Published in final edited form as:

Microcirculation. 2017 August ; 24(6): . doi:10.1111/micc.12359.

A conceptual framework for predicting and addressing the consequences of disease-related microvascular dysfunction

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Abstract

Objective: A growing body of evidence indicates that impaired microvascular perfusion plays a pathological role in a number of diseases. This manuscript aims to better define which aspects of microvascular perfusion are important, what mass transport processes (eg, insulin action, tissue oxygenation) may be impacted, and what therapies might reverse these pathologies.

Methods: We derive a theory of microvascular perfusion and solute flux drawing from established relationships in mass transport and anatomy. We then apply this theory to predict relationships between microvascular perfusion parameters and microvascular solute flux.

Results: For convection-limited exchange processes (eg, pulmonary oxygen uptake), our model predicts that bulk blood flow is of primary importance. For diffusion-limited exchange processes (eg, insulin action), our model predicts that perfused capillary density is of primary importance. For convection/diffusion co-limited exchange processes (eg, tissue oxygenation), our model predicts that various microvascular perfusion parameters interact in a complex, context-specific manner. We further show that our model can predict established mass transport defects in disease (eg, insulin resistance in diabetes).

Conclusions: The contributions of microvascular perfusion parameters to tissue-level solute flux can be described using a minimal mathematical model. Our results hold promise for informing therapeutic interventions targeting microvascular perfusion.

Keywords

blood flow; insulin resistance; mathematical modeling; microvascular perfusion; oxygen delivery

1 | INTRODUCTION

Research investigating microvascular perfusion has traditionally focused on the quantity of blood flow supplied by upstream arteries. The scientific literature increasingly shows that the distribution of this blood flow within the microcirculation is also physiologically important. Evidence of microvascular dysfunction contributing to tissue hypoxia has been reported in the metabolic syndrome^{1,2} and sepsis.^{3,4} Similar microvascular defects have been reported in Alzheimer's disease,⁵ inflammatory bowel disease,⁶ and hypertension and obesity.⁷ Hints of a mechanism linking impaired microvascular perfusion to tissue hypoxia can be found in simulation studies of ischemic heart disease⁸ and cerebrovascular disease.⁹ Tissue hypoxia is the most common focus of microvascular perfusion studies, but other solute exchange processes are also influenced by microvascular perfusion. For example, animal models of insulin resistance show that the primary barrier to glucose flux in the insulin-resistant state lies in the extracellular (ie, vascular to interstitial) step of glucose and insulin delivery.¹⁰ The sheer variety of diseases in which microvascular perfusion defects occur and the variety of consequences related to impaired perfusion suggests that microvascular perfusion is a critical physiological process in its own right.

This manuscript will discuss the importance of three distinct perfusion parameters: the amount of blood flowing through a microvascular network (bulk blood flow), its distribution at microvascular bifurcations (perfusion heterogeneity), and the number of capillaries accessible to flowing blood (perfused capillary density). It is unclear which solute exchange processes (eg, oxygen delivery, lactate clearance) are impacted by which specific microvascular perfusion parameters and how perfusion abnormalities might be therapeutically targeted. The importance of bulk blood flow is illustrated by diseases that substantially decrease blood supply (eg, heart failure, peripheral arterial disease). Perfusion heterogeneity has been empirically demonstrated to modulate oxygen flux in skeletal muscle^{1,2} and is also thought to contribute to ischemic heart disease and cerebrovascular disease.^{8,9} Perfused capillary density is modulated both by long-term changes in anatomical capillary density (density of capillaries present within the tissue, perfused or not) and by short-term changes in the fraction of capillaries present that are actually perfused. Long-term changes in anatomical capillary density can be stimulated by diseases such as T2DM¹¹ and physiological stressors such as exercise training.¹² Short-term changes in perfused capillary density can be caused by stimuli such as endothelial glycocalyx degradation, as is observed in sepsis and hyperglycemia.^{13,14} Consideration of these parameters in combination rather than in isolation allows inference of their relative contextual importance.

In addition to developing a generalized theory of microvascular perfusion and solute flux, we will also attempt to persuade the reader of its utility in identifying therapeutic strategies. This is perhaps best illustrated by successful examples of therapies targeting perfused capillary density. For example, one major cause of reductions in perfused capillary density is plugging of capillaries by adherent leukocytes or microemboli.^{15,16} Persistent reduction in perfused capillary density is a major determinant of organ failure and mortality in sepsis.¹⁷ As would be expected from these findings, therapies that reduce adhesive interactions in the microvasculature (and thus unplug blocked capillaries) also improve patient outcomes.⁴ Anatomical capillary density and hemostatic status are also major determinants of insulin

sensitivity in T2DM.^{18–22} Insulin-sensitizing drugs such as metformin tend to also reduce adhesive interactions in the microvasculature,²³ and this may be part of their mechanism of action. In both sepsis and T2DM, therapies that improve microvascular function served to treat pathologies that are not generally discussed as microvascular defects. In both cases, some element of trial and error was required to even identify the microcirculation as a potential issue, and again to determine which aspects of microvascular perfusion were most relevant. Trial and error was then required to identify therapies to exploit these parameters, and often this mechanism of action was discovered post hoc. The analysis included in this manuscript aims to enable translational researchers to bypass much of this confusion by accurately predicting which microvascular parameters are relevant to their research and how they might be targeted.

