc.12336.

474. Sakao S, Taraseviciene-Stewart L, Lee JD, Wood K, Cool CD, Voelkel NF. Initial apoptosis is followed by increased proliferation of apoptosis-resistant endothelial cells. *FASEB J* 19: 1178–1180, 2005.

475. Santhanam L, Christianson DW, Nyhan D, Berkowitz DE. Arginase and vascular aging. *J Appl Physiol (1985)* 105: 1632–1642, 2008. doi:10.1152/japplphysiol.90627.2008.

476. Sasaki N, Yamashita T, Takaya T, Shinohara M, Shiraki R, Takeda M, Emoto N, Fukatsu A, Hayashi T, Ikemoto K, Nomura T, Yokoyama M, Hirata K, Kawashima S. Augmentation of vascular remodeling by uncoupled endothelial nitric oxide synthase in a mouse model of diabetes mellitus. *Arterioscler Thromb Vasc Biol* 28: 1068–1076, 2008. doi:10.1161/ATVBAHA.107.160754.

477. Sathanoori R, Rosi F, Gu BJ, Wiley JS, Müller CE, Olde B, Erlinge D. Shear stress modulates endothelial KLF2 through activation of P2X4. *Purinergic Signal* 11: 139–153, 2015. doi:10.1007/s11302-014-9442-3.

478. Sawada N, Jiang A, Takizawa F, Safdar A, Manika A, Tesmenitsky Y, Kang KT, Bischoff J, Kalwa H, Sartoretto JL, Kamei Y, Benjamin LE, Watada H, Ogawa Y, Higashikuni Y, Kessinger CW, Jaffer FA, Michel T, Sata M, Croce K, Tanaka R, Arany Z. Endothelial PGC-1α mediates vascular dysfunction in diabetes. *Cell Metab* 19: 246–258, 2014. doi:10.1016/j.cmet.2013.12.014.

479. Schalkwijk CG, Stehouwer CD. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin Sci (Lond)* 109: 143–159, 2005. doi:10.1042/CS20050025.

480. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, Zecchin A, Cantelmo AR, Christen S, Goveia J, Heggermont W, Goddë L, Vinckier S, Van Veldhoven PP, Eelen G, Schoonjans L, Gerhardt H, Dewerchin M, Baes M, De Bock K, Ghesquière B, Lunt SY, Fendt SM, Carmeliet P. Corrigendum: fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 526: 144, 2015. doi:10.1038/nature14624.

481. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, Zecchin A, Cantelmo AR, Christen S, Goveia J, Heggermont W, Goddé L, Vinckier S, Van Veldhoven PP, Eelen G, Schoonjans L, Gerhardt H, Dewerchin M, Baes M, De Bock K, Ghesquière B, Lunt SY, Fendt SM, Carmeliet P. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* 520: 192–197, 2015. doi:10.1038/nature14362.

482. Schoors S, Cantelmo AR, Georgiadou M, Stapor P, Wang X, Quaegebeur A, Cauwenberghs S, Wong BW, Bifari F, Decimo I, Schoonjans L, De Bock K, Dewerchin M, Carmeliet P. Incomplete and transitory decrease of glycolysis: a new paradigm for anti-angiogenic therapy? *Cell Cycle* 13: 16–22, 2014. doi:10.4161/cc.27519.

483. Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquière B, Cauwenberghs S, Kuchnio A, Wong BW, Quaegebeur A, Goveia J, Bifari F, Wang X, Blanco R, Tembuyser B, Cornelissen I, Bouché A, Vinckier S, Diaz-Moralli S, Gerhardt H, Telang S,

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

Cascante M, Chesney J, Dewerchin M, Carmeliet P. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* 19: 37–48, 2014. doi:10.1016/j.cmet.2013.11.008.

484. Schunkert H, König IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, Preuss M, Stewart AF, Barbalic M, Gieger C, Absher D, Aherrahrou Z, Allayee H, Altshuler D, Anand SS, Andersen K, Anderson JL, Ardissino D, Ball SG, Balmforth AJ, Barnes TA, Becker DM, Becker LC, Berger K, Bis JC, Boekholdt SM, Boerwinkle E, Braund PS, Brown MJ, Burnett MS, Buysschaert I, Carlquist JF, Chen L, Cichon S, Codd V, Davies RW, Dedoussis G, Dehghan A, Demissie S, Devaney JM, Diemert P, Do R, Doering A, Eifert S, Mokhtari NE, Ellis SG, Elosua R, Engert JC, Epstein SE, de Faire U, Fischer M, Folsom AR, Freyer J, Gigante B, Girelli D, Gretarsdottir S, Gudnason V, Gulcher JR, Halperin E, Hammond N, Hazen SL, Hofman A, Horne BD, Illig T, Iribarren C, Jones GT, Jukema JW, Kaiser MA, Kaplan LM, Kastelein JJ, Khaw KT, Knowles JW, Kolovou G, Kong A, Laaksonen R, Lambrechts D, Leander K, Lettre G, Li M, Lieb W, Loley C, Lotery AJ, Mannucci PM, Maouche S, Martinelli N, McKeown PP, Meisinger C, Meitinger T, Melander O, Merlini PA, Mooser V, Morgan T, Mühleisen TW, Muhlestein JB, Münzel T, Musunuru K, Nahrstaedt J, Nelson CP, Nöthen MM, Olivieri O, Patel RS, Patterson CC, Peters A, Peyvandi F, Qu L, Quyyumi AA, Rader DJ, Rallidis LS, Rice C, Rosendaal FR, Rubin D, Salomaa V, Sampietro ML, Sandhu MS, Schadt E, Schäfer A, Schillert A, Schreiber S, Schrezenmeir J, Schwartz SM, Siscovick DS, Sivananthan M, Sivapalaratnam S, Smith A, Smith TB, Snoep JD, Soranzo N, Spertus JA, Stark K, Stirrups K, Stoll M, Tang WH, Tennstedt S, Thorgeirsson G, Thorleifsson G, Tomaszewski M, Uitterlinden AG, van Rij AM, Voight BF, Wareham NJ, Wells GA, Wichmann HE, Wild PS, Willenborg C, Witteman JC, Wright BJ, Ye S, Zeller T, Ziegler A, Cambien F, Goodall AH, Cupples LA, Quertermous T, März W, Hengstenberg C, Blankenberg S, Ouwehand WH, Hall AS, Deloukas P, Thompson JR, Stefansson K, Roberts R, Thorsteinsdottir U, O'Donnell CJ, McPherson R, Erdmann J, Samani NJ; Cardiogenics; CARDIoGRAM Consortium. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet* 43: 333–338, 2011. doi:10.1038/ng.784.

485. Scott A, Fruttiger M. Oxygen-induced retinopathy: a model for vascular pathology in the retina. *Eye (Lond)* 24: 416–421, 2010. doi:10.1038/eye.2009.306.

486. Seaman S, Stevens J, Yang MY, Logsdon D, Graff-Cherry C, St Croix B. Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell* 11: 539–554, 2007. doi:10.1016/j.ccr.2007.04.017.

