Theoretical Models for Blood Flow Regulation in Heterogeneous Microvascular Networks

by

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Signed: _________ Brendan Fry _________
DEDICATION

For my parents and Mandi
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Abstract

Proper distribution of blood flow in the microcirculation is necessary to match changing oxygen demands in various tissues. How this coordination of perfusion and consumption occurs in heterogeneous microvascular networks remains incompletely understood. Theoretical models are powerful tools that can help bridge this knowledge gap by simulating a range of conditions difficult to obtain experimentally. Here, an algorithm is first developed to estimate blood flow rates in large microvascular networks. Then, a theoretical model is presented for metabolic blood flow regulation in a realistic heterogeneous network structure, derived from experimental results from hamster cremaster muscle in control and dilated states. The model is based on modulation of arteriolar diameters according to the length-tension characteristics of vascular smooth muscle. Responses of smooth muscle cell tone to myogenic, shear-dependent, and metabolic stimuli are included. Blood flow is simulated including unequal hematocrit partition at diverging vessel bifurcations. Convective and diffusive oxygen transport in the network is simulated, and oxygen-dependent metabolic signals are assumed to be conducted upstream from distal vessels to arterioles. Simulations are carried out over a range of tissue oxygen demand. With increasing demand, arterioles dilate, blood flow increases, and the numbers of flowing arterioles and capillaries, as defined by red-blood-cell flux above a small threshold value, increase. Unequal hematocrit partition at diverging bifurcations contributes to capillary recruitment and enhances tissue oxygenation. The results imply that microvessel recruitment can occur as a consequence of local control of arteriolar tone. The effectiveness of red-blood-cell-dependent and independent mechanisms for the metabolic response of local blood flow regulation is examined over a range of tissue oxygen demands. Model results suggest that although a red-blood-cell-independent mechanism is most effective in increasing flow and preventing hypoxia, the addition of a red-blood-cell-dependent
mechanism leads to a higher median tissue oxygen level, indicating distinct roles for
the two mechanisms. In summary, flow rates in large microvessel networks can be
estimated with the proposed algorithm, and the theoretical model for flow regulation
predicts a mechanism for capillary recruitment, as well as roles for red-blood-cell-
dependent and independent mechanisms in the metabolic regulation of blood flow in
heterogeneous microvascular networks.
Chapter 1

Introduction

1.1 The microcirculation

The microcirculation is the part of the circulation consisting of the smallest blood vessels in the body. In the microcirculation, vessels are densely spaced with small diameters, creating a large surface area for diffusion. As a result, it is the location of the majority of oxygen exchange between vessels and tissue. Microvessels range in diameter from a few hundred microns down to just a few microns, and there are three types of vessels in the microcirculation: arterioles, which carry oxygenated blood from the arteries; capillaries, which are the smallest vessels in the body; and venules, which carry de-oxygenated blood to the veins.

The structure of the microcirculation is very heterogeneous (See Figure 1.1), with wide variation in vessel size, spacing, density, and path length [76]. The network on the top of Figure 1.1 was imaged in the rat mesentery, which is a thin sheet covering the abdomen [79]. The network on the bottom of Figure 1.1 was traced in the hamster cremaster skeletal muscle [4]. Though completely different tissues, both the mesentery and cremaster microvascular networks exhibit the structural heterogeneity characteristic of the microcirculation. This heterogeneous structure leads to a heterogeneous distribution of blood flow and transport of solutes. In addition, demand for oxygen and other solutes in surrounding tissue is non-uniform. For instance, areas of tissue with low surrounding oxygen levels tend to consume less oxygen [36]. In skeletal muscle, contraction and relaxation occur in microvascular units, so that adjacent muscle fibers can be at rest and maximally working, respectively. How oxygen is carried in the blood will thus impact how well supply and demand can be met in
Figure 1.1. Two microvascular networks. On the top is a microvascular network imaged in the rat mesentery [74]; on the bottom is a microvessel network traced from the hamster cremaster muscle [4]. Both networks exhibit structural heterogeneity characteristic of the microcirculation.
the microcirculation.

1.2 Oxygen transport in the blood and tissue

The composition of blood is around 55-60% plasma and 40-45% red blood cells (RBCs), on average [77]. However, only 2-3% of oxygen in the arterial blood is dissolved in the blood plasma; the rest is carried in the RBCs [7]. The RBCs contain a concentrated solution of the protein hemoglobin, and each hemoglobin molecule can bind up to four oxygen molecules. One can define a parameter called the oxyhemoglobin saturation, which is the fraction of the binding sites occupied by oxygen. The saturation is a sigmoidal function of the partial pressure of oxygen in the blood (abbreviated as the blood $PO_2$) – which is proportional to the concentration of dissolved oxygen – due to the cooperative binding of oxygen to hemoglobin. The oxygen carried in the blood bound to hemoglobin accounts for the remaining 97-98% of the blood oxygen.

To function properly, every tissue in the body consumes oxygen. To get oxygen from the air into the tissue cells that consume it, air is first inhaled and transported into tiny air sacs in the lungs – called alveoli – which are surrounded by small blood vessels. Due to the oxygen concentration gradient, oxygen diffuses from the alveoli into these vessels, so that blood leaving the lungs is very well-oxygenated. This oxygenated blood travels down into the heart, where it is pressurized and subsequently ejected out into the arteries, which are the large blood vessels coming out of the heart. Oxygen is then carried convectively in the blood via the arteries, which branch into smaller and smaller vessels down into the tissue. Oxygen then diffuses out from the vessels into the tissue, again due to the concentration gradient.