2 | MATERIALS AND METHODS

2.1 | Governing equations

We begin our analysis with a widely used formulation of microvascular solute flux derived from Fick's laws of diffusion²⁴:

$$J_s = Q \times (C_a - C_i) \times \left(1 - e^{-\frac{P \times A}{Q}} \right). \quad (1)$$

Here J_s is solute flux across the endothelium, Q is capillary blood flow, C_a is arterial concentration, C_i is interstitial concentration, P is a metric of permeability to the solute of interest, and A is capillary surface area. Note that S is used instead for capillary surface area in traditional representations of this formula. We elected to use A to denote surface area in this analysis so as not to conflict with subsequent use of S to denote sensitivity. For purposes of subsequent sensitivity analyses, we further specify the definition of permeability using the following equation:

$$P = \frac{V \times D}{R_d}. \quad (2)$$

Here V is capillary volume, D is a diffusion rate constant, and R_d is the effective radius of diffusion. Using this definition of permeability, a more complete formulation of solute flux is:

$$J_s = Q \times (C_a - C_i) \times \left(1 - e^{-\frac{D \times A \times V}{R_d \times Q}} \right). \quad (3)$$

For simplicity of presentation in subsequent analyses, we will also introduce the dimensionless diffusion/convection matching parameter β (Equation 4). Values of β much

greater than 1 reflect an excess of diffusion capacity relative to bulk blood flow, while those much less than 1 reflect an excess of bulk blood flow relative to diffusion capacity.

$$\beta = \frac{D \times A \times V}{R_d \times Q}. \quad (4)$$

Incorporating this simplified notation into Equations 1–3 yields:

$$J_s = Q \times (C_a - C_i) \times (1 - e^{-\beta}). \quad (5)$$

This governing equation will subsequently be applied to determine the effects of bulk blood flow (Sections 3.1 and 3.2), diffusion capacity (Sections 3.1 and 3.3), perfused capillary density (Section 3.4), and perfusion heterogeneity (Section 3.5), along with simulations of the consequences of experimentally defined phenotypes including alterations in each of these parameters on microvascular solute flux (Section 3.6).

2.2 | Fractional equilibration and diffusion/convection matching

Individual determinants of diffusion/convection matching (eg, capillary blood volume, capillary surface area, diffusion capacity) are difficult to measure. However, their physical consequences can be empirically observed using fractional equilibration (ϵ) of the solute of interest between capillary blood and the interstitium, as defined by Eugene Renkin.²⁵ Fractional equilibration is defined as the ratio of arteriovenous concentration difference to arterial-interstitial concentration difference (Equation 6, Figure 1A). Determining fractional equilibration requires measurement of solute concentration in three compartments—arterial blood (C_a), venous blood (C_v), and interstitial fluid (C_i). Plausible reference values of C_a , C_v , C_i , and ϵ for compounds of physiological interest are included in Figure 2B.

$$\epsilon = \frac{C_a - C_v}{C_a - C_i}. \quad (6)$$

Fractional equilibration is related to the diffusion/convection matching parameter β by following transformation:

$$\epsilon = (1 - e^{-\beta}). \quad (7)$$

ϵ approaches 1 in cases where diffusion capacity exceeds convective delivery (eg, pulmonary oxygen flux) and ϵ approaches zero in cases where convective delivery exceeds diffusion capacity (eg, insulin delivery to skeletal muscle).

2.3 | Blood flow distribution

To determine the influence of arteriolar perfusion heterogeneity on microvascular solute flux, we simulated blood flow distribution within an idealized arteriolar network as illustrated in Figure 2. Each arteriole bifurcates into two smaller daughter vessels (Figure 2A) for 15 consecutive vessel generations for a total of 2^{15} simulated capillaries. Distribution of blood flow at each bifurcation is described by the parameter γ , which is defined as the fraction of parent vessel blood flow directed to the higher-flow daughter vessel (Figure 2B). Thus, $\gamma=0.5$ represents homogenous perfusion, and increasing deviation from 0.5 represents increasingly heterogeneous perfusion.

3 | RESULTS

3.1 | Effects of blood flow and diffusion capacity

The effects of bulk blood flow on microvascular solute flux are visualized in Figure 3A. The effects of diffusion capacity on microvascular solute flux are visualized in Figure 3B. These results reflect the predictions of Equation 8. Increases in blood flow and diffusion capacity both result in saturable increases in microvascular solute flux. The level at which increasing blood flow yields diminishing returns increases with increasing diffusion capacity, and vice versa. For diffusion-limited, low fractional equilibration molecules (eg, insulin), microvascular solute flux increases robustly with increasing diffusion capacity but increases minimally with increasing blood flow. For convection-limited, high equilibration molecules (eg, oxygen in the lung), microvascular solute flux increases minimally with increasing diffusion capacity but increases robustly with increasing blood flow. For diffusion/convection co-limited, intermediate equilibration molecules (eg, oxygen in skeletal muscle), both diffusion capacity and blood flow limit the rate of microvascular solute flux.