487. Sen S, Roy S, Bandyopadhyay G, Scott B, Xiao D, Ramadoss S, Mahata SK, Chaudhuri G. γ-Aminobutyric Acid Is Synthesized and Released by the Endothelium: Potential Implications. *Circ Res* 119: 621–634, 2016. doi:10.1161/CIRCRESAHA.116.308645.

488. Sena CM, Matafome P, Crisóstomo J, Rodrigues L, Fernandes R, Pereira P, Seiça RM. Methylglyoxal promotes oxidative stress and endothelial dysfunction. *Pharmacol Res* 65: 497–506, 2012. doi:10.1016/j.phrs.2012.03.004.

489. Sengupta R, Holmgren A. Thioredoxin and glutaredoxin-mediated redox regulation of ribonucleotide reductase. *World J Biol Chem* 5: 68–74, 2014. doi:10.4331/wjbc.v5.i1.68.

490. Serra H, Chivite I, Angulo-Urarte A, Soler A, Sutherland JD, Arruabarrena-Aristorena A, Ragab A, Lim R, Malumbres M, Fruttiger M, Potente M, Serrano M, Fabra À, Viñals F, Casanovas O, Pandolfi PP, Bigas A, Carracedo A, Gerhardt H, Graupera M. PTEN mediates Notch-dependent stalk cell arrest in angiogenesis. *Nat Commun* 6: 7935, 2015. doi:10.1038/ncomms8935.

491. Sessa WC, García-Cardeña G, Liu J, Keh A, Pollock JS, Bradley J, Thiru S, Braverman IM, Desai KM. The Golgi association of endothelial nitric oxide synthase is necessary for the efficient synthesis of nitric oxide. *J Biol Chem* 270: 17641–17644, 1995. doi:10.1074/jbc.270.30.17641.

492. Shao B, Bayraktutan U. Hyperglycaemia promotes human brain microvascular endothelial cell apoptosis via induction of protein kinase C-ßI and prooxidant enzyme NADPH oxidase. *Redox Biol* 2: 694–701, 2014. doi:10.1016/j.redox.2014.05.005.

493. Shaul PW. Regulation of endothelial nitric oxide synthase: location, location, location. *Annu Rev Physiol* 64: 749–774, 2002. doi:10.1146/annurev.physiol.64.081501.155952.

494. Sheetz MJ, King GL. Molecular understanding of hyperglycemia's adverse effects for diabetic complications. *JAMA* 288: 2579–2588, 2002. doi:10.1001/jama.288.20.2579.

495. Shenouda SM, Widlansky ME, Chen K, Xu G, Holbrook M, Tabit CE, Hamburg NM, Frame AA, Caiano TL, Kluge MA, Duess MA, Levit A, Kim B, Hartman ML, Joseph L, Shirihai OS, Vita JA. Altered mitochondrial dynamics contributes to endothelial dysfunction in diabetes mellitus. *Circulation* 124: 444–453, 2011. doi:10.1161/CIRCULATIONAHA.110.014506.

496. Shevalye H, Watcho P, Stavniichuk R, Dyukova E, Lupachyk S, Obrosova IG. Metanx alleviates multiple manifestations of peripheral neuropathy and increases intraepidermal nerve fiber density in Zucker diabetic fatty rats. *Diabetes* 61: 2126–2133, 2012. doi:10.2337/db11-1524.

497. Shinohara M, Thornalley PJ, Giardino I, Beisswenger P, Thorpe SR, Onorato J, Brownlee M. Overexpression of glyoxalase-I in bovine endothelial cells inhibits intracellular advanced glycation endproduct formation and prevents hyperglycemia-induced increases in macromolecular endocytosis. *J Clin Invest* 101: 1142–1147, 1998. doi:10.1172/JCI119885.

498. Shinozaki K, Kashiwagi A, Nishio Y, Okamura T, Yoshida Y, Masada M, Toda N, Kikkawa R. Abnormal biopterin metabolism is a major cause of impaired endothelium-dependent relaxation through nitric oxide/O_2^- imbalance in insulin-resistant rat aorta. *Diabetes* 48: 2437–2445, 1999. doi:10.2337/diabetes.48.12.2437.

499. Shirai T, Nazarewicz RR, Wallis BB, Yanes RE, Watanabe R, Hilhorst M, Tian L, Harrison DG, Giacomini JC, Assimes TL, Goronzy JJ, Weyand CM. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J Exp Med* 213: 337–354, 2016. doi:10.1084/jem.20150900.

500. Shiraishi T, Verdone JE, Huang J, Kahlert UD, Hernandez JR, Torga G, Zarif JC, Epstein T, Gatenby R, McCartney A, Elisseeff JH, Mooney SM, An SS, Pienta KJ. Glycolysis is the primary bioenergetic pathway for cell motility and cytoskeletal remodeling in human prostate and breast cancer cells. *Oncotarget* 6: 130–143, 2015. doi:10.18632/oncotarget.2766.

501. Shrikhande GV, Scali ST, da Silva CG, Damrauer SM, Csizmadia E, Putheti P, Matthey M, Arjoon R, Patel R, Siracuse JJ, Maccariello ER, Andersen ND, Monahan T, Peterson C, Essayagh S, Studer P, Guedes RP, Kocher O, Usheva A, Veves A, Kaczmarek E, Ferran C. *O*-glycosylation regulates ubiquitination and degradation of the anti-inflammatory protein A20 to accelerate atherosclerosis in diabetic ApoE-null mice. *PLoS One* 5: e14240, 2010. doi:10.1371/journal.pone.0014240.

502. Siegerink B, Maas R, Vossen CY, Schwedhelm E, Koenig W, Böger R, Rothenbacher D, Brenner H, Breitling LP. Asymmetric and symmetric dimethylarginine and risk of secondary cardiovascular disease events and mortality in patients with stable coronary heart disease: the KAROLA follow-up study. *Clin Res Cardiol* 102: 193–202, 2013. doi:10.1007/s00392-012-0515-4.

503. Singh A, Ramnath RD, Foster RR, Wylie EC, Fridén V, Dasgupta I, Haraldsson B, Welsh GI, Mathieson PW, Satchell SC. Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx. *PLoS One* 8: e55852, 2013. doi:10.1371/journal.pone.0055852.

504. Siu KL, Gao L, Cai H. Differential Roles of Protein Complexes NOX1-NOXO1 and NOX2-p47phox in Mediating Endothelial Redox Responses to Oscillatory and Unidirectional Laminar Shear Stress. *J Biol Chem* 291: 8653–8662, 2016. doi:10.1074/jbc.M115.713149.

505. Slawson C, Copeland RJ, Hart GW. *O*-GlcNAc signaling: a metabolic link between diabetes and cancer? *Trends Biochem Sci* 35: 547–555, 2010. doi:10.1016/j.tibs.2010.04.005.

506. Sonveaux P, Copetti T, De Saedeleer CJ, Végran F, Verrax J, Kennedy KM, Moon EJ, Dhup S, Danhier P, Frérart F, Gallez B, Ribeiro A, Michiels C, Dewhirst MW, Feron O. Targeting the lactate transporter MCT1 in endothelial cells inhibits lactate-induced HIF-1 activation and tumor angiogenesis. *PLoS One* 7: e33418, 2012. doi:10.1371/journal.pone.0033418.