In short, oxygen is carried long distances in the blood vessels via convection and is delivered short distances to surrounding tissue via diffusion. The reason for this is that the process of diffusion is only efficient over these short distances. In heavily
working skeletal muscle, the maximum distance oxygen can diffuse from the vessel into tissue is on the order of 50 µm – less than the width of the average human hair. Thus, to adequately deliver oxygen from vessels to tissue, blood vessels must be spaced very closely together, as occurs in the microcirculation.

1.2.1 Green’s function method for modeling oxygen transport

Modeling oxygen transport in the microcirculation dates back to 1919, with the work of Krogh [48, 49]. In his model (referred to as a Krogh-type model), it was assumed that each vessel was surrounded by a thin tissue sleeve, and the tissue was only delivered oxygen by the vessel it surrounded. In addition, the tissue was allowed to extract oxygen from the vessels until the vessel was completely deprived of oxygen. This simplified oxygen transport model is still widely used today, but has significant limitations. From experiment, it is known that tissue receives oxygen from all nearby vessels [19], that oxygen can diffuse among vessels [24], and that tissue oxygen consumption declines when vessel oxygen gets very low, so that oxygen can never be fully extracted from vessels [91]. The assumptions of the model thus lead to an overestimation of the number of vessels with very low oxyhemoglobin saturation levels.

The method used in the subsequent chapters, which will be referred to as the Green’s function method, attempts to resolve the issues created by the Krogh-type model, allowing for the diffusive exchange of oxygen between tissue points and all vessels in the network, as well as among vessels [40, 86]. The vessel segments in the network are represented as discrete oxygen sources, and the PO$_2$ field in the tissue is represented as the superposition of the fields resulting from those sources. One can consider the steady-state diffusion equation with oxygen consumption:

$$D\alpha \nabla^2 P = M(P)$$  \hspace{1cm} (1.2.1)

$$= \text{complex distribution of sinks and sources}, \hspace{1cm} (1.2.2)$$
where $D$ is the diffusion coefficient, $\alpha$ is the solubility, $P$ is the $PO_2$, and $M(P)$ is the tissue oxygen consumption rate (which depends on the $PO_2$). Equation (1.2.1) can be formulated in terms of Green’s function as follows:

$$D\alpha \nabla^2 G(x, x_i) = \delta(x - x_i),$$

(1.2.3)

where the right-hand side of the equation is the multi-dimensional Dirac delta function. Using the above formulation, the solution can be written in terms of a sum of Green’s functions as follows:

$$P = \sum_i G(x, x_i)q_i,$$

(1.2.4)

where $q_i$ is a weight term for the $i$th Green’s function [86]. Since the form of the Green’s function solution to equation (1.2.1) is known, the unknowns in this formulation amount to the weights in front of each $G(x, x_i)$ term. Since the form of the solution is known, there is not a requirement for a very fine spacing of tissue points near the vessel wall (despite the steep oxygen gradient), as would be the case for a finite difference or finite element method. This greatly reduces the number of unknowns in the problem, which allows for much faster calculation of the solution. In addition, much of the computation time for this method is in solving systems of equations, which can be done in parallel. With the recent emergence of general purpose computing on graphical-processing-unit-based systems, the computation time of this algorithm has been further reduced.

1.3 Blood flow in networks

The microvessel networks used in this work are shown in Figure 1.2, and are derived from studies in the rat mesentery [79] and the hamster cremaster muscle [4]. They are represented as directed graphs, where each edge of the graph represents a blood vessel segment. Each segment has a corresponding diameter and length, and pressure-driven
flow in each vessel is assumed to be governed by Poiseuille’s law:

\[ Q = \frac{\pi \Delta P \cdot D^4}{128 \mu L} \]  

(1.3.1)

where \( Q \) is blood flow rate, \( \Delta P \) is the pressure drop across the vessel, \( D \) is the diameter, \( L \) is the length, and \( \mu \) is the apparent viscosity. Thus, there is a sensitive dependence of flow rate on diameter. Also, due to the often comparable size of RBCs to the microvessels in which they are flowing, flow cannot be considered Newtonian. Apparent viscosity is used in Equation (1.3.1), and is defined as the viscosity of a Newtonian fluid that would give the same blood flow rate for a given vessel geometry and driving pressure \[74\]. Studies of flow in the microcirculation have demonstrated that \( \mu \) is a function of vessel diameter and RBC volume fraction \[72\], and parameterizations of this dependence have been fit to experimental data \[80\].