3.2 | Sensitivity of solute flux to blood flow

To quantify the sensitivity of microvascular solute flux to blood flow, the modified convection/diffusion matching parameter β' was defined as a function of fraction of baseline flow rate (f), which alters convection/diffusion matching solely through its interaction with blood flow at baseline (Q_0):

$$\beta' = \frac{D \times A \times V}{R_d \times Q'} = \frac{D \times A \times V}{R_d \times Q_0 \times f} = \frac{\beta_0}{f}. \quad (8)$$

Combining Equations 8 and 5 while accounting for increased blood flow yields:

$$J'_s = f \times Q_0 \times (C_a - C_i) \times \left(1 - e^{-\frac{\beta_0}{f}}\right). \quad (9)$$

The sensitivity of microvascular solute flux to changes in blood flow (S_f) is defined as the partial derivative of microvascular solute flux (j'_s) with respect to fraction of baseline blood flow (f) normalized to baseline microvascular solute flux ($J_{s,0}$):

$$S_f = \left(\frac{dJ'_s}{df} \right) / j_{s,0}. \quad (10)$$

Baseline blood flow (Q_0) and arterial-interstitial concentration gradient ($C_a - C_i$) cancel out when performing this calculation, and so a transformation between fractional equilibration and convection/diffusion matching at baseline (β_0) can be used to solve for S_f as a function of fractional equilibration at baseline (ϵ_0):

$$S_f = \frac{(1 - \epsilon_0) \times \left(\frac{1}{1 - \epsilon_0} - \ln \left(\frac{1}{1 - \epsilon_0} \right) - 1 \right)}{\epsilon_0}. \quad (11)$$

The results of this calculation are visualized in Figure 4. Note that fractional equilibration is also influenced by circumstances that change solute flux, and so ϵ_0 is not necessarily equal to ϵ' . For convection-limited compounds which undergo near-complete equilibration with the interstitium (eg, oxygen in the lung), flux increases or decreases near-proportionally with increasing/decreasing bulk blood flow. For diffusion-limited compounds which undergo minimal equilibration with the interstitium (eg, insulin), flux changes minimally with changes in bulk blood flow. Flux of compounds at intermediate values of baseline fractional equilibration undergoes subproportional changes in response to changing blood flow.

3.3 | Sensitivity of solute flux to diffusion rate constant

To determine the sensitivity of microvascular solute flux to diffusion capacity, we defined the modified convection/diffusion matching parameter β' as a function of fraction of baseline diffusion rate constant (k), which alters convection/diffusion matching solely through its interaction with diffusion capacity at baseline (D_0):

$$\beta' = \frac{D' \times S \times V}{R_d \times Q} = \frac{D_0 \times k \times S \times V}{R_d \times Q} = \beta_0 \times k. \quad (12)$$

Combining Equations 12 and 5 yields:

$$J'_s = Q \times (C_a - C_i) \times (1 - e^{(-\beta_0 \times k)}). \quad (13)$$

The sensitivity of microvascular solute flux to changes in diffusion rate constant (S_k) is defined as the partial derivative of modified micro-vascular solute flux (S_k) with respect to fraction of baseline diffusion rate constant (k) normalized to baseline microvascular solute flux ($J_{s,0}$):

$$S_k = \left(\frac{dJ'_s}{dk} \right) / J_{s,0} \quad (14)$$

Baseline blood flow (Q_0) and arterial-interstitial concentration gradient ($C_a - C_i$) cancel out when performing this calculation, and so the transformation between fractional equilibration and convection/diffusion matching parameter at baseline (β_0) can be used to solve for S_k as a function of fractional equilibration at baseline (ϵ_0):

$$S_k = \frac{\ln\left(\frac{1}{1-\epsilon_0}\right) \times (1 - \epsilon_0)}{\epsilon_0} \quad (15)$$

The results of this calculation are visualized in Figure 5. For convection-limited compounds which undergo near-complete equilibration with the interstitium (eg, oxygen in the lung), flux changes minimally with changes in diffusion rate constant. For diffusion-limited compounds which undergo minimal equilibration with the interstitium during capillary transit (eg, insulin), flux increases or decreases near-proportionally with increasing/decreasing diffusion rate constant. Flux of compounds at intermediate values of baseline fractional equilibration undergoes subproportional changes in response to changes in diffusion rate constant.

3.4 | Sensitivity of solute flux to perfused capillary density

To determine the sensitivity of microvascular solute flux to perfused capillary density, we defined the modified convection/diffusion matching parameter β' as a function of fraction of baseline perfused capillary density (d). Capillary density influences diffusion/convection matching through a variety of parameters. In this analysis, two modes of interaction with perfused capillary density were considered. In the first case (Equations 16–19), the concentration gradient within the interstitium is substantial and the solute of interest diffuses freely across the endothelium (eg, lactate clearance). In this case, capillary density modulates the effective diffusion radius ($R_{d,0}$) along with capillary surface area (A_0) and blood volume (V_0):

$$\beta' = \frac{D \times A' \times V'}{R'_d \times Q} = \frac{D \times A_0 \times d \times V_0 \times d}{(R_{d,0}/d) \times Q} = \beta_0 \times d^3 \quad (16)$$

Combining Equations 16 and 5 yields:

$$J'_s = Q \times (C_a - C_i) \times (1 - e^{(-\beta_0 \times d^3)}) \quad (17)$$

The sensitivity of microvascular solute flux to relative change in perfused capillary density (S_d) is defined as the partial derivative of modified microvascular solute flux (J'_s) with respect to fraction of baseline perfused capillary density (d) normalized to baseline microvascular solute flux ($J_{s,0}$):

$$S_d = \left(\frac{dJ'_s}{dd} \right) / J_{s,0}. \quad (18)$$

Blood flow (Q) and arterial-interstitial concentration gradient ($C_a - C_i$) cancel out when performing this calculation, and so the transformation between fractional equilibration and convection/diffusion matching at baseline (β_0) can be used to solve for S_d as a function of baseline fractional equilibration at baseline (ϵ_0):

$$S_d = \frac{3 \times \ln\left(\frac{1}{1-\epsilon_0}\right) \times (1 - \epsilon_0)}{\epsilon_0}. \quad (19)$$