507. Spolarics Z, Lang CH, Bagby GJ, Spitzer JJ. Glutamine and fatty acid oxidation are the main sources of energy for Kupffer and endothelial cells. *Am J Physiol Gastrointest Liver Physiol* 261: G185–G190, 1991.

508. Srinivas SR, Gopal E, Zhuang L, Itagaki S, Martin PM, Fei YJ, Ganapathy V, Prasad PD. Cloning and functional identification of slc5a12 as a sodium-coupled low-affinity transporter for monocarboxylates (SMCT2). *Biochem J* 392: 655–664, 2005. doi:10.1042/BJ20050927.

509. Sriram G, Tan JY, Islam I, Rufaihah AJ, Cao T. Efficient differentiation of human embryonic stem cells to arterial and venous endothelial cells under feeder- and serum-free conditions. *Stem Cell Res Ther* 6: 261, 2015. doi:10.1186/s13287-015-0260-5.

510. St Croix B, Rago C, Velculescu V, Traverso G, Romans KE, Montgomery E, Lal A, Riggins GJ, Lengauer C, Vogelstein B, Kinzler KW. Genes expressed in human tumor endothelium. *Science* 289: 1197–1202, 2000. doi:10.1126/science.289.5482.1197.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

511. Stanczuk L, Martinez-Corral I, Ulvmar MH, Zhang Y, Laviña B, Fruttiger M, Adams RH, Saur D, Betsholtz C, Ortega S, Alitalo K, Graupera M, Mäkinen T. cKit Lineage Hemogenic Endothelium-Derived Cells Contribute to Mesenteric Lymphatic Vessels. *Cell Reports* 10: 1708–1721, 2015. doi:10.1016/j.celrep.2015.02.026.

512. Storkebaum E, Lambrechts D, Carmeliet P. VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection. *BioEssays* 26: 943–954, 2004. doi:10.1002/bies.20092.

513. Strasser GA, Kaminker JS, Tessier-Lavigne M. Microarray analysis of retinal endothelial tip cells identifies CXCR4 as a mediator of tip cell morphology and branching. *Blood* 115: 5102–5110, 2010. doi:10.1182/blood-2009-07-230284.

514. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 321: 405–412, 2000. doi:10.1136/bmj.321.7258.405.

515. Su Y, Qadri SM, Hossain M, Wu L, Liu L. Uncoupling of eNOS contributes to redox-sensitive leukocyte recruitment and microvascular leakage elicited by methylglyoxal. *Biochem Pharmacol* 86: 1762–1774, 2013. doi:10.1016/j.bcp.2013.10.008.

516. Su Y, Qadri SM, Wu L, Liu L. Methylglyoxal modulates endothelial nitric oxide synthase-associated functions in EA.hy926 endothelial cells. *Cardiovasc Diabetol* 12: 134, 2013. doi:10.1186/1475-2840-12-134.

517. Suematsu N, Ojaimi C, Kinugawa S, Wang Z, Xu X, Koller A, Recchia FA, Hintze TH. Hyperhomocysteinemia alters cardiac substrate metabolism by impairing nitric oxide bioavailability through oxidative stress. *Circulation* 115: 255–262, 2007. doi:10.1161/CIRCULATIONAHA.106.652693.

518. Sugimoto K, Ishibashi T, Sawamura T, Inoue N, Kamioka M, Uekita H, Ohkawara H, Sakamoto T, Sakamoto N, Okamoto Y, Takuwa Y, Kakino A, Fujita Y, Tanaka T, Teramoto T, Maruyama Y, Takeishi Y. LOX-1-MT1-MMP axis is crucial for RhoA and Rac1 activation induced by oxidized low-density lipoprotein in endothelial cells. *Cardiovasc Res* 84: 127–136, 2009. doi:10.1093/cvr/cvp177.

519. Sukumar P, Viswambharan H, Imrie H, Cubbon RM, Yuldasheva N, Gage M, Galloway S, Skromna A, Kandavelu P, Santos CX, Gatenby VK, Smith J, Beech DJ, Wheatcroft SB, Channon KM, Shah AM, Kearney MT. Nox2 NADPH oxidase has a critical role in insulin resistance-related endothelial cell dysfunction. *Diabetes* 62: 2130–2134, 2013. doi:10.2337/db12-1294.

520. Sun X, Kumar S, Sharma S, Aggarwal S, Lu Q, Gross C, Rafikova O, Lee SG, Dasarathy S, Hou Y, Meadows ML, Han W, Su Y, Fineman JR, Black SM. Endothelin-1 induces a glycolytic switch in pulmonary arterial endothelial cells via the mitochondrial translocation of endothelial nitric oxide synthase. *Am J Respir Cell Mol Biol* 50: 1084–1095, 2014. doi:10.1165/rcmb.2013-0187OC.

521. Sutendra G, Michelakis ED. The metabolic basis of pulmonary arterial hypertension. *Cell Metab* 19: 558–573, 2014. doi:10.1016/j.cmet.2014.01.004.

522. Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. *J Cell Biol* 209: 13–22, 2015. doi:10.1083/jcb.201412052.

523. Takahashi T, Harris RC. Role of endothelial nitric oxide synthase in diabetic nephropathy: lessons from diabetic eNOS knockout mice. *J Diabetes Res* 2014: 590541, 2014. doi:10.1155/2014/590541.

524. Takizawa F, Mizutani S, Ogawa Y, Sawada N. Glucose-independent persistence of PAI-1 gene expression and H3K4 tri-methylation in type 1 diabetic mouse endothelium: implication in metabolic memory. *Biochem Biophys Res Commun* 433: 66–72, 2013. doi:10.1016/j.bbrc.2013.02.064.

525. Talati M, Hemnes A. Fatty acid metabolism in pulmonary arterial hypertension: role in right ventricular dysfunction and hypertrophy. *Pulm Circ* 5: 269–278, 2015. doi:10.1086/681227.

526. Talati MH, Brittain EL, Fessel JP, Penner N, Atkinson J, Funke M, Grueter C, Jerome WG, Freeman M, Newman JH, West J, Hemnes AR. Mechanisms of Lipid Accumulation in the Bone Morphogenetic Protein Receptor Type 2 Mutant Right Ventricle. *Am J Respir Crit Care Med* 194: 719–728, 2016. doi:10.1164/rccm.201507-1444OC.

527. Tammali R, Reddy AB, Srivastava SK, Ramana KV. Inhibition of aldose reductase prevents angiogenesis in vitro and in vivo. *Angiogenesis* 14: 209–221, 2011. doi:10.1007/s10456-011-9206-4.

528. Tanaka J, Qiang L, Banks AS, Welch CL, Matsumoto M, Kitamura T, Ido-Kitamura Y, DePinho RA, Accili D. Foxo1 links hyperglycemia to LDL oxidation and endothelial nitric oxide synthase dysfunction in vascular endothelial cells. *Diabetes* 58: 2344–2354, 2009. doi:10.2337/db09-0167.

529. Tang X, Luo YX, Chen HZ, Liu DP. Mitochondria, endothelial cell function, and vascular diseases. *Front Physiol* 5: 175, 2014. doi:10.3389/fphys.2014.00175.

530. Tapiero H, Mathé G, Couvreur P, Tew KD. I. Arginine. *Biomed Pharmacother* 56: 439–445, 2002. doi:10.1016/S0753-3322(02)00284-6.

