Understanding the Causes and Implications of Endothelial Metabolic Variation in Cardiovascular Disease through Genome-Scale Metabolic Modeling

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High-throughput biochemical profiling has led to a requirement for advanced data interpretation techniques capable of integrating the analysis of gene, protein, and metabolic profiles to shed light on genotype-phenotype relationships. Herein, we consider the current state of knowledge of endothelial cell (EC) metabolism and its connections to cardiovascular disease (CVD) and explore the use of genome-scale metabolic models (GEMs) for integrating metabolic and genomic data. GEMs combine gene expression and metabolic data acting as frameworks for their analysis and, ultimately, afford mechanistic understanding of how genetic variation impacts metabolism. We demonstrate how GEMs can be used to investigate CVD-related genetic variation, drug resistance mechanisms, and novel metabolic pathways in ECs. The application of GEMs in personalized medicine is also highlighted. Particularly, we focus on the potential of GEMs to identify metabolic biomarkers of endothelial dysfunction and to discover methods of stratifying treatments for CVDs based on individual genetic markers. Recent advances in systems biology methodology, and how these methodologies can be applied to understand EC metabolism in both health and disease, are thus highlighted.

Keywords: endothelium, metabolism, personalized/precision medicine, metabolomics, metabolic modeling, genetics

INTRODUCTION

Cardiovascular disease (CVD) includes acute and chronic conditions, such as stroke and coronary heart disease (1). CVD results in a shortened life span and is the biggest cause of death worldwide (1–3). The endothelium is the single cell layer that lines blood vessels and lymphatic system and its dysfunction contributes to the development of CVD (4, 5). Endothelial cells (ECs) play an important role in controlling vascular tone and by secreting or expressing surface molecules, they ensure appropriate regulation of blood flow, countering intravascular activation of platelets, and coagulation (6, 7). Moreover, cardiac ECs have been shown to affect the ventricular myocardium. Thus, the
force-frequency response of cardiac muscle in the presence of increased cardiac workload is blunted after damage to the cardiac endothelium (8).

A vascular surface that normally is thromboresistant, anti-inflammatory, vasodilatory, and anti-proliferative can turn into a surface that is thrombogenic, pro-inflammatory, vasoconstrictive, and stimulatory of smooth muscle cell proliferation. Often this change is reactive and transient restoring vascular homeostasis. However, in diseases such as atherosclerosis, hypertension, and diabetes mellitus (DM) such changes, known as endothelial dysfunction, may be prolonged and critical for disease progression. The extent of pathological metabolic perturbation is determined by an interaction of lifestyle factors, such as diet and exercise with underlying genetic factors (9–12). Consequently, health-care interventions may be more effective if adapted to an individual.

Metabolic modeling offers insights into cellular metabolism (13). Below, we consider endothelial metabolic alterations, their contribution to endothelial dysfunction, and integrated analysis of this information with genome-scale metabolic models (GEMs) to advance personalized health care.

ENDOTHELIAL METABOLISM

Endothelial cell metabolism has been investigated in multiple contexts including angiogenesis, hypoxia, shear stress, glycemia, and response to perturbations with mediators of vascular health including thrombin, sphingosine-1-phosphate, and more (14–19). The endothelium operates with variable nutrient availability and oxygen partial pressures in a manner that is EC subtype specific (20) and results in altered synergy in the oxidation of its core nutrients glucose, fatty acids, and amino acids (17, 21–23) that are reviewed specifically elsewhere (24, 25) but considered collectively here and illustrated in Figure 1.