Alternately, interstitial concentration gradients may be negligible relative to trans-endothelial concentration gradients (eg, oxygen in skeletal muscle). In this case, the effective radius of diffusion (R_d) is not appreciably altered by perfused capillary density. Repeating the same procedure as in Equations 16–19 with this modification yields:

$$\beta' = \frac{D \times A' \times V'}{R_d \times Q} = \frac{D \times A_0 \times d \times V_0 \times d}{R_d \times Q} = \beta_0 \times d^2, \quad (20)$$

$$J'_s = Q \times (1 - e^{(-\beta_0 \times d^2)}), \quad (21)$$

$$S_d = \left(\frac{dJ'_s}{dk} \right) / J_{s,0}, \quad (22)$$

$$S_d = \frac{2 \times \ln\left(\frac{1}{1-\epsilon_0}\right) \times (1 - \epsilon_0)}{\epsilon_0}. \quad (23)$$

The results of these calculations are visualized in Figure 6. Although most compounds will be co-limited by trans-endothelial and interstitial diffusion processes, the strictly endothelium-limited and strictly interstitium-limited results shown here comprise the lower/

upper bounds of sensitivity to perfused capillary density. Flux of convection-limited compounds, which undergoes near-complete equilibration with the interstitium (eg, oxygen in the lung), changes minimally with changes in perfused capillary density. Flux of diffusion-limited compounds, which undergo minimal equilibration with the interstitium (eg, insulin), will undergo increases or decreases between 2× and 3× larger than the corresponding incremental change in perfused capillary density. Flux of compounds at intermediate values of baseline fractional equilibration undergoes somewhat smaller but still supra-proportional changes in response to changes in diffusion rate constant.

Note that the sensitivity of microvascular solute flux to perfused capillary density is geometrically identical to the sensitivity to diffusion rate constant, only scaled up 2×–3×. Thus, a 1% decrease in perfused capillary density could be fully compensated by a 2%–3% increase in diffusion rate constant, and vice versa.

3.5 | Sensitivity of solute flux to microvascular perfusion heterogeneity

To define the influence of perfusion heterogeneity on microvascular solute flux, we defined the total microvascular solute flux across a capillary network (J_{tot}) based on the mean capillary flow rate ($\bar{Q}$) and the fraction of mean flow rate within each capillary (f_i):

$$J_{\text{tot}} = \sum \bar{Q} \times f_i \times (C_a - C_i) \times \left(1 - e^{-\frac{\bar{\beta}}{f_i}} \right). \quad (24)$$

Because the degree of perfusion heterogeneity (γ) was found to influence the degree of sensitivity to perfusion heterogeneity (ie, flux does not vary linearly with γ), it is useful to discuss the sensitivity of micro-vascular solute flux to perfusion heterogeneity in terms of the relative (%) change in solute flux between two representative perfusion states (see Section 2.3 for discussion of perfusion distribution simulation):

$$\% \Delta J_{\text{tot}, 1 \rightarrow 2} = 100 \times \frac{J_{\text{tot}, 1} - J_{\text{tot}, 2}}{J_{\text{tot}, 1}}. \quad (25)$$

Mean capillary blood flow ($\bar{Q}$) and arterial-interstitial concentration gradient ($C_a - C_i$) cancel out when performing this calculation, and so $\% J_{\text{tot}, 1 \rightarrow 2}$ can be expressed as a function of mean solute convection/diffusion matching ($\bar{\beta}$) and blood flow distribution (as determined by γ):

$$\% \Delta J_{\text{tot}, 1 \rightarrow 2} = 100 \times \frac{\sum f_{1i} \times \left(1 - e^{-\frac{\bar{\beta}}{f_{1i}}}\right) - \sum f_{2i} \times \left(1 - e^{-\frac{\bar{\beta}}{f_{2i}}}\right)}{\sum f_{1i} \times \left(1 - e^{-\frac{\bar{\beta}}{f_{1i}}}\right)}. \quad (26)$$

For illustration purposes, we chose to simulate the differences in microvascular solute flux between LZRs ($\gamma=0.52$) and OZRs (animal model of T2DM, $\gamma=0.59$), as differences in arteriolar perfusion heterogeneity in these models have been extensively characterized in previous studies.^{1,2,26–28} Figure 7 shows the predicted % decrease in solute flux in OZR relative to LZR as a function of fractional equilibration in the LZR. Compounds whose flux is entirely limited by diffusion or convection are not appreciably impacted by perfusion heterogeneity. However, intermediate equilibration compounds whose flux is co-limited by diffusion and convection (eg, oxygen in skeletal muscle) are sensitive to perfusion heterogeneity because over-perfused capillaries cannot fully compensate for under-perfused capillaries. For these molecules, losses in capillary perfusion are more impactful than are equivalent gains.