531. Tapiero H, Mathé G, Couvreur P, Tew KD. II. Glutamine and glutamate. *Biomed Pharmacother* 56: 446–457, 2002. doi:10.1016/S0753-3322(02)00285-8.

532. Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. *J Intern Med* 280: 97–113, 2016. doi:10.1111/joim.12465.

533. Tărlungeanu DC, Deliu E, Dotter CP, Kara M, Janiesch PC, Scalise M, Galluccio M, Tesulov M, Morelli E, Sonmez FM, Bilguvar K, Ohgaki R, Kanai Y, Johansen A, Esharif S, Ben-Omran T, Topcu M, Schlessinger A, Indiveri C, Duncan KE, Caglayan AO, Gunel M, Gleeson JG, Novarino G. Impaired Amino Acid Transport at the Blood Brain Barrier Is a Cause of Autism Spectrum Disorder. *Cell* 167: 1481–1494, 2016. doi:10.1016/j.cell.2016.11.013.

534. Teixeira G, Szyndralewiez C, Molango S, Carnesecchi S, Heitz F, Wiesel P, Wood JM. Therapeutic potential of NADPH oxidase 1/4 inhibitors. *Br J Pharmacol* 174: 1647–1669, 2017. doi:10.1111/bph.13532.

535. Telang S, O'Neal J, Tapolsky G, Clem B, Kerr A, Imbert-Ferndandez Y, Chesney J. Discovery of a PFKFB3 inhibitor for phase I trial testing that synergizes with the B-Raf inhibitor vemurafenib. *Cancer Metab* 2, Suppl 1: 14, 2014. doi:10.1186/2049-3002-2-S1-P14.

536. Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 106: 2781–2786, 2002. doi:10.1161/01.CIR.0000039526.42991.93.

537. Thenappan T, Duprez D. Pulmonary arterial hypertension. In: *Arterial Disorders*. New York: Springer, 2015, p. 313–332.

538. Thiam AR, Farese RV, Jr, Walther TC. The biophysics and cell biology of lipid droplets. *Nat Rev Mol Cell Biol* 14: 775–786, 2013. doi:10.1038/nrm3699.

539. Tian X, Shi Y, Liu N, Yan Y, Li T, Hua P, Liu B. Upregulation of DAPK contributes to homocysteine-induced endothelial apoptosis via the modulation of Bcl2/Bax and activation of caspase3. *Mol Med Rep* 14: 4173–4179, 2016. doi:10.3892/mmr.2016.5733.

540. Torres-Vázquez J, Kamei M, Weinstein BM. Molecular distinction between arteries and veins. *Cell Tissue Res* 314: 43–59, 2003. doi:10.1007/s00441-003-0771-8.

541. Toth P, Tarantini S, Tucsek Z, Ashpole NM, Sosnowska D, Gautam T, Ballabh P, Koller A, Sonntag WE, Csiszar A, Ungvari Z. Resveratrol treatment rescues neurovascular coupling in aged mice: role of improved cerebromicrovascular endothelial function and downregulation of NADPH oxidase. *Am J Physiol Heart Circ Physiol* 306: H299–H308, 2014. doi:10.1152/ajpheart.00744.2013.

542. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, McDowell EP, Lazo-Kallanian S, Williams IR, Sears C, Armstrong SA, Passegué E, DePinho RA, Gilliland DG. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell* 128: 325–339, 2007. doi:10.1016/j.cell.2007.01.003.

543. Tousoulis D, Kampoli AM, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. *Curr Vasc Pharmacol* 10: 4–18, 2012. doi:10.2174/157016112798829760.

544. Tsai KL, Huang YH, Kao CL, Yang DM, Lee HC, Chou HY, Chen YC, Chiou GY, Chen LH, Yang YP, Chiu TH, Tsai CS, Ou HC, Chiou SH. A novel mechanism of coenzyme Q10 protects against human endothelial cells from oxidative stress-induced injury by modulating NO-related pathways. *J Nutr Biochem* 23: 458–468, 2012. doi:10.1016/j.jnutbio.2011.01.011.

545. Tsuchiya K, Accili D. Liver sinusoidal endothelial cells link hyperinsulinemia to hepatic insulin resistance. *Diabetes* 62: 1478–1489, 2013. doi:10.2337/db12-1296.

546. Tsuchiya K, Tanaka J, Shuiqing Y, Welch CL, DePinho RA, Tabas I, Tall AR, Goldberg IJ, Accili D. FoxOs integrate pleiotropic actions of insulin in vascular endothelium to protect mice from atherosclerosis. *Cell Metab* 15: 372–381, 2012. doi:10.1016/j.cmet.2012.01.018.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

547. Tulsiani DR, Touster O. Studies on dehydrogenases of the glucuronate-xylulose cycle in the livers of diabetic mice and rats. *Diabetes* 28: 793–798, 1979. doi:10.2337/diab.28.9.793.

548. Tyagi N, Ovechkin AV, Lominadze D, Moshal KS, Tyagi SC. Mitochondrial mechanism of microvascular endothelial cells apoptosis in hyperhomocysteinemia. *J Cell Biochem* 98: 1150–1162, 2006. doi:10.1002/jcb.20837.

549. Ubezio B, Blanco RA, Geudens I, Stanchi F, Mathivet T, Jones ML, Ragab A, Bentley K, Gerhardt H. Synchronization of endothelial Dll4-Notch dynamics switch blood vessels from branching to expansion. *eLife* 5: e12167, 2016. doi:10.7554/eLife.12167.

549a. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352: 837–853, 1998. doi:10.1016/S0140-6736(98)07019-6.

550. Unterluggauer H, Mazurek S, Lener B, Hütter E, Eigenbrodt E, Zwerschke W, Jansen-Dürr P. Premature senescence of human endothelial cells induced by inhibition of glutaminase. *Biogerontology* 9: 247–259, 2008. doi:10.1007/s10522-008-9134-x.

551. Vaisman N, Gospodarowicz D, Neufeld G. Characterization of the receptors for vascular endothelial growth factor. *J Biol Chem* 265: 19461–19466, 1990.

552. Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M. PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res* 66: 562–573, 2005. doi:10.1016/j.cardiores.2005.01.026.

553. Van Beijnum JR, Dings RP, van der Linden E, Zwaans BM, Ramaekers FC, Mayo KH, Griffioen AW. Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature. *Blood* 108: 2339–2348, 2006. doi:10.1182/blood-2006-02-004291.

554. Van de Veire S, Stalmans I, Heindryckx F, Oura H, Tijeras-Raballand A, Schmidt T, Loges S, Albrecht I, Jonckx B, Vinckier S, Van Steenkiste C, Tugues S, Rolny C, De Mol M, Dettori D, Hainaud P, Coenegrachts L, Contreres JO, Van Bergen T, Cuervo H, Xiao WH, Le Henaff C, Buysschaert I, Kharabi Masouleh B, Geerts A, Schomber T, Bonnin P, Lambert V, Haustraete J, Zacchigna S, Rakic JM, Jiménez W, Noël A, Giacca M, Colle I, Foidart JM, Tobelem G, Morales-Ruiz M, Vilar J, Maxwell P, Vinores SA, Carmeliet G, Dewerchin M, Claesson-Welsh L, Dupuy E, Van Vlierberghe H, Christofori G, Mazzone M, Detmar M, Collen D, Carmeliet P. Further pharmacological and genetic evidence for the efficacy of PlGF inhibition in cancer and eye disease. *Cell* 141: 178–190, 2010. doi:10.1016/j.cell.2010.02.039.