Glycolysis Affects Endothelial Proliferation and Angiogenesis

Endothelial cells oxidize glucose largely by glycolysis, allowing maximal availability of oxygen for transendothelial transport to perivascular cells (26–29). Carbons from glucose are primarily excreted as lactate with only 1 in 200 pyruvate equivalents contributing to oxidative phosphorylation (26). Laminar shear stress, the frictional force created by blood flow, promotes anti-inflammatory, anti-thrombotic, and anti-oxidative properties in ECs and helps to maintain quiescence largely via the transcription factor Kruppel-like factor 2 (30) that acts to repress phosphofructokinase-2/fructose-2,6-bisphosphatase-3 (PFKFB3) thereby promoting a quiescent phenotype (16).

In response to angiogenic factors induced by injury or in pathological conditions such as hypoxia, nutrient deprivation, or tissue damage, ECs quickly form new vasculature by sprouting. During vessel sprouting, glycolysis is increased further, mediated by increased activity of PFKFB3, the loss of which impairs vessel formation (26). Increased glycolysis without oxidation of pyruvate relies on lactate dehydrogenase to supply NAD⁺, and the activity of PFKFB3 is reflected in both intracellular and secreted lactate of ECs (31). Furthermore, lactate is involved in PFKFB3-mediated endothelial proliferation, tube formation, and Akt activation providing a plausible explanation for PFKFB3-mediated angiogenesis (31). Lactate dehydrogenase activity also increases with EC subtype proliferation rate. In pulmonary microvascular ECs, rapid angiogenesis is dependent on lactate dehydrogenase A expression (14).

Endothelial-dependent vascular function correlates with blood glucose levels (32–37). In hyperglycemia, glycolaldehyde-3-phosphate dehydrogenase is inactivated, impeding glycolysis (38). A build-up of fructose-6-phosphate, a glycolytic intermediate, impacts hexosamine biosynthesis generating N-acetylglucosamine that glycosylates and modifies angiogenic proteins including Notch and vascular endothelial growth factor receptor 2 (39–45) and, inhibits eNOS (46). Excess glucose also enters the polyol pathway, producing excess advanced glycation end products (AGEs) (47, 48). AGEs alter the binding of erythrocytes and platelets to the endothelium (49, 50), and clinical arterial responsiveness correlates negatively with the ratio of AGEs to soluble receptor of AGEs (51).

Fatty Acid and Amino Acids Metabolism

Fatty acid-binding protein 4 (FABP4) is an intracellular fatty acid chaperone protein that impacts the peroxisome proliferator-activated receptor transcription pathway (52). Circulating levels of FABP4 are associated with endothelial dysfunction in DM patients (53) and increased risk of atherosclerosis and cerebrovascular malformations (54, 55).

Fatty acid oxidation (FAO) accounts for roughly 14% of ATP production in cultured EC (22). Carnitine palmitoyl transferase (CPT1A), a long-chain fatty acid shuttle protein regulated by...
AMP-activated protein kinase, is a key point of FAO regulation (22, 56, 57). Palmitate has been shown to contribute carbons to nucleotide formation via the tricarboxylic acid (TCA) cycle. When CPT1A was knocked down in vitro, vessel sprouting was impaired due to low levels of deoxy ribonucleotides. CPT1A knockdown in mice produced impaired retinal vessel formation (58).

In addition to glucose and fatty acids, amino acids contribute to EC metabolism and function (59). Specifically glutamine fuels anaplerotic reactions via the TCA cycle (23, 29, 60). Internalization of glutamine occurs via solute carrier family 1 member 5 (23, 29), and inhibition of glutaminase causes premature senescence and reduced proliferation in ECs (61). The most intensely investigated amino acid with respect to endothelial dysfunction is, however, arginine in the context of its conversion to the vasorelaxant nitric oxide (NO) by endothelial nitric oxide synthase (eNOS).