3.6 | Defining the effects of complex phenotypes on microvascular solute flux

The procedure for determining the effects of perfusion heterogeneity outlined in Equations 24–26 can also be used to determine the effects of a complex phenotype involving several alterations to microvascular perfusion. For this analysis, we introduced parameters for fraction of healthy bulk blood flow through the entire microvascular network (fQ) and fraction of healthy perfused capillary density (fD):

$$J_{\text{tot}} = \sum fQ \times \bar{Q}_0 \times f_i \times (C_a - C_i) \times \left(1 - e^{-\frac{\bar{\beta}_0 \times fD^3}{fQ \times f_i}}\right), \quad (27)$$

$$\% \Delta J_{\text{tot}, 1 \rightarrow 2} = 100 \times \frac{\sum f_{1i} \times \left(1 - e^{-\frac{\bar{\beta}_0}{f_{1i}}}\right) - \sum fQ \times f_{2i} \times \left(1 - e^{-\frac{\bar{\beta}_0 \times fD^3}{fQ \times f_{2i}}}\right)}{\sum f_{1i} \times \left(1 - e^{-\frac{\bar{\beta}_0}{f_{1i}}}\right)}. \quad (28)$$

For demonstration purposes, we characterized the effects of microvascular perfusion defects on solute flux in T2DM (OZR model), sepsis, and acute hyperglycemia or glycocalyx degradation (acute hyperglycemia causes glycocalyx degradation and related perfusion defects¹⁴). In the OZR model of T2DM, bulk blood flow is reduced by ~20%, capillary density is reduced by ~20%, and perfusion heterogeneity is increased from $\gamma=0.52$ to $\gamma=0.59$.¹ In sepsis, distribution of blood flow to meet metabolic demands is impaired, perfused capillary density is markedly reduced, and microvascular perfusion heterogeneity is readily visible under microscopic observation.²⁹ To simulate a worst-case perfusion scenario, we assumed a 20% reduction in bulk blood flow, a 50% reduction in perfused capillary density, and an increase in γ from 0.52 to 0.7. In cases of acute glycocalyx degradation or hyperglycemia, bulk blood flow is not significantly altered, perfused capillary density is reduced by ~30%, and perfusion heterogeneity is increased, although the precise degree of this increase is unclear.¹³ For purposes of this analysis, perfusion heterogeneity during hyper-glycemia was assumed be similar to that in the OZR. The results of this analysis are shown in Figure 8 below. Our model predicts that micro-vascular solute flux will be impaired in all three phenotypes, but that the precise etiology of this impairment varies.

4 | DISCUSSION

In this manuscript, we derive a generalized theory of microvascular perfusion and solute flux in branching microvascular networks building from the single-capillary analysis of Eugene Renkin.²⁵ This theory enables a number of physiological predictions, which we will discuss beginning with associations between physiological parameters, progressing to the implications of complex phenotypes, and eventually moving on to therapies effective for acute treatment of microvascular perfusion defects. Throughout our discussion, we will use exercise capacity (or tissue oxygenation where appropriate) and insulin action as model solute exchange processes. We encourage the reader to follow along with another, self-relevant exchange process (eg, drug delivery) in mind. Where the predictions we draw this model have been tested, both the causes and the solutions of impaired microvascular flux can typically be predicted from our model. In addition, we will outline the limitations of our model for the reader's consideration when applying our theory.

4.1 | Physiological associations

We begin with predictions of physiological associations. The first is that pulmonary oxygen uptake (a convection-limited exchange process) should vary proportionally with bulk blood flow. Consistent with this prediction, cardiac output is the primary determinant of pulmonary oxygen uptake.³⁰ Conversely, our model predicts that increasing bulk blood flow without correcting its distribution would do little to enhance insulin action in skeletal muscle (a diffusion-limited process). This prediction is plausible—animal models of insulin resistance often display increased cardiac output³¹ and regional blood flow diverges from regional glucose uptake.³² Further, our model predicts that capillary density (a determinant of diffusion capacity) would be a major determinant of insulin action. The observation that capillary density and insulin sensitivity are associated in both human and animal models supports this prediction.^{18,22} Our model also predicts that capillary density would be related,

albeit weakly, to skeletal muscle oxygen uptake (a diffusion/convection co-limited process). This prediction is consistent with the observations that capillary density and $\text{VO}_{2\text{max}}$ are correlated in peripheral arterial disease,³³ and capillary density increases with aerobic exercise training.¹² Finally, our model predicts that pulmonary capillary density (a determinant of diffusion capacity) would have little impact on pulmonary oxygen uptake (convection-limited) except in extreme cases. It is unclear that this prediction has been directly tested, but it is telling that canonical descriptions of diseases that interfere with pulmonary oxygen diffusion do not involve capillary rarefaction.³⁴

4.2 | Disease phenotype predictions

We will now shift to discussion of specific microvascular perfusion phenotypes. For this purpose, we will employ three physiological states: (i) T2DM, (ii) sepsis, and (iii) acute glycocalyx degradation. Both T2DM (Figure 8A) and sepsis (Figure 8B) decrease perfused capillary density and increase perfusion heterogeneity. Our model therefore predicts insulin resistance in both disease states as a result of diffusion limitations. These same diffusion limitations would be expected to cause impaired exercise capacity in T2DM and impaired tissue oxygenation in sepsis. These predictions are consistent with literature reports in both disease states.^{35–38} Assuming a causal role for reduced perfused capillary density and microvascular perfusion heterogeneity in limiting diffusion capacity, one would predict that exercise capacity and insulin sensitivity in T2DM are correlated, as well as tissue oxygenation and insulin sensitivity in sepsis. Exercise capacity and insulin sensitivity in T2DM are indeed correlated.³⁹ The possibility of a correlation between insulin resistance and tissue hypoxia in sepsis has not been directly tested, but insulin resistance, tissue hypoxia, and impaired capillary perfusion are all thought to contribute to mortality,⁴⁰ suggesting that this correlation would be observed if were to be tested.