555. Vander Heiden MG. Exploiting tumor metabolism: challenges for clinical translation. *J Clin Invest* 123: 3648–3651, 2013. doi:10.1172/JCI72391.

556. Vedantham S, Noh H, Ananthakrishnan R, Son N, Hallam K, Hu Y, Yu S, Shen X, Rosario R, Lu Y, Ravindranath T, Drosatos K, Huggins LA, Schmidt AM, Goldberg IJ, Ramasamy R. Human aldose reductase expression accelerates atherosclerosis in diabetic apolipoprotein E-/- mice. *Arterioscler Thromb Vasc Biol* 31: 1805–1813, 2011. doi:10.1161/ATVBAHA.111.226902.

557. Végran F, Boidot R, Michiels C, Sonveaux P, Feron O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-κB/IL-8 pathway that drives tumor angiogenesis. *Cancer Res* 71: 2550–2560, 2011. doi:10.1158/0008-5472.CAN-10-2828.

558. Vendrov AE, Hakim ZS, Madamanchi NR, Rojas M, Madamanchi C, Runge MS. Atherosclerosis is attenuated by limiting superoxide generation in both macrophages and vessel wall cells. *Arterioscler Thromb Vasc Biol* 27: 2714–2721, 2007. doi:10.1161/ATVBAHA.107.152629.

559. Vicent D, Ilany J, Kondo T, Naruse K, Fisher SJ, Kisanuki YY, Bursell S, Yanagisawa M, King GL, Kahn CR. The role of endothelial insulin signaling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 111: 1373–1380, 2003. doi:10.1172/JCI15211.

560. Vigetti D, Viola M, Karousou E, De Luca G, Passi A. Metabolic control of hyaluronan synthases. *Matrix Biol* 35: 8–13, 2014. doi:10.1016/j.matbio.2013.10.002.

561. Vinores SA, Xiao WH, Aslam S, Shen J, Oshima Y, Nambu H, Liu H, Carmeliet P, Campochiaro PA. Implication of the hypoxia response element of the Vegf promoter in mouse models of retinal and choroidal neovascularization, but not retinal vascular development. *J Cell Physiol* 206: 749–758, 2006. doi:10.1002/jcp.20525.

562. Viswambharan H, Yuldasheva NY, Sengupta A, Imrie H, Gage MC, Haywood N, Walker AM, Skromna A, Makova N, Galloway S, Shah P, Sukumar P, Porter KE, Grant PJ, Shah AM, Santos CX, Li J, Beech DJ, Wheatcroft SB, Cubbon RM, Kearney MT. Selective Enhancement of Insulin Sensitivity in the Endothelium In Vivo Reveals a Novel Proatherosclerotic Signaling Loop. *Circ Res* 120: 784–798, 2017. doi:10.1161/CIRCRESAHA.116.309678.

563. Vizán P, Sánchez-Tena S, Alcarraz-Vizán G, Soler M, Messeguer R, Pujol MD, Lee WN, Cascante M. Characterization of the metabolic changes underlying growth factor angiogenic activation: identification of new potential therapeutic targets. *Carcinogenesis* 30: 946–952, 2009. doi:10.1093/carcin/bgp083.

564. Vokes SA, Krieg PA. Endoderm is required for vascular endothelial tube formation, but not for angioblast specification. *Development* 129: 775–785, 2002.

565. Vokes SA, Krieg PA. Vascular molecular embryology. In: *PanVascular Medicine*, edited by Lanzer P. Berlin: Springer-Verlag, 2015, p. 27–51. doi:10.1007/978-3-642-37078-6_2.

566. Vorum H, Brodersen R, Kragh-Hansen U, Pedersen AO. Solubility of long-chain fatty acids in phosphate buffer at pH 7.4. *Biochim Biophys Acta* 1126: 135–142, 1992. doi:10.1016/0005-2760(92)90283-2.

567. Wacker A, Gerhardt H. Endothelial development taking shape. *Curr Opin Cell Biol* 23: 676–685, 2011.

568. Walenta S, Schroeder T, Mueller-Klieser W. Lactate in solid malignant tumors: potential basis of a metabolic classification in clinical oncology. *Curr Med Chem* 11: 2195–2204, 2004. doi:10.2174/0929867043364711.

569. Walsh TP, Winzor DJ, Clarke FM, Masters CJ, Morton DJ. Binding of aldolase to actin-containing filaments. Evidence of interaction with the regulatory proteins of skeletal muscle. *Biochem J* 186: 89–98, 1980. doi:10.1042/bj1860089.

570. Wang L, Xiao Y, Ping Y, Li J, Zhao H, Li F, Hu J, Zhang H, Deng Y, Tian J, Li X. Integrating multi-omics for uncovering the architecture of cross-talking pathways in breast cancer. *PLoS One* 9: e104282, 2014. doi:10.1371/journal.pone.0104282.

571. Wang R, Szabo C, Ichinose F, Ahmed A, Whiteman M, Papapetropoulos A. The role of H2S bioavailability in endothelial dysfunction. *Trends Pharmacol Sci* 36: 568–578, 2015. doi:10.1016/j.tips.2015.05.007.

572. Wang W, Wang Y, Long J, Wang J, Haudek SB, Overbeek P, Chang BH, Schumacker PT, Danesh FR. Mitochondrial fission triggered by hyperglycemia is mediated by ROCK1 activation in podocytes and endothelial cells. *Cell Metab* 15: 186–200, 2012. doi:10.1016/j.cmet.2012.01.009.

573. Wang XH, Qian RZ, Zhang W, Chen SF, Jin HM, Hu RM. MicroRNA-320 expression in myocardial microvascular endothelial cells and its relationship with insulin-like growth factor-1 in type 2 diabetic rats. *Clin Exp Pharmacol Physiol* 36: 181–188, 2009. doi:10.1111/j.1440-1681.2008.05057.x.

574. Wang XR, Zhang MW, Chen DD, Zhang Y, Chen AF. 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: E1135–E1145, 2011. doi:10.1152/ajpendo.00001.2011.

575. Wang Y, Zhang Z, Sun W, Tan Y, Liu Y, Zheng Y, Liu Q, Cai L, Sun J. Sulforaphane attenuation of type 2 diabetes-induced aortic damage was associated with the upregulation of Nrf2 expression and function. *Oxid Med Cell Longev* 2014: 123963, 2014. doi:10.1155/2014/123963.

576. Wang ZV, Deng Y, Gao N, Pedrozo Z, Li DL, Morales CR, Criollo A, Luo X, Tan W, Jiang N, Lehrman MA, Rothermel BA, Lee AH, Lavandero S, Mammen PPA, Ferdous A, Gillette TG, Scherer PE, Hill JA. Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell* 156: 1179–1192, 2014. doi:10.1016/j.cell.2014.01.014.