The blood vessel hematocrit (abbreviated \( H_D \)) is the fraction of the blood volume occupied by red blood cells; as noted previously, normal \( H_D \) is 40-45\% \[77\]. This is an average throughout the vessels in the body, however, and does not necessarily indicate that most individual vessels have this hematocrit. In fact, this is not the case. Studies in the rat mesenteric microcirculation have indicated that at vessel bifurcations (where one parent vessel branches into two daughter vessels), the parent and daughter vessels do not have the same hematocrit, and the differences are often significant. The daughter vessel with the larger flow rate tends to have a larger hematocrit than the parent vessel, and the daughter vessel with the smaller flow rate tends to have a smaller hematocrit than the parent vessel \[71\]. This occurs in large part because of the presence of a cell-free or cell-depleted layer adjacent to the vessel wall, where red blood cells do not flow. The cell-free layer is relatively constant in width throughout the microcirculation, so that it takes up a larger fraction of the blood flow in smaller vessels \[74\]. Because of this unequal hematocrit partition at vessel bifurcations, the distribution of RBCs in individual microvessels varies greatly. This in turn has a significant impact on the delivery of oxygen with vessels and to
Figure 1.2. Networks used in simulations. On the left is a microvascular network derived from the rat mesentery [82]; on the right is a microvessel network derived from the hamster cremaster muscle [28].
surrounding tissue.

1.3.1 Estimating blood flow rates

Modeling flow in networks requires information on pressure-flow relationships in each vessel and boundary conditions on the vessels entering and leaving the network. To overcome the difficulties in obtaining measured flow rates in many individual boundary microvessels, a tool was developed to estimate blood flow rates in large microvascular networks based on incomplete boundary conditions [27]. Using independent information on the typical distribution of wall shear stresses and pressures in microvessels, the algorithm resolves the indeterminacy from having one or more unknown boundary conditions, by minimizing the deviation of pressures and wall shear stresses from target values, using a constrained optimization approach with Lagrange multipliers (similar to the technique of simulated annealing [44]). It was tested on experimental flow data from microvascular networks in the rat mesentery, and with very few boundary conditions specified, predicted flows showed very small errors in most vessel segments. This algorithm provides a basis for deducing functional properties of microvessel networks.

1.4 Blood flow regulation

Blood flow is regulated (distributed) according to the demands of the tissue. The distribution of flow throughout the body varies widely under different conditions; for example, the amount of blood flowing to skeletal muscle can vary over orders of magnitude from rest to maximal exertion [87]. This regulation of blood flow occurs not just at the level of organs, but within organs as well. Microvascular blood flow is very tightly regulated according to tissue needs at the micron scale. This regulation occurs mainly via the contraction and relaxation of the vascular smooth muscle (VSM) that surrounds the arterioles. A multitude of factors influence the level of VSM tone,
which determines how much blood is sent down particular pathways. Interestingly, the capillaries do not have any VSM, so they cannot actively control the amount of blood flow they receive.

Multiple mechanisms contribute to local blood flow regulation. Arteriolar VSM tone changes in response to changes in intraluminal pressure, a mechanism known as the *myogenic response*. There are three distinct phases to this mechanism. For low pressures, the arterioles passively dilate in response to pressure increases. In the range of around 20 to 120 mmHg (which encompasses the normal physiological range), increases in pressure result in arteriolar constriction due to active myogenic VSM contraction. For pressures greater than 140 mmHg, the VSM tone is maximal, and so the arterioles again passively dilate in response to pressure increases [10, 41].

Increases in blood flow result in increases in shear stresses along vessel walls, causing the release of endothelial factors such as nitric oxide (NO) and endothelial-derived hyperpolarizing factor (EDHF). The release of these factors cause VSM relaxation and vessel dilation, and can induce responses that travel along the endothelium, altering flow rates in vessels proximal to the site of vasodilator release [50, 68]. This is known as the *shear-dependent response*.

In response to an increase in metabolic demand, arterioles will dilate; this is called the *metabolic response*. This increase in flow after an increase in metabolic demand is called functional hyperemia, and occurs both locally and up to millimeters upstream [87]. This coordinated change in vascular resistance along a large network pathway allows for the tight matching of oxygen consumption by oxygen delivery. The focus in the present study is on the mechanism(s) of the metabolic response, which will be described in more detail below.

As noted above, the capillaries cannot actively change their diameters in response to metabolic changes. Somehow downstream vessels must communicate metabolic needs to upstream arterioles, which must constrict or dilate, to send less or more blood down the pathway.
Experiments have shown one way in which this happens in the microcirculation: the RBCs, which carry and deliver most of the oxygen in the blood, release the molecule adenosine triphosphate (ATP) at a rate inversely related to the amount of hemoglobin-bound oxygen. The ATP then diffuses from the RBCs to the vessel wall, initiating an electrochemical cell-to-cell conduction along the endothelium, resulting in contraction or relaxation of the VSM surrounding the upstream arteriole [23].

Other potential mechanisms for metabolic blood flow regulation are independent of RBCs. In response to decreases in local PO$_2$, the vessel wall itself may release vasodilators such as nitric oxide [17]. Alternatively, the tissue cells release various substances that diffuse to nearby vessels and cause vasodilation [6].

Proper distribution of blood flow is essential in the skeletal muscle, which can exhibit a wide range of tissue oxygen consumption and blood flow rates [83, 87]. It is not fully understood how this tight matching of supply to demand occurs, but it has been suggested that this is achieved at least in part by a process called capillary recruitment [5, 46], whereby vessels which are not flowing at rest become flowing in response to an increase in metabolic demand. The mechanism of this recruitment, however, is not completely known. For the better part of the 20th century, the active control of flow in individual capillaries was thought to be at the level of the individual capillary [42]; in particular, it was believed that a “pre-capillary sphincter” was located at the entrance of each capillary, and possessed enough VSM tone to close off flow [29]. While this would provide an explanation for experimental results that have demonstrated sequential opening of individual capillaries in response to decreases in oxygen levels [55], these pre-capillary sphincters have never been observed in skeletal muscle in experiment [14].