Mechanistically, our model predicts that the exercise impairment in T2DM results from a combination of impaired muscle oxygenation and impaired clearance of metabolic wastes such as lactate and CO_2 . Consistent with these hypotheses, muscle oxygenation during contractions is impaired in animal models of T2DM⁴¹ and muscle pH decreases more robustly during exercise in human subjects with T2DM than in healthy controls.⁴² Similarly, our model predicts impaired clearance of metabolic wastes in sepsis. This prediction is consistent with decreased interstitial pH (both lactate and CO_2 are acidic) and interstitial hypercapnia in sepsis.^{38,43} Likewise, our model predicts that the mechanism of insulin resistance in T2DM would be impaired diffusion of insulin. Consistent with this hypothesis, the limiting stage in insulin action occurs at the extracellular (ie, vascular-interstitial) levels,¹⁰ and diet-induced obesity (a precursor to T2DM) causes impaired insulin access to the interstitium.⁴⁴ In sepsis, the influences of microvascular perfusion on solute diffusion are confounded by a robust increase in vascular permeability,⁴⁵ which is not taken into account in our analysis.

In hyperglycemia- or enzymatic-induced glycocalyx degradation (Figure 8C), our model predicts both impaired exercise capacity and insulin resistance, again resulting from diffusion limitations secondary to reduced perfused capillary density and heterogeneous perfusion. The interaction between glycocalyx degradation and exercise capacity has not

been tested. As relates to insulin sensitivity, however, our model predictions are correct.^{46,47} The mechanisms that have been previously investigated for hyperglycemia-induced insulin resistance often involve oxidative signaling processes rather than mass transport effects.⁴⁸ Our model predicts that insulin resistance in these states is accounted for, at least in part, by impaired diffusion of glucose and insulin. These possibilities have not been tested.

4.3 | Recommendations for scientific practice

We have observed and participated in a general trend in the medical literature that can be formulated as follows: (i) tissue-level flux of a molecule of interest is impaired in a disease of interest, (ii) investigators suspect a vascular/microvascular contribution to this defect, (iii) subsequent studies determine that bulk blood flow does not fully account for the defect, (iv) subsequent studies determine that tissue-level production/demand does not fully account for the defect, and then finally, (v) subsequent studies demonstrate a contribution from microvascular perfusion. Many scholarly publications spanning many years of investigation are often required before research of putative microvascular therapies can even begin. In the interest of helping the reader to avoid this trap, we have created a flowchart (Figure 9A) to guide investigations of this sort.

The rationale underlying our flowchart questions stem from basic qualitative attributes of our theory and are reflected in the interpretations of flowchart results provided in Figure 9B. Microvascular perfusion-related transport defects of any etiology are predicted to have similar influences on the flux of molecules with similar equilibration properties (see Figures 4–7). Thus, if a particular molecule is *uniquely* impacted by an observed flux defect, it is likely that the cause of the defect is specific to that molecule (eg, tissue-level demand, cell membrane transporters). If interstitial concentration of a molecule of interest equilibrates slowly with the bloodstream (eg, insulin), any microvascular contribution to reduced flux of this molecule is likely to involve diffusion-limiting effects such as capillary dropout, capillary rarefaction, or perfusion heterogeneity. For rapidly equilibrating molecules (eg, oxygen), both diffusion- and convection-limiting effects may impact tissue-level flux. Thus, if larger, more slowly equilibrating molecules are not also affected, reduced bulk blood flow (a convection-limiting effect) is a plausible culprit. Conversely, if tissue-level flux is impaired across a wide range of equilibration rates, diffusion-limiting perfusion defects such as perfusion heterogeneity and capillary dropout are plausible contributors.

Typical scientific practice in the past has been to bypass these considerations and assay bulk blood flow alone. Our theory predicts that this strategy will often generate confusion. Even for convection-limited or convection/diffusion co-limited transport processes such as tissue oxygenation, heterogeneous blood flow distribution and capillary dropout may recapitulate the effects of reduced blood flow.

We will now provide a demonstration of these considerations using oxygen delivery to skeletal muscle in T2DM as an example. Like oxygen, blood glucose equilibrates near-fully with the interstitium during capillary transit.⁴⁴ Glucose delivery to skeletal muscle during hyper-insulinemia is also impaired in T2DM,¹⁰ and the degree to which the capacity for glucose flux (ie, insulin sensitivity) is reduced correlates to the degree to which the capacity for oxygen flux (ie, $\text{VO}_{2\text{max}}$) is reduced.³⁹ These observations provide an answer of “yes”

to the first flowchart question. Oxygen and glucose are both rapidly equilibrating molecules, thus providing an answer of “yes” to the second flowchart question. Delivery of insulin (a large, diffusion-limited molecule) to the muscle interstitium is also impaired in diet-induced obesity (a precursor to T2DM),⁴⁴ thus providing an answer of “yes” to the third flow chart question. The degree of skeletal hypoxia during exercise reported in T2DM is often severe,⁴¹ while the insulin transport defect is more subtle. These observations provide an answer of “lesser” to the final flowchart question, yielding a recommendation of “assay capillary perfusion.” True to form, studies of microvascular and capillary perfusion in multiple models of T2DM reveal substantial capillary dropout and perfusion heterogeneity,^{1,2,26,27,49,50} and we now know that reversal of these defects can acutely improve skeletal muscle oxygenation in at least one animal model.^{1,28} With the benefit of hindsight and a theoretical understanding of microvascular solute flux, many years of trial and error could have been avoided using the approach recommended here.