577. Wautier JL, Schmidt AM. Protein glycation: a firm link to endothelial cell dysfunction. *Circ Res* 95: 233–238, 2004. doi:10.1161/01.RES.0000137876.28454.64.

578. Wedgwood S, Black SM. Role of reactive oxygen species in vascular remodeling associated with pulmonary hypertension. *Antioxid Redox Signal* 5: 759–769, 2003. doi:10.1089/152308603770380061.

579. Wei X, Schneider JG, Shenouda SM, Lee A, Towler DA, Chakravarthy MV, Vita JA, Semenkovich CF. De novo lipogenesis maintains vascular homeostasis through endothelial nitric-oxide synthase (eNOS) palmitoylation. *J Biol Chem* 286: 2933–2945, 2011. doi:10.1074/jbc.M110.193037.

580. Wei X, Song H, Semenkovich CF. Insulin-regulated protein palmitoylation impacts endothelial cell function. *Arterioscler Thromb Vasc Biol* 34: 346–354, 2014. doi:10.1161/ATVBAHA.113.302848.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

581. Weis SM, Cheresh DA. Tumor angiogenesis: molecular pathways and therapeutic targets. *Nat Med* 17: 1359–1370, 2011. doi:10.1038/nm.2537.

582. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324: 1076–1080, 2009. doi:10.1126/science.1164097.

583. Welti J, Loges S, Dimmeler S, Carmeliet P. Recent molecular discoveries in angiogenesis and antiangiogenic therapies in cancer. *J Clin Invest* 123: 3190–3200, 2013. doi:10.1172/JCI70212.

584. Wenceslau CF, McCarthy CG, Szasz T, Spitler K, Goulopoulou S, Webb RC; Working Group on DAMPs in Cardiovascular Disease. Mitochondrial damage-associated molecular patterns and vascular function. *Eur Heart J* 35: 1172–1177, 2014. doi:10.1093/eurheartj/ehu047.

585. Wigle JT, Harvey N, Detmar M, Lagutina I, Grosveld G, Gunn MD, Jackson DG, Oliver G. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. *EMBO J* 21: 1505–1513, 2002. doi:10.1093/emboj/21.7.1505.

586. Wilhelm K, Happel K, Eelen G, Schoors S, Oellerich MF, Lim R, Zimmermann B, Aspalter IM, Franco CA, Boettger T, Braun T, Fruttiger M, Rajewsky K, Keller C, Brüning JC, Gerhardt H, Carmeliet P, Potente M. FOXO1 couples metabolic activity and growth state in the vascular endothelium. *Nature* 529: 216–220, 2016. doi:10.1038/nature16498.

587. Wilson AM, Harada R, Nair N, Balasubramanian N, Cooke JP. L-Arginine supplementation in peripheral arterial disease: no benefit and possible harm. *Circulation* 116: 188–195, 2007. doi:10.1161/CIRCULATIONAHA.106.683656.

588. Wilting J, Chao TI. Integrated vascular anatomy. In: *PanVascular Medicine*, edited by Lanzer P. Berlin: Springer, 2015, p. 193–241. doi:10.1007/978-3-642-37078-6_252

589. Winegrad AI, Burden CL. L-Xylulose metabolism in diabetes mellitus. *N Engl J Med* 274: 298–305, 1966. doi:10.1056/NEJM196602102740602.

590. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci USA* 105: 18782–18787, 2008. doi:10.1073/pnas.0810199105.

591. Wong BW, Wang X, Zecchin A, Thienpont B, Cornelissen I, Kalucka J, García-Caballero M, Missiaen R, Huang H, Brüning U, Blacher S, Vinckier S, Goveia J, Knobloch M, Zhao H, Dierkes C, Shi C, Hägerling R, Moral-Dardé V, Wyns S, Lippens M, Jessberger S, Fendt SM, Luttun A, Noel A, Kiefer F, Ghesquière B, Moons L, Schoonjans L, Dewerchin M, Eelen G, Lambrechts D, Carmeliet P. The role of fatty acid β-oxidation in lymphangiogenesis. *Nature* 542: 49–54, 2017. doi:10.1038/nature21028.

592. Woods A, Dickerson K, Heath R, Hong SP, Momcilovic M, Johnstone SR, Carlson M, Carling D. Ca^{2+}/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells. *Cell Metab* 2: 21–33, 2005. doi:10.1016/j.cmet.2005.06.005.

593. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA* 290: 2159–2167, 2003. doi:10.1001/jama.290.16.2159.

594. Wu C, Huang RT, Kuo CH, Kumar S, Kim CW, Lin YC, Chen YJ, Birukova A, Birukov KG, Dulin NO, Civelek M, Lusis AJ, Loyer X, Tedgui A, Dai G, Jo H, Fang Y. Mechanosensitive PPAP2B Regulates Endothelial Responses to Atherorelevant Hemodynamic Forces. *Circ Res* 117: e41–e53, 2015. doi:10.1161/CIRCRESAHA.117.306457.

595. Wu G, Haynes TE, Li H, Yan W, Meininger CJ. Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. *Biochem J* 353: 245–252, 2001. doi:10.1042/bj3530245.

596. Wu G, Haynes TE, Yan W, Meininger CJ. Presence of glutamine:fructose-6-phosphate amidotransferase for glucosamine-6-phosphate synthesis in endothelial cells: effects of hyperglycaemia and glutamine. *Diabetologia* 44: 196–202, 2001. doi:10.1007/s001250051599.

597. Wu L, Wary KK, Revskoy S, Gao X, Tsang K, Komarova YA, Rehman J, Malik AB. Histone Demethylases KDM4A and KDM4C Regulate Differentiation of Embryonic Stem Cells to Endothelial Cells. *Stem Cell Rep* 5: 10–21, 2015. doi:10.1016/j.stemcr.2015.05.016.

598. Wu S, Gao X, Yang S, Meng M, Yang X, Ge B. The role of endoplasmic reticulum stress in endothelial dysfunction induced by homocysteine thiolactone. *Fundam Clin Pharmacol* 29: 252–259, 2015. doi:10.1111/fcp.12101.

599. Xia Y, Tsai AL, Berka V, Zweier JL. Superoxide generation from endothelial nitric-oxide synthase. A Ca^{2+}/calmodulin-dependent and tetrahydrobiopterin regulatory process. *J Biol Chem* 273: 25804–25808, 1998. doi:10.1074/jbc.273.40.25804.

600. Xiao Y, Huang W, Zhang J, Peng C, Xia M, Ling W. Increased plasma *S*-adenosylhomocysteine-accelerated atherosclerosis is associated with epigenetic regulation of endoplasmic reticulum stress in apoE-/- mice. *Arterioscler Thromb Vasc Biol* 35: 60–70, 2015. doi:10.1161/ATVBAHA.114.303817.

601. Xiao Y, Su X, Huang W, Zhang J, Peng C, Huang H, Wu X, Huang H, Xia M, Ling W. Role of *S*-adenosylhomocysteine in cardiovascular disease and its potential epigenetic mechanism. *Int J Biochem Cell Biol* 67: 158–166, 2015. doi:10.1016/j.biocel.2015.06.015.