Given the lack of observational evidence for control of capillary flow at the individual capillary level, it seemed likely that control should reside in the vessels immediately upstream of the capillaries – the terminal arterioles [42]. That is, the constriction and dilation of the terminal arteriole controls flow in a group of capillar-
ies which it feeds. Experiments have indeed shown that flow in groups of capillaries is controlled by varying the VSM tone in the feeding terminal arteriole [4, 5, 15, 59]; one might expect then that simultaneous recruitment of all capillaries fed by a given terminal arteriole would occur upon dilation of the arteriole [85]. However, Lindbom and Arfors [55] demonstrated that capillaries fed by the same terminal arteriole could open sequentially in response to decreases in oxygen levels. Explanations for this behavior have been suggested based on mechanical properties of RBCs flowing through small vessels [85], but the issue remains unresolved. With the control of capillary flow likely residing at the level of the terminal arterioles, however, there is evidence for a potential connection between capillary recruitment and arteriolar blood flow regulation.

1.5 Modeling blood flow regulation

Theoretical models are powerful tools that can be used to quantitatively assess biological processes for which experiments can merely provide qualitative observations. Within the context of microvascular blood flow regulation, a significant advantage of such a model is that individual regulatory mechanisms can be turned on and off, to examine what effects each one has on the whole system. This idea of so-called “theoretical knockouts” opens up possibilities that are not available to experimentalists, as it is not often the case that these mechanisms can be individually modulated in the lab.

Previous studies have modeled arteriolar constriction and dilation in individual vessels, based on changes in vessel wall tension [11, 33]; Cornelissen et al. [16] extended these ideas in a model that included the effects of myogenic, flow-dependent, and metabolic responses on flow control in a compartmental vessel network. The responses, however, were assessed individually, rather than in combination.

The work of Arciero et al. [1] and Carlson et al. [10] expanded this work to
include the combined effects of these three regulation mechanisms. They assumed
the metabolic signal was generated by an RBC-dependent mechanism, and showed
that the three responses working together can provide metabolic regulation of blood
flow. Their models, however, divided up the microcirculatory network into seven
equivalent compartments of parallel vessels, connected in series. This simplification
does not take into account the heterogeneity of actual microvessel networks.

Roy et al. [82] advanced these models to include heterogeneous structure, replac-
ing the middle compartments from the network in [1, 10] with a structure derived
from a digitized image of an actual rat mesenteric microvascular network. Using this
model, the authors were able to assess the role that the unequal hematocrit parti-
tion at vessel bifurcations may play in the regulation of microvascular blood flow.
Namely, the RBC partitioning led to an instability in the RBC-derived metabolic
signal, whereby low-flow (and thus low-hematocrit) channels were unable to gen-
erate a sufficiently large signal to effectively direct more blood downstream. This
instability was avoided if the metabolic signal was assumed to be generated by a
RBC-independent mechanism. The model used in [82] was limited, however, by the
simplified Krogh-type oxygen transport model used. In addition, the model was based
on a network from the mesenteric microcirculation, a tissue that shows little or no
active regulation of blood flow.

1.6 Questions addressed

The subsequent chapters attempt to address the following questions, using theoretical
models:

Chapter 2

How can blood flow rates be estimated in large microvascular networks when
only a small fraction of the boundary flows or pressures are known?
Chapter 3

What is the mechanism of capillary recruitment?

Chapter 4

What combinations of wall-derived and RBC-derived mechanisms are most effective in the metabolic regulation of microvascular blood flow?
Chapter 2

Estimation of blood flow rates in large microvascular networks

2.1 Introduction

Methods for structural imaging of microvascular networks have developed rapidly in recent years and can provide detailed information on three-dimensional networks containing thousands of segments. Available methods include confocal imaging [12], reconstruction from serial sections [31, 43, 62, 89], and micro-CT imaging [3, 30, 37]. These methods can be used to visualize and quantify the complete vascular structure within tissue volumes of several cubic millimeters. When combined with appropriate automated image processing algorithms [52, 66], such data can be used to derive quantitative structural information in terms of the positions, lengths, diameters, and connectivity of the vessel segments forming the network.

The distribution of blood flow rates in microvascular networks fundamentally influences perfusion, solute transport, flow regulation, and growth and adaptation in the vascular system. However, available methods for direct observation of blood flow within individual vessels, such as intravital microscopy or endocardial probes, are largely limited to sheet-like or surface vascular networks. Approaches that can be used to measure microvascular flow in thicker tissues, such as laser-Doppler flowmetry, Doppler optical coherence tomography, or magnetic resonance perfusion imaging, do not resolve individual microvessels, and yield spatially-averaged measurements. The ability to analyze and predict the functional properties of observed microvascular network structures is thus hindered by the lack of correspondingly detailed flow information.