4.4 | Implications for therapy

We will now introduce interventions that have been shown to improve each of the microvascular perfusion parameters considered in our analysis. Acute reductions in perfused capillary density are often caused by adhesive interactions such as leukocyte adhesion or microembolism.^{15,51} Certain anticoagulant drugs would therefore be expected to improve capillary perfusion, and this effect has indeed been observed.⁴ Microvascular perfusion heterogeneity is less well understood, but likely involves impaired vessel function within arteriolar networks. This defect could plausibly be mitigated by antioxidant treatment. Antioxidant treatment can also reduce adhesive interactions (thus potentially improving perfused capillary density) and arteriolar perfusion is mechanically coupled to capillary perfusion (ie, capillary plugging causes perfusion heterogeneity), so antioxidants would be expected to acutely improve both perfusion heterogeneity and perfused capillary density. Sure enough, acute antioxidant treatment does help to improve microvascular perfusion in certain contexts.⁴ Bulk blood flow, meanwhile, is controlled by vascular tone and can be increased using vasodilators.

For diffusion-limited processes such as insulin action, our model predicts that the correct anticoagulants and antioxidants would be sufficient to acutely improve insulin sensitivity in insulin-resistant individuals. As regards antioxidants, this hypothesis has been repeatedly validated.^{52–54} The possibility that anticoagulants improve insulin sensitivity has not been directly tested, but its plausibility is supported by associations of a variety of hemostatic parameters with insulin resistance.^{19–21}

In sepsis, our simulations suggest that diffusion limitations caused by reduced perfused capillary density are a major cause of impaired substrate delivery and metabolite clearance. More importantly, impaired capillary perfusion is thought to be a major cause of organ failure and death in sepsis.^{3,4,29,55} Certain anticoagulants (eg, activated protein C) and certain antioxidants (eg, Vitamin E) markedly improve microvascular perfusion in sepsis.⁴ True to form, activated protein C substantially improves patient outcomes,⁵⁶ as do certain antioxidants.⁵⁷ These reports are consistent with our model predictions. Although the interactions of these therapies with microvascular solute flux have not been widely

investigated, perfused capillary density and interstitial hypercapnia (a marker of impaired CO₂ clearance) are inversely associated and interstitial hypercapnia can be acutely improved by capillary perfusion rescue.⁴³

For oxygen delivery in T2DM (see Figure 9A), our model predicts that a complex mixture of reduced bulk blood flow, reduced capillary density, and increased perfusion heterogeneity is required to explain the phenotype. Consequently, our model predicts that a drug combination consisting of anticoagulants, antioxidants, and vasodilators (or equivalent) would be necessary to reverse the oxygenation defect. In the OZR model of T2DM, these predictions have been tested in the perfused hindlimb precisely; this combination of drugs is effective in acutely restoring normal oxygen flux.¹

Our model accurately predicts effective therapeutic targets for acute intervention in each case where model predictions have been explicitly tested, but except in the OZR model of T2DM, the proposed mechanisms have not yet been validated. Additionally, other compounds targeting microvascular perfusion may be more suitable for long-term use. It is tempting to speculate that the cardiovascular protective effects of aspirin,⁵⁸ for example, relate to its anticoagulant and antioxidant properties, or that the apparent ability of metformin to extend lifespan⁵⁹ relates to its microvascular perfusion benefits.²³ Future studies will be required to investigate these possibilities.

4.5 | Model limitations

Our model accurately predicts many physiological associations, predicts which physiological parameters and solute exchange processes will be impaired in a variety of disease states, and predicts the therapies that will normalize these phenotypes, yet there are key limitations. One limitation of our model is its simplicity. In reality, capillaries are heterogeneous,⁶⁰ and we neglect this source of heterogeneity. Another consideration is that changes in endothelial or interstitial permeability were largely neglected. Our rationale for this simplification was twofold: (i) due to the selective permeability of the endothelium and interstitium, changes in permeability will be different for each molecule and cannot readily be generalized, and (ii) capillary permeability is highly dynamic and does not lend itself to description by a single parameter. This limitation will thus require testing of specific molecules in different physiological settings to determine their individual properties. However, as evidenced by the fact that those model predictions which have previously been tested were correct, neglecting changes in capillary permeability does not compromise the general utility of the model. Based on the noted limitations, our model accurately reflects the *contribution* of microvascular perfusion changes to the disease phenotype and that these *contributions* occur within the larger context of the local microenvironment.

4.6 | Conclusions

The relationship between bulk blood flow and tissue-level solute flux is dissociated except in cases of convection-limited transport processes such as pulmonary oxygen uptake. Our simulations suggest that microvascular perfusion is adequately described by our model to explain the observed dissociation. In instances where relevant experimental data exist, our model successfully predicts which solutes are most profoundly influenced by microvascular

perfusion and which microvascular parameters are most strongly associated with solute flux. These results may account for literature reports in a variety of disease states and underscore the importance of a theoretical understanding of the microvascular parameters influencing microvascular exchange processes. Our model facilitates this theoretical understanding and even allows some prediction of the therapies required to repair microvascular perfusion defects. Future work will be required both to further validate the predictive power of our model and to develop and test therapies targeting microvascular perfusion independently from bulk blood flow.

5 | PERSPECTIVES

A growing body of evidence indicates that impaired microvascular perfusion, as opposed to reduced blood flow, is a critical process in the pathology of a variety of diseases. The physiological role of microvascular perfusion is poorly understood, and as a result, therapies specifically targeting the microcirculation are limited. This manuscript provides a theoretical and conceptual framework for understanding the role of the microcirculation and how it might be therapeutically targeted in a context-specific manner.

Acknowledgments

Funding information

Funding for this study was provided by VA Merit, and the Center for Women's Health Research and Department of Bioengineering at the University of Colorado.