602. Xu H, Hertzel AV, Steen KA, Wang Q, Suttles J, Bernlohr DA. Uncoupling lipid metabolism from inflammation through fatty acid binding protein-dependent expression of UCP2. *Mol Cell Biol* 35: 1055–1065, 2015. doi:10.1128/MCB.01122-14.

603. Xu J, Zou MH. Molecular insights and therapeutic targets for diabetic endothelial dysfunction. *Circulation* 120: 1266–1286, 2009. doi:10.1161/CIRCULATIONAHA.108.835223.

604. Xu L, Kanasaki K, Kitada M, Koya D. Diabetic angiopathy and angiogenic defects. *Fibrogenesis Tissue Repair* 5: 13, 2012. doi:10.1186/1755-1536-5-13.

605. Xu S, Liu Z, Liu P. Targeting hydrogen sulfide as a promising therapeutic strategy for atherosclerosis. *Int J Cardiol* 172: 313–317, 2014. doi:10.1016/j.ijcard.2014.01.068.

606. Xu W, Kaneko FT, Zheng S, Comhair SA, Janocha AJ, Goggans T, Thunnissen FB, Farver C, Hazen SL, Jennings C, Dweik RA, Arroliga AC, Erzurum SC. Increased arginase II and decreased NO synthesis in endothelial cells of patients with pulmonary arterial hypertension. *FASEB J* 18: 1746–1748, 2004. doi:10.1096/fj.04-2317fje.

607. Xu W, Koeck T, Lara AR, Neumann D, DiFilippo FP, Koo M, Janocha AJ, Masri FA, Arroliga AC, Jennings C, Dweik RA, Tuder RM, Stuehr DJ, Erzurum SC. Alterations of cellular bioenergetics in pulmonary artery endothelial cells. *Proc Natl Acad Sci USA* 104: 1342–1347, 2007. doi:10.1073/pnas.0605080104.

608. Xu Y, An X, Guo X, Habtetsion TG, Wang Y, Xu X, Kandala S, Li Q, Li H, Zhang C, Caldwell RB, Fulton DJ, Su Y, Hoda MN, Zhou G, Wu C, Huo Y. Endothelial PFKFB3 plays a critical role in angiogenesis. *Arterioscler Thromb Vasc Biol* 34: 1231–1239, 2014. doi:10.1161/ATVBAHA.113.303041.

609. Xu Y, Zhang Z, Hu J, Stillman IE, Leopold JA, Handy DE, Loscalzo J, Stanton RC. Glucose-6-phosphate dehydrogenase-deficient mice have increased renal oxidative stress and increased albuminuria. *FASEB J* 24: 609–616, 2010. doi:10.1096/fj.09-135731.

610. Xue M, Qian Q, Adaikalakoteswari A, Rabbani N, Babaei-Jadidi R, Thornalley PJ. Activation of NF-E2-related factor-2 reverses biochemical dysfunction of endothelial cells induced by hyperglycemia linked to vascular disease. *Diabetes* 57: 2809–2817, 2008. doi:10.2337/db06-1003.

611. Xue M, Rabbani N, Momiji H, Imbasi P, Anwar MM, Kitteringham N, Park BK, Souma T, Moriguchi T, Yamamoto M, Thornalley PJ. Transcriptional control of glyoxalase 1 by Nrf2 provides a stress-responsive defence against dicarbonyl glycation. *Biochem J* 443: 213–222, 2012. doi:10.1042/BJ20111648.

612. Yadav UC, Srivastava SK, Ramana KV. Prevention of VEGF-induced growth and tube formation in human retinal endothelial cells by aldose reductase inhibition. *J Diabetes Complications* 26: 369–377, 2012. doi:10.1016/j.jdiacomp.2012.04.017.

613. Yalcin A, Clem BF, Imbert-Fernandez Y, Ozcan SC, Peker S, O'Neal J, Klarer AC, Clem AL, Telang S, Chesney J. 6-Phosphofructo-2-kinase (PFKFB3) promotes cell cycle progression and suppresses apoptosis via Cdk1-mediated phosphorylation of p27. *Cell Death Dis* 5: e1337, 2014. doi:10.1038/cddis.2014.292.

614. Yalcin A, Clem BF, Simmons A, Lane A, Nelson K, Clem AL, Brock E, Siow D, Wattenberg B, Telang S, Chesney J. Nuclear targeting of 6-phosphofructo-2-kinase (PFKFB3) increases proliferation via cyclin-dependent kinases. *J Biol Chem* 284: 24223–24232, 2009. doi:10.1074/jbc.M109.016816.

615. Yamamoto K, Ohga N, Hida Y, Maishi N, Kawamoto T, Kitayama K, Akiyama K, Osawa T, Kondoh M, Matsuda K, Onodera Y, Fujie M, Kaga K, Hirano S, Shinohara N,

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.

Shindoh M, Hida K. Biglycan is a specific marker and an autocrine angiogenic factor of tumour endothelial cells. *Br J Cancer* 106: 1214–1223, 2012. doi:10.1038/bjc.2012.59.

616. Yamanishi S, Katsumura K, Kobayashi T, Puro DG. Extracellular lactate as a dynamic vasoactive signal in the rat retinal microvasculature. *Am J Physiol Heart Circ Physiol* 290: H925–H934, 2006. doi:10.1152/ajpheart.01012.2005.

617. Yamashita J, Itoh H, Hirashima M, Ogawa M, Nishikawa S, Yurugi T, Naito M, Nakao K, Nishikawa S. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature* 408: 92–96, 2000. doi:10.1038/35040568.

618. Yan SF, Ramasamy R, Naka Y, Schmidt AM. Glycation, inflammation, and RAGE: a scaffold for the macrovascular complications of diabetes and beyond. *Circ Res* 93: 1159–1169, 2003. doi:10.1161/01.RES.0000103862.26506.3D.

619. Yanes O, Clark J, Wong DM, Patti GJ, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G. Metabolic oxidation regulates embryonic stem cell differentiation. *Nat Chem Biol* 6: 411–417, 2010. doi:10.1038/nchembio.364.

620. Yang F, Qi X, Gao Z, Yang X, Zheng X, Duan C, Zheng J. Homocysteine injures vascular endothelial cells by inhibiting mitochondrial activity. *Exp Ther Med* 12: 2247–2252, 2016. doi:10.3892/etm.2016.3564.

621. Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev* 68: 270–279, 2010. doi:10.1111/j.1753-4887.2010.00282.x.

622. Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I, Magistretti PJ. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc Natl Acad Sci USA* 111: 12228–12233, 2014. doi:10.1073/pnas.1322912111.

623. Yang XL, Tian J, Liang Y, Ma CJ, Yang AN, Wang J, Ma SC, Cheng Y, Hua X, Jiang YD. Homocysteine induces blood vessel global hypomethylation mediated by LOX-1. *Genet Mol Res* 13: 3787–3799, 2014. doi:10.4238/2014.May.16.2.

624. Yang Y, Oliver G. Development of the mammalian lymphatic vasculature. *J Clin Invest* 124: 888–897, 2014. doi:10.1172/JCI71609.

625. Yang Y, Wu Z, Meininger CJ, Wu G. L-Leucine and NO-mediated cardiovascular function. *Amino Acids* 47: 435–447, 2015. doi:10.1007/s00726-014-1904-y.