\(^1\)Published version in [27].
Previous studies have provided a basis for predicting the distribution of blood flow in microvascular networks \cite{53, 77}, taking into account effects resulting from the particulate nature of blood. In these studies, the apparent viscosity of blood has typically been characterized as a function of microvessel diameter and hematocrit using an empirical model \cite{73, 80}. At diverging bifurcations, red blood cells are generally distributed such that daughter vessels at diverging vessel bifurcations receive different hematocrits. Typically, the daughter vessel with the higher flow rate has a larger discharge hematocrit. This unequal distribution of hematocrit has been described by empirical models that depend on the diameters and flow rates in the parent and daughter vessels and the hematocrit in the parent vessel \cite{71, 73}. These relationships are key components of models to predict the distribution of flows and hematocrits in a network, given information on the diameters, lengths, and topological arrangement of all segments \cite{56, 79}.

However, simulations of blood flow in a network also require boundary conditions, in the form of flow or pressure values at all boundary nodes of the network and hematocrit values at all nodes receiving inflows to the network. The large network structures that have been experimentally observed typically have many boundary nodes. This is because the boundaries of the region of observation do not coincide with tissue boundaries, meaning that they intersect with a large number of vessel segments. The flows in the major feeding and draining vessels can potentially be measured directly or deduced from measured or typical values of tissue perfusion, but the flows in the numerous smaller microvessels forming the network boundary are generally unknown, and must be estimated by some means. One approach is to assign pressure or flow values to boundary vessels, based on typical values for vessels with similar diameters or types \cite{64, 69}. This approach inevitably involves some arbitrary assumptions, and may result in unrealistic uniformity in parameter values, for instance, if all boundary capillaries are assigned equivalent flows or pressures. Therefore, an alternative approach is proposed here. This approach involves minimizing the
sum, over all segments in the network, of the squared deviations of wall shear stresses and pressures from target values derived from independent information about typical network hemodynamic properties. The goal of the present study was to develop and test a new mathematical and computational algorithm based on this approach.

2.2 Methods

2.2.1 Network Hemodynamics

The flow rate $Q_j$ in segment $j$ of the network is assumed to be governed by Poiseuille’s law. For each segment, a positive flow direction is defined, from start node to end node. The relationship between the nodal pressures $p_k$ and the segment flows can be expressed in matrix form as

$$Q_j = \sum_{k \in N} M_{jk} p_k, \quad (2.2.1)$$

where $N$ is the set of all nodes (junctions) in the network and

$$M_{jk} = \begin{cases} 
+\pi r_j^4/(8\mu_j \ell_j), & \text{if } k \text{ is the start node of segment } j; \\
-\pi r_j^4/(8\mu_j \ell_j), & \text{if } k \text{ is the end node of segment } j; \\
0, & \text{otherwise}. 
\end{cases} \quad (2.2.2)$$

where $r_j$, $\ell_j$, and $\mu_j$ denote segment radius, length, and effective viscosity of segment $j$. By conservation of mass, the sum of the flows at each interior node is zero. This condition can be combined with the conditions on the boundary nodes to give

$$\sum_{j \in S} L_{ij} Q_j + Q_{0i} = 0 \text{ for } i \in N, \quad (2.2.3)$$

where $S$ denotes all segments in the network, and

$$L_{ij} = \begin{cases} 
-1, & \text{if } i \text{ is the start node of segment } j; \\
+1, & \text{if } i \text{ is the end node of segment } j; \\
0, & \text{otherwise}. 
\end{cases} \quad (2.2.4)$$
If node \( i \) is a boundary node, then \( Q_{0i} \) is the inflow (or outflow, if negative). At interior nodes, \( Q_{0i} = 0 \). Combining (2.2.1) and (2.2.3) yields

\[
\sum_{k \in N} K_{ik} p_k = -Q_{0i} \quad \text{for} \ i \in N,
\]

where

\[
K_{ik} = \sum_{j \in S} L_{ij} M_{jk}.
\]

A pressure boundary condition can be imposed at node \( i \) by replacing the \( i \)th row of the matrix \( K \) with a single diagonal entry of 1 and replacing \(-Q_{0i}\) by the prescribed pressure. If the pressure or flow is known at every boundary node (Figure 2.1A), then the system (2.2.5) is fully determined and can be solved by standard methods [56, 79].

The effective viscosity \( \mu_j \) in segment \( j \) is determined as a function of the radius \( r_j \) and the hematocrit \( H_{Dj} \) of each segment, using an empirical \textit{in vivo} viscosity relationship [80]. The hematocrits can be computed from the flow rates \( Q_j \), using empirical relationships for hematocrit partition at diverging bifurcations [71, 73]. To satisfy these relationships, an iterative procedure is required to solve for the flows, hematocrits, and effective viscosities in each vessel. Specifically, the flow in each vessel is calculated with initial values for discharge hematocrit. These flow values are then used to update \( H_{Dj} \) in each vessel. Effective viscosity is then recalculated using the new hematocrits. These steps are repeated until convergence is reached for \( Q_j \), \( H_{Dj} \), and \( \mu_j \) in each vessel.

### 2.2.2 Estimation of Flows with Incomplete Boundary Conditions

We now address cases for which boundary conditions, in the form of specified flow or pressure at each boundary node, are known only at a subset of the boundary nodes (Figure 2.1B). If the boundary conditions are not all known, the system (2.2.5)
(A) If boundary conditions (pressure or flow, including at least one pressure boundary condition) are specified at all boundary nodes of a network, and the flow resistances of all segments are known, then the flow rates in all segments of the network are fully determined (solid lines). (B) With incomplete boundary data, the equations governing flow in the network lead to an underdetermined linear system, and flows in some segments remain as unknowns (dashed lines).