**Endothelial Nitric Oxide Is Important to Vascular Function and Its Production Is Affected by Genetic and Metabolic Factors**

In addition to causing vasorelaxation, NO affects smooth muscle cell proliferation, aggregation and adhesion of platelets and leukocytes, important processes to atherosclerosis and other CVD (62, 63). When eNOS has insufficient arginine, a result of competition with arginase, and/or lacks the cofactor tetrahydrobiopterin, it produces reactive oxygen species (ROS) instead of the products NO and citrulline – in a pathological state known as uncoupling (64–71). Furthermore, the pressure of \( O_2 \) causes rapid inactivation of endothelium-derived NO (72). Indeed, arginase and eNOS activities and genotypes in addition to tetrahydrobiopterin levels have all been linked to endothelial function (73–76).

Altered NOS activity due to inhibition by asymmetric dimethylarginine (ADMA) encourages NO uncoupling leading to endothelial dysfunction. ADMA levels, and the ratio of ADMA to arginine, have been connected to several aspects of CVD risk (77–80).

Genetic variation in eNOS affects some measures of recovery of blood flow control in acute myocardial infarction (73). Inhibiting arginase activity, which reduces eNOS uncoupling, is helpful in restoring endothelial function in both coronary artery disease and after ischemia–reperfusion injury (64, 65). Genetic variation in NOS1 has also been linked with CVD in various studies (75, 76). Furthermore, the ROS scavenger methionine sulfoxide reductase A, important to reducing the effect of uncoupled NOS and other ROS, is affected by genetic variation relevant to coronary artery disease risk (81, 82).

Interestingly, the extracellular presence of certain amino acids – ornithine, l-lysine, l-homoarginine, l-glutamine, l-leucine, or l-serine – decreases NO and increases endothelium-dependent vascular resistance. This effect is reversible by adding arginine to the medium and was shown to be dependent on \( y^+L \) and \( y^+ \) family amino acid transporters (83).

**DECODING ENDOTHELIAL METABOLISM AND FUNCTION THROUGH COMPUTATIONAL MODELING**

The previous section highlights the complexity of the contribution of metabolism to endothelial dysfunction. Importantly, some of the most common human metabolic gene alterations impact enzymes that are of importance to endothelial metabolic phenotypes. These include pyruvate kinase (84) glucose-6-phosphate dehydrogenase, which alters CVD risk (85), in addition to those already mentioned above. The variability of the effect of these mutations on cardiovascular phenotypes highlights the problem of untangling complex genetic diseases (12). This complexity is aggravated by lifestyle choices that impact the expression and activity of these genes (9, 86–89). How altered gene expression and the environment combine to advance CVD can, however, be explored on the metabolic level, through metabolic systems analysis using genome-scale models of endothelial metabolism.

For CVD research, genome-scale modeling promises to contribute to the definition of endothelial metabolism under different physiological conditions, allow the differentiation of individual endothelial metabolic phenotypes that can be related to CVD states and ultimately contribute to individualized therapy. In the following sections, we explain the concept of GEMs, their current and potential applications toward increasing the understanding of endothelial metabolism, and how this could lead to novel discoveries to combat CVD on the individual level.

**GEMs Provide Snapshots of Metabolism**

Genome-scale metabolic models are computational models that can be used to describe and investigate the metabolic flux phenotype of a cell based on disparate biochemical information. GEMs are built from biochemical component knowledge-bases, also termed biochemical network reconstructions (90). Reconstructions are organism specific and account for genetic, and biochemical components, and their interactions, based on annotated biological information sourced from literature. All metabolic reactions and metabolites contained within a reconstruction can be represented as a numerical matrix, which is comprised of the stoichiometric factors of reactants and products of each metabolic reaction. In this format, the metabolome is subject to computational research allowing metabolic reaction flux at steady state through metabolic pathways to be computed (91).

Genome-scale metabolic reconstructions aim to account for as many as possible biochemical interactions that have been described in an organism (e.g., a human). While reconstructions afford a mechanistic description of genotype–phenotype relationships, they are not context specific. However, when constrained with cell or context-specific data, for example gene expression information of ECs, reconstructions afford GEMs that are descriptive of the biological event and cell of interest. Gene expression data of a HUVEC cells at normoxia vs. hypoxia would for instance generate two GEMs based on the same reconstruction thereby providing two snapshots descriptive of metabolic flux through reactions as defined by the two expression datasets. Essentially, reconstructions define the biochemical components...
of a particular cell or cellular event. Genomic, proteomic, and/or metabolomic fingerprints can thus be analyzed and compared within the context of GEMs (92).