Abbreviations:

LZR	Lean Zucker Rat
OZR	Obese Zucker Rat
T2DM	type 2 diabetes mellitus

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FIGURE 1. Solutes vary in their degree fractional equilibration with the interstitium during capillary transit. (A) Visualization of the concept of equilibration. Complete equilibration implies that venous solute concentration is equal to interstitial solute concentration, while partial equilibration implies that venous concentration lies somewhere between arterial and interstitial concentrations. (B) Examples of physiologically relevant compounds and plausible values for their fractional equilibration

FIGURE 2.

Simulated distribution of blood flow in an idealized microvascular network. (A) The simulated microvascular network consisted of a series of arterioles terminating in a symmetric bifurcation of two smaller arterioles. Fifteen vessel generations were simulated for a total of 2^{15} individual capillaries; only four vessel generations are illustrated to preserve clarity. (B) Distribution of flow rate at each microvascular bifurcation was defined by the parameter γ , which is defined as the fraction of parent vessel flow directed to the higher-flow daughter vessel. Figure recreated with permissions from Butcher et al. 2013²⁶

FIGURE 3.

Effects of blood flow and diffusion capacity on microvascular solute flux. (A) Solute flux increases asymptotically with increasing flow. Increasing diffusion capacity increases the threshold of diminishing returns for increasing flow. (B) Solute flux increases asymptotically with increasing diffusion capacity. Increasing flow increases the threshold of diminishing returns for increasing diffusion capacity

FIGURE 4.

Relationship between baseline fractional equilibration and sensitivity to blood flow. Convection-limited compounds, which equilibrate completely or near-completely with the interstitium during capillary transit, undergo a proportional increase/decrease in flux in response to an increase/decrease in blood flow. Diffusion-limited compounds, which equilibrate minimally with the interstitium during capillary transit, are minimally sensitive to changes in blood flow

FIGURE 5.

Relationship between baseline fractional equilibration and sensitivity to diffusion capacity. Convection-limited compounds, which equilibrate completely or near-completely with the interstitium during capillary transit, are minimally sensitive to changes in diffusion capacity. Diffusion-limited compounds, which equilibrate minimally with the interstitium during capillary transit, undergo a proportional increase/decrease in flux in response to an increase/decrease in diffusion capacity

FIGURE 6.

Relationship between baseline fractional equilibration and sensitivity to perfused capillary density. Convection-limited compounds, which equilibrate completely or near-completely with the interstitium during capillary transit, are minimally sensitive to changes in perfused capillary density. Diffusion-limited compounds, which equilibrate minimally with the interstitium during capillary transit, undergo a 2×–3× proportional increase/decrease in flux in response to an incremental increase/decrease in perfused capillary density. The magnitude of the effect of perfused capillary density further depends upon whether the primary site of resistance to solute diffusion lies at the endothelium or within the interstitium, interstitium-limited compounds being more sensitive to perfused capillary density than endothelium-limited compounds

FIGURE 7.

Relationship between baseline fractional equilibration and sensitivity to arteriolar perfusion heterogeneity. Compounds whose flux is limited almost entirely by convection or almost entirely by diffusion are minimally affected by perfusion heterogeneity. Compounds whose flux is co-limited by diffusion and convection undergo reduced microvascular flux under conditions of microvascular perfusion heterogeneity. The range of fractional equilibration most affected by perfusion heterogeneity gradually shifts toward relatively diffusion-limited compounds with an increasing degree of perfusion heterogeneity (see Figure 8). The curve shown reflects the impacts of perfusion heterogeneity on solute flux in the OZR relative to the LZR

FIGURE 8.

Complex phenotypes influence solute flux through a variety of mechanisms. (A) Solute flux in diabetes is predicted to be affected primarily by perfused capillary density in the range of interest for insulin action, and by a complex mixture of bulk blood flow, perfusion heterogeneity, and capillary density effects in the range of interest for exercise capacity. (B) Solute flux in sepsis is predicted to be affected primarily by perfused capillary density and perfusion heterogeneity in the range of interest for both insulin action and exercise capacity. (C) Solute flux in hyperglycemia or glycocalyx degradation is predicted to be affected

primarily by perfused capillary density in the range of interest for insulin action, and by a combination of capillary density and perfusion heterogeneity in the range of interest for exercise capacity

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(B)

	What to Look For	Interpretation
The observed defect is likely specific to X	Reduced production, reduced consumption, reduced membrane transport, etc., of X	Microvascular perfusion is probably NOT contributing to the defect.
Assay blood flow.	Reduced blood flow.	If blood flow is reduced, this change is a likely cause for the defect. If blood flow is NOT reduced, assay capillary perfusion.
Assay capillary perfusion.	Capillary dropout, perfusion heterogeneity, and reduced flow velocity.	If perfusion is altered, this change is a likely cause for the defect. If perfusion is NOT altered, assay capillary perfusion AND density.
Assay capillary perfusion AND density.	Capillary dropout, perfusion heterogeneity, reduced flow velocity and/or reduced capillary density.	If perfusion is altered, this change is a likely cause for the defect. If capillary density is reduced, this change is a likely cause for the defect.

FIGURE 9. Flowchart of recommended steps to test for microvascular contributions to solute flux defects. (A) This flow chart is intended to help investigators determine an appropriate experimental design to assess the possibility that tissue-level flux of a molecule of interest (X) is impaired due to a vascular/microvascular defect. Direct observation of capillary perfusion is advisable in most cases. (B) Interpretation of flowchart destinations. In general, if a transport defect is solute specific, it is probably not caused by a microcirculatory issue.

Alterations in blood flow, capillary density, and capillary blood flow distribution are all probable causes of transport defects

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