Figure 2.1.

becomes

$$\sum_{k \in N} K_{ik}p_k = -Q_{0i}, \quad i \in I \cup B,$$

where $I$ is the set of interior nodes and $B$ is the set of boundary nodes with known boundary conditions. This system is underdetermined and has multiple solutions. To obtain a unique solution, additional assumptions are required. In the present model, it is assumed that information is available about the typical distributions of pressure and wall shear stress in the network, such that a target pressure and wall shear stress can be defined for each segment. The specification of these target values is discussed further below. Using these distributions, a solution is sought that minimizes the total
squared deviation $D$ from the target values of pressures and wall shear stresses:

$$D = \frac{1}{2} k_p \sum_{k \in N} w_k (p_k - p_{0k})^2 + \frac{1}{2} k_r \sum_{j \in S} \ell_j (\tau_j - \tau_{0j})^2. \quad (2.2.8)$$

Here, $\tau_j$ is the wall shear stress in segment $j$, $\tau_{0j}$ is the corresponding target shear stress, $p_{0k}$ is the target pressure at node $k$, $k_p$ and $k_r$ are weighting factors associated with the pressure and shear deviations from the target values, and $w_k$ is the vessel length associated with node $k$, defined as

$$w_k = \frac{1}{2} \sum \ell_j, \quad (2.2.9)$$

where the sum is over the segments $j$ connected to node $k$. This constrained optimization problem can be formulated in terms of Lagrangian objective function

$$L = \frac{1}{2} k_p \sum_{k \in N} w_k (p_k - p_{0k})^2 + \frac{1}{2} k_r \sum_{j \in S} \ell_j (\tau_j - \tau_{0j})^2 + \sum_{i \in I \cup B} \lambda_i \left( \sum_{k \in N} K_{ik} p_k + Q_0 i \right), \quad (2.2.10)$$

where $\lambda_i$ are Lagrange multipliers. At the solution, $L$ is stationary with respect to the unknowns $p_i$ and $\lambda_i$. In each segment, the shear stress is proportional to the flow rate:

$$\tau_j = c_j Q_j = c_j \sum_{k \in N} M_{jk} p_k, \quad (2.2.11)$$

where $c_j = 4\mu_j/(\pi r_j^3)$ is held constant in the optimization. Setting $\partial L/\partial p_i = 0$ gives

$$k_r \sum_{k \in N} H_{ik} p_k + k_p w_i (p_i - p_{0i}) + \sum_{k \in I \cup B} K_{ki} \lambda_k = k_r \sum_{j \in S} \tau_{0j} c_j M_{ji} \ell_j, \quad i \in N, \quad (2.2.12)$$

where

$$H_{ik} = \sum_{j \in S} c_j^2 M_{ji} M_{jk} \ell_j. \quad (2.2.13)$$

Setting $\partial L/\partial \lambda_i = 0$ recovers equation $(2.2.7)$. Equations $(2.2.7)$ and $(2.2.12)$ form a square linear system with unknowns $p_i$ and $\lambda_i$, which can be solved using standard methods.
2.2.3 Target Pressures and Shear Stresses

In this approach, the target pressures at each node and target shear stresses in each segment are assumed to be constants or functions of other variables in the computation. The assumed values may be obtained from observations of hemodynamic characteristics in sub-regions of the experimental preparation accessible to direct measurements, e.g., by intravital microscopy, or derived from experimental estimates and/or empirical correlations in similar experimental systems. Prior information about values of pressures and shear stresses in the specific network under consideration is not required.

In the simulations presented here, networks derived from morphological data from the rat mesentery are considered [76]. Two approaches for setting the magnitudes of the target wall shear stresses in these networks are examined. The first approach is to use a fixed value for all segments. In this tissue, the frequency distribution of segment pressures, considering all vessel types (arterioles, venules, and capillaries), has a maximum at \( \sim 21 \) mmHg (see Figure 1A of [76], relative pressure \( \sim 0.1 \)). The average wall shear stress for segments with this pressure is \( \sim 15 \) dyn/cm\(^2\) [75]. Therefore, a target value \( |\tau_{0j}| = 15 \) dyn/cm\(^2\) was used. For one network, further simulations were carried out to test the sensitivity of the results to this value.

The second approach is based on experimental observations and simulations of blood flow in microvascular networks that showed a systematic trend of increasing wall shear stress with increasing intravascular pressure [75]. This trend was attributed to the structural adaptation of microvessels in response to hemodynamic stimuli. Specifically, it has been observed that increased wall shear stress results in active remodeling of the vessel wall to increase vessel lumen diameter, whereas increased pressure causes inward remodeling of the vessel wall and a reduction in lumen diameter. The combined effect of these two responses is considered to underlie the observed correlation between pressure and shear stress [78]. The target shear stress was cal-
culated as a function of the actual midpoint pressure \( p_j \) in each segment, using the empirical pressure-shear relationship observed in mesenteric microvascular networks [75]:

\[
|\tau_{0j}| = 100 - 86 \cdot \exp \left\{ -5000 \cdot [\log(\log(p_j))]^{5.4} \right\},
\]

(2.2.14)

where shear stress and pressure are expressed in units of dyn/cm\(^2\) and mmHg, respectively. As the target values \( |\tau_{0j}| \) depend on the current values of the segment midpoint pressures \( p_j \), an iterative procedure was used in which segment pressures \( p_j \) were estimated based on a given set of \( |\tau_{0j}| \), and then \( |\tau_{0j}| \) were updated according to equation (2.2.14).