626. Yao D, Brownlee M. Hyperglycemia-induced reactive oxygen species increase expression of the receptor for advanced glycation end products (RAGE) and RAGE ligands. *Diabetes* 59: 249–255, 2010. doi:10.2337/db09-0801.

627. Yeh WL, Lin CJ, Fu WM. Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. *Mol Pharmacol* 73: 170–177, 2008. doi:10.1124/mol.107.038851.

628. Yeo H, Lyssiotis CA, Zhang Y, Ying H, Asara JM, Cantley LC, Paik JH. FoxO3 coordinates metabolic pathways to maintain redox balance in neural stem cells. *EMBO J* 32: 2589–2602, 2013. doi:10.1038/emboj.2013.186.

629. Yizhak K, Chaneton B, Gottlieb E, Ruppin E. Modeling cancer metabolism on a genome scale. *Mol Syst Biol* 11: 817, 2015. doi:10.15252/msb.20145307.

630. Yu CW, Liang X, Lipsky S, Karaaslan C, Kozakewich H, Hotamisligil GS, Bischoff J, Cataltepe S. Dual role of fatty acid-binding protein 5 on endothelial cell fate: a potential link between lipid metabolism and angiogenic responses. *Angiogenesis* 19: 95–106, 2016. doi:10.1007/s10456-015-9491-4.

631. Yun H, Lee M, Kim SS, Ha J. Glucose deprivation increases mRNA stability of vascular endothelial growth factor through activation of AMP-activated protein kinase in DU145 prostate carcinoma. *J Biol Chem* 280: 9963–9972, 2005. doi:10.1074/jbc.M412994200.

632. Zala D, Hinckelmann MV, Yu H, Lyra da Cunha MM, Liot G, Cordelières FP, Marco S, Saudou F. Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell* 152: 479–491, 2013. doi:10.1016/j.cell.2012.12.029.

633. Zeng G, Nystrom FH, Ravichandran LV, Cong LN, Kirby M, Mostowski H, Quon MJ. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* 101: 1539–1545, 2000. doi:10.1161/01.CIR.101.13.1539.

634. Zhang E, Guo Q, Gao H, Xu R, Teng S, Wu Y. Metformin and Resveratrol Inhibited High Glucose-Induced Metabolic Memory of Endothelial Senescence through SIRT1/p300/p53/p21 Pathway. *PLoS One* 10: e0143814, 2015. doi:10.1371/journal.pone.0143814.

635. Zhang H, Liu Z, Ma S, Zhang H, Kong F, He Y, Yang X, Wang Y, Xu H, Yang A, Tian J, Zhang M, Cao J, Jiang Y, Guo X. Ratio of *S*-adenosylmethionine to *S*-adenosylhomocysteine as a sensitive indicator of atherosclerosis. *Mol Med Rep* 14: 289–300, 2016. doi:10.3892/mmr.2016.5230.

636. Zhang L, Conejo-Garcia JR, Yang N, Huang W, Mohamed-Hadley A, Yao W, Benencia F, Coukos G. Different effects of glucose starvation on expression and stability of VEGF mRNA isoforms in murine ovarian cancer cells. *Biochem Biophys Res Commun* 292: 860–868, 2002. doi:10.1006/bbrc.2002.6710.

637. Zhang QJ, Wang Z, Chen HZ, Zhou S, Zheng W, Liu G, Wei YS, Cai H, Liu DP, Liang CC. Endothelium-specific overexpression of class III deacetylase SIRT1 decreases atherosclerosis in apolipoprotein E-deficient mice. *Cardiovasc Res* 80: 191–199, 2008. doi:10.1093/cvr/cvn224.

638. Zhang Z, Apse K, Pang J, Stanton RC. High glucose inhibits glucose-6-phosphate dehydrogenase via cAMP in aortic endothelial cells. *J Biol Chem* 275: 40042–40047, 2000. doi:10.1074/jbc.M007505200.

639. Zhang Z, Wang S, Zhou S, Yan X, Wang Y, Chen J, Mellen N, Kong M, Gu J, Tan Y, Zheng Y, Cai L. Sulforaphane prevents the development of cardiomyopathy in type 2 diabetic mice probably by reversing oxidative stress-induced inhibition of LKB1/AMPK pathway. *J Mol Cell Cardiol* 77: 42–52, 2014. doi:10.1016/j.yjmcc.2014.09.022.

640. Zhao YD, Courtman DW, Deng Y, Kugathasan L, Zhang Q, Stewart DJ. Rescue of monocrotaline-induced pulmonary arterial hypertension using bone marrow-derived endothelial-like progenitor cells: efficacy of combined cell and eNOS gene therapy in established disease. *Circ Res* 96: 442–450, 2005. doi:10.1161/01.RES.0000157672.70560.7b.

641. Zheng PP, van der Weiden M, van der Spek PJ, Vincent AJ, Kros JM. Intratumoral, not circulating, endothelial progenitor cells share genetic aberrations with glial tumor cells. *J Cell Physiol* 228: 1383–1390, 2013. doi:10.1002/jcp.24309.

642. Zheng Z, Chen H, Li J, Li T, Zheng B, Zheng Y, Jin H, He Y, Gu Q, Xu X. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. *Diabetes* 61: 217–228, 2012. doi:10.2337/db11-0416.

643. Zhou S, Chen HZ, Wan YZ, Zhang QJ, Wei YS, Huang S, Liu JJ, Lu YB, Zhang ZQ, Yang RF, Zhang R, Cai H, Liu DP, Liang CC. Repression of P66Shc expression by SIRT1 contributes to the prevention of hyperglycemia-induced endothelial dysfunction. *Circ Res* 109: 639–648, 2011. doi:10.1161/CIRCRESAHA.111.243592.

644. Zhou S, Zhang Z, Xu G. Notable epigenetic role of hyperhomocysteinemia in atherogenesis. *Lipids Health Dis* 13: 134, 2014. doi:10.1186/1476-511X-13-134.

645. Zhou X, Chen M, Zeng X, Yang J, Deng H, Yi L, Mi MT. Resveratrol regulates mitochondrial reactive oxygen species homeostasis through Sirt3 signaling pathway in human vascular endothelial cells. *Cell Death Dis* 5: e1576, 2014. doi:10.1038/cddis.2014.530.

646. Zhou X, Li Q, Wang Y, Huang S, Jiang L, Zhou J, Cao Y. A single fusion signal for t(14;18)(q32;q21) translocation is present in both the follicular lymphoma and local endothelial cells. *J Huazhong Univ Sci Technolog Med Sci* 32: 324–327, 2012. doi:10.1007/s11596-012-0056-0.

647. Zhou Y, Fujisawa I, Ino K, Matsue T, Shiku H. Metabolic suppression during mesodermal differentiation of embryonic stem cells identified by single-cell comprehensive gene expression analysis. *Mol Biosyst* 11: 2560–2567, 2015. doi:10.1039/C5MB00340G.

648. Zhu J, Shi Z, Wang J, Zhang B. Empowering biologists with multi-omics data: colorectal cancer as a paradigm. *Bioinformatics* 31: 1436–1443, 2015. doi:10.1093/bioinformatics/btu834.

Downloaded from journals.physiology.org/journal/physrev (106.051.226.007) on August 4, 2022.