The methodology of building, curating, and analyzing reconstructions and GEMs is commonly referred to as constraint-based analysis. Various software has been developed to facilitate constraint-based analysis including the COBRA and RAVEN toolboxes for Matlab, Merlin and CORDA (93–96). Detailed protocols describing the necessary stages of building and curation are established (90, 97–99). Ultimately, constraint-based analysis of GEMs allows holistic exploration of metabolic phenotypes in silico and affords realistic hypotheses of biochemical mechanisms (92). In the past 5 years, multiple applications of GEMs descriptive of human metabolism have materialized that may contribute to the understanding of how genetic and environmental factors collectively contribute to CVD disease phenotypes when applied to endothelial metabolic research.

**GEMs Differentiate between Metabolic Phenotypes**

In the context of CVD, GEMs that are descriptive of healthy and CVD endothelial metabolism can be produced. As recently reviewed in Väremo et al. (100), GEMs of various tissues have been built and applied to the investigation of CVD-related disorders, including DM and metabolic syndrome, although not yet endothelium (101–104). Transcriptional changes in cardiomyocytes of DM patients have been analyzed using the myocyte-specific GEM, iMyocyte2419, revealing deregulation of metabolic pathways ultimately linked to dihydro-lipoamide dehydrogenase, a unique characteristic of myocyte response in DM (101).

Genome-scale metabolic models serve as a biomarker discovery tool, and a tool to discover potentially “druggable” metabolic (105). Computational techniques exist that predict the pathways likely to be responsible for differences between two metabolic states, identifying these differences allows reactions, linked to genes in a GEM, to be selected as drug targets, for example in hepatocellular carcinoma and Alzheimer’s, or metabolites to be identified as potential biomarkers for example for drug resistance in ovarian cancer (100, 106–108). Changes due in FAO in ECs leading to alterations in EC permeability – clinically important to sepsis – have been detected using a GEM. Altering FAO using drugs was shown to alter permeability, which may be clinically useful (109), future discoveries of this type may be linked to NO synthesis or clotting factor production useful for modulating CVD risk factors.

**GEMs Can Define Endothelial Metabolism**

Genome-scale metabolic models that are descriptive of core endothelial metabolism have already been produced. Patella et al recently used endothelial proteomic data to constrain the human reconstruction, Recon 1 (110), to generate a GEM that describes EC core metabolism during tube formation in matrigel (109). FAO was identified as an area of metabolism that is altered during tube formation. CPT1A inhibition affects ATP production via the TCA cycle and oxidative phosphorylation.

Downstream, this alters Ca^{2+} signaling and junctional proteins via phospho-signaling to alter endothelial permeability, which were partially reversed by pyruvate supplementation (109). Automated GEMs have also been generated for colon and cerebral cortex ECs (111), though these models were not applied to CVD research.

Although automatically generated GEMs of EC metabolism have been used to reveal basal endothelial metabolic pathway usage, further curation and validation of EC GEMs would be beneficial. Investigations of vascular endothelial metabolism in different conditions and with different genetic backgrounds could be achieved, allowing genetic variation outside the context of core energy metabolism to be queried. For example, due to the inherent connectivity of metabolic reactions within GEMs, alterations in the release of sphingosine-1-P (a sphingolipid involved in vascular and immune signaling pathways) from ECs could be hypothesized and related to alterations in core energy metabolism induced by global metabolic expression profiles. The release of sphingosine-1-P from ECs and its contribution to individual vascular health could thus be proposed on biochemical alterations on the systems level as opposed to mutations in sphingosine kinase alone.