The target pressure is set to a fixed value in all segments, \( p_{0k} = p_0 = 31 \) mmHg, which is the average of the feeding and draining pressure of rat mesenteric microvascular networks [76]. The term proportional to \( k_p \) in the objective function \( L \) biases the node pressures toward this value. The results of the procedure depend on \( k_p \) and \( k_\tau \) only through their ratio, and \( k_p \) was arbitrarily set to 0.1. In practice, \( k_\tau/k_p > 1 \), and so the bias of node pressures toward \( p_0 \) is relatively small. Nonetheless, this term is necessary to constrain the nodal pressures to lie in a realistic range. For one network, additional simulations were carried out to test the sensitivity of the results to the target pressure.

As the flow directions in the network are not known a priori, it is necessary also to specify the direction (sign) of the target wall shear stress in each segment. In principle, this could be done by performing the optimization over all possible choices of target directions, but this is not feasible for networks with hundreds or thousands of segments. Therefore, the following heuristic strategy was employed. Initial flow directions were chosen randomly, and an optimization was performed with a very small value \( (10^{-4}) \) of the weight \( k_\tau \) associated with the shear stress term in the objective function, with \( |\tau_{0j}| \) set to a constant value \( (5 \) dyn/cm\(^2\)\). The resulting set of pressures and flow directions was used to re-assign \( \tau_{0j} \) in each segment, with the
sign of $\tau_{0j}$ corresponding to the last computed flow direction. This process was then repeated until two consecutive iterations gave the same flow directions in all segments. The value of $k_r$ was then doubled, and the flow directions from the previous iteration were used to determine $\tau_{0j}$ for this new $k_r$ value. The above process was then carried out iteratively for a sequence of geometrically increasing values of $k_r$. The final shear stress weight, $k_{r,\text{final}}$, was chosen to minimize the number of segments for which predicted flow directions were opposite to observed flow directions.

The rationale for this procedure is that if $k_r$ were set to $k_{r,\text{final}}$ at the outset, without this iterative procedure, the flow directions would be strongly biased to the initial, randomly assigned directions and would not readjust to achieve a lower overall error, due to the large weight of $k_r$ relative to $k_p$. However, when $k_r$ is set initially to a small value, the bias toward the initially assigned flow directions is small, allowing changes in flow directions (Figure 2.2).

With increasing $k_r$, flow directions eventually stabilize, having achieved better estimates (i.e., lower values of $L$) than would be achieved by simply fixing a $k_r$ value. This process is analogous to the well-known optimization technique of simulated annealing [44].

2.3 Results

2.3.1 Identification of Flow Directions, Estimation of $k_{r,\text{final}}$

The model was tested using experimental data from four networks of the rat mesentery, for which flow rates were previously measured in all segments [79]. In these networks, the number of segments ranged from 383 to 547, and the number of boundary conditions ranged from 22 to 40. In the initial testing of the method, two boundary conditions were prescribed in network I, for the main feeding arteriole and the main draining venule. In networks II, III, and IV, two main venules drained the network and three boundary conditions were therefore prescribed, for the main ar-
Figure 2.2. Schematic illustration of optimization algorithm. The two graphs represent the dependence of the objective function on flow rate in one segment of the network. The objective function consists of a component dependent on the flow direction and a component independent of the flow direction, but dependent on the deviation of the shear stress from the target value. (A) When $k_t$ is small, the flow directions can change readily, such that the objective function can approach the global minimum. (B) As $k_t$ increases, the flow directions become fixed according to the result at preceding optimizations with smaller values of $k_t$, but the flow rates are increasingly determined by the minimization of the deviation of shear stress from the target value.

Figure 2.3 shows the results of the optimization process for the capillaries and the two main venules. In each case, the remaining boundary conditions were left unknown. In networks I and II, the number of reversed segments reached a minimum near $k_t = 0.4096$, while in the other two networks, no clear minimum was reached (Figure 2.3). These results were obtained using a fixed target wall shear stress, 15 dyn/cm². Based on these results, the final shear stress weight was set to...
\( k_{r,\text{final}} = 0.4096 \) in all four networks. With this value, the algorithm gave an average of 9.2\% of the segments with predicted flow directions reversed (incorrect) relative to the flow directions obtained when the correct boundary conditions were applied to all boundary nodes. These results are summarized in Table 2.1. For one of the networks (number I), the segments with reversed flows are identified in Figure 2.4. In this network containing 547 segments, with 2 of 36 boundary conditions specified, the algorithm resulted in incorrect flow directions in a total of 28 segments, including only 5 of the remaining 34 boundary segments. Many of the segments with reversed flow lie near the boundaries of the network. This is to be expected, because the flow in a segment near the boundaries is sensitive to a few unknown boundary conditions. Flow in a segment far from the boundaries depends on many boundary conditions, and is therefore not as sensitive to errors in individual boundary conditions.

<table>
<thead>
<tr>
<th>Network</th>
<th>Number of segments</th>
<th>Number of boundary conditions</th>
<th>Number of specified boundary conditions</th>
<th>Percentage of reversed flows</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2810</td>
<td>547</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>1502</td>
<td>389</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>III</td>
<td>1008</td>
<td>383</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>IV</td>
<td>1508</td>
<td>392</td>
<td>40</td>
<td>3</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.1. Percentage of reversed flows in mesenteric networks when two or three boundary conditions are specified.