**GEMs Can Be Personalized to Account for Individual Genetic Variation**

Computational modeling can contribute to decisions regarding the suitability of a treatment for individual patients. GEMs could be produced for individuals based on genomics and subsequently used to stratify patients and personalize medical interventions for CVD. GEMs maybe based on generalized transcriptomic data from a pool of samples from a cell type (112) or a set of models may be created from individual samples and comparing the metabolic phenotypes predicted by each, allowing links between metabolism and broader phenotype, such as drug resistance in cancer cells, to be explored and may lead to insights about predictive biomarkers and druggable targets (108, 113, 114). Various algorithms for selecting active reactions for context-specific models based on transcriptomic and proteomic data are available including INIT and IMAT. These approaches have differing strengths and weaknesses that have been described and compared elsewhere (98).

Individualized hepatocellular carcinoma models have been used to predict patient outcomes based on the predicted production of acetate, identified as a key metabolic pathway for survival (114). Twenty-four individualized GEMs of erythrocytes were created based on genetic and metabolic data. These captured altered dynamics of erythrocyte metabolism and allowed the identification of individuals at risk to drug-induced anemia based upon their genomic sequence (115). These examples highlight a potential workflow, exemplified in Figure 2, to contribute to the personalization and stratification of medical treatments in the clinic. In the future, it is envisioned that an EC GEM could be used in a similar fashion by comparing GEMs CVD patients and healthy individuals to identify key metabolic changes to CVD for example those that increase production of atherosclerotic plaques.
CONCLUSION

Developing personalized CVD therapeutic interventions relies on the ability to account for genotypic and phenotypic variation. Variability in disease phenotypes can be captured and understood in the context of GEMs to facilitate this process.

Genome-scale metabolic models provide an integrated approach in studying EC metabolism. They allow analysis of the multiple factors affecting ECs in the body, facilitating the exploration of the relationship of genotype to metabolic phenotype. This offers the possibility of producing personalized predictions of CVD risk and treatment, that account for both genetic and lifestyle factors. Currently, GEMs are the only biochemical model type that can account for both of these factors within a predictive modeling framework (92).

Genome-scale metabolic models are only one type of model used to account for EC function. Focused and mechanistic computational models of various aspects of vascular biology have also been made. These address some important biophysical parameters that are currently outside the scope of GEM modeling. This includes assessing the effects of shear stress on blood vessel reactivity and growth as well as the effects on blood cell/endothelium interactions of flow (116–122). Models describing the effects of circulation on endothelial metabolites have also been built (123). Endothelial NO interactions (124–126), Ca²⁺ signaling (127) along with protein (128) and mechanical (119) signaling have also been addressed with computational modeling. Models have been individualized using patient data and have explored the effects of stenting on blood flow (129–132).

Integrating biophysical and signaling parameters with GEMs would generate a more complete understanding of the role of endothelial metabolism for CVD. In addition, these future GEMs would allow retrospective analysis of biophysical and genomic data that have been generated in the last few decades from population studies (86, 133), whose analysis is currently confined to multivariate statistical and comparative analysis techniques for the identification of CVD risk factors. Such an effort could allow, for example, in silico querying of the effect of LDL deposition on global endothelial metabolism. Indeed, computational analysis of LDL metabolism has already proposed novel approaches to combat CVD (134–136).

Realistic computational predictions of the effects of genetic and environmental perturbations on endothelial metabolism are possible and benefical. There has been some exploration of CVD with GEMs and analysis of EC metabolism with GEMs; however, the full potential of this technique is only just beginning to be explored. Existing and future models will allow clinicians and researchers to investigate variable endothelial function in silico in a data-driven manner, to optimize future clinical interventions.

AUTHOR CONTRIBUTIONS

SM and OR wrote the manuscript and conceived the ideology. HH, SP, and PJ conceived the ideology and contributed to writing of the manuscript.

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