2.3.2 Estimation of Flows and Pressures

The segment flows and nodal pressures predicted for network I with two specified boundary conditions are compared in Figure 2.5 with the corresponding estimates with all boundary conditions specified. The results for the two sets of estimates are strongly correlated, although large discrepancies exist in a relatively small subset of the segments. These results suggest that the flow distributions estimated by this
Figure 2.3. Dependence of the number of reversed flows on the weighting factor $k_r$ for shear stress in the optimization. A general trend toward reduction in number of reversed flows was found as $k_r$ was sequentially increased. However, this trend was reversed at large values of $k_r$ in two networks. The vertical dashed line corresponds to $k_r = 0.4096$, the value used in the subsequent application of the method. (A) Network I, (B) network II, (C) network III, and (D) network IV.

Figure 2.6 shows the effect of increasing the number of known boundary conditions on the normalized root mean square deviation ($D_{NRMS}$) between segment flows predicted with partial boundary information ($Q_j$) and those predicted when all boundary
Figure 2.4. Computer-generated map of network I. Flow directions predicted with two known boundary conditions are compared with flow directions with all boundary conditions known. Black: correct flow direction. Red: reversed flow direction.
conditions are known \((Q_{j0})\), defined as

\[
D_{NRMS} = \left[ \frac{\sum_{j \in S} (Q_j - Q_{j0})^2}{\sum_{j \in S} Q_{j0}^2} \right]^{1/2}.
\]  (2.3.1)

Figure 2.5. Node pressures and segment flows in each segment of network I. Values predicted with two known boundary conditions (B.C.s) are plotted against values with all boundary conditions known. (A) Log(flows), and (B) node pressures.

Boundary conditions were successively imposed in order of decreasing magnitude of observed flow. As would be expected, \(D_{NRMS}\) approaches zero as more boundary conditions are specified.

To test the dependence of the results on the randomly chosen initial flow directions, we performed simulations with six different randomized starting conditions, for network I. The results with zero boundary conditions specified showed large variations (Figure 2.6), but with one or more boundary conditions specified, the results were essentially independent of the assumed starting conditions. We also computed results corresponding to Figures 2.5A and 2.6 for red blood cell fluxes. The results (not shown) were essentially indistinguishable from those for blood flow rate.
2.3.3 Sensitivity of Results to Assumed Target Values

The computations described above were repeated using a pressure-dependent target wall shear stress $|\tau_{0j}|$ in each segment, according to equation (2.2.14). The results (not shown) did not differ significantly from those obtained using $|\tau_{0j}| = 15$ dyn/cm$^2$ for all segments. Using a fixed $|\tau_{0j}|$ is simpler and requires less detailed hemodynamic information, and is therefore preferable. When the simulation was repeated for network I with $|\tau_{0j}| = 30$ dyn/cm$^2$, the number of incorrect flow directions and $D_{NRMS}$ with two boundary conditions specified were almost doubled. This indicates that $|\tau_{0j}|$ is an important parameter in the method. Increasing the target pressure $p_{0k}$ from...
31 to 41 mmHg did not significantly change the number of incorrect flow directions or $D_{NRMS}$. However, decreasing $p_{0k}$ to 21 mmHg led to a large increase in both the number of incorrect flow directions and $D_{NRMS}$.

2.4 Discussion

Methods for imaging the three-dimensional structure of microvascular networks have advanced in recent years. However, the interpretation of the results of such studies in terms of the functional properties of the microvasculature remains a challenge to be addressed. Basic morphological measures such as vascular length density, vascular volume fractions, or vessel-vessel spacing provide very limited information about the ability of a network to perfuse a tissue and to deliver an adequate supply of oxygen and other nutrients. Networks with similar values of these parameters may nonetheless have entirely different functional characteristics because of different connectivity or diameter distributions. For example, in tumor networks, high levels of perfusion and vascular density may coexist with significant tissue hypoxia because of functional shunting of blood flow through short pathways [70]. Ideally, assessment of microcirculatory function should thus be based on information on both structure and flow distribution. However, detailed flow information is technically difficult to obtain in many cases of interest.

The theoretical method presented in this study is intended to address this lack of experimental information. In this method, the distribution of flows in a network is computed based on (i) known physical principles governing blood flow in microvascular networks; (ii) limited information about the flows or pressures at a few major vessels feeding and draining the network; and (iii) independently derived empirical information about the distributions of pressures and flows in the network. The algorithm uses a constrained optimization approach to generate estimates of segment flows based on this incomplete information. These estimates are shown to correlate well
with the results obtained when full information on boundary conditions is available. In some of the networks considered, blood flow velocities were previously measured experimentally and flow rates were estimated [76, 80]. While these estimates could, in principle, be used as an alternative standard for comparison with the results of the present method, they are subject to experimental errors [80], leading to significant violation of flow conservation at bifurcations. Consequently, the simulation cannot possibly reproduce the experimentally observed flow rates. The goal of the algorithm is to estimate segment flow rates in the absence of a full set of boundary conditions. Therefore, the appropriate standard of comparison is the computed flow distribution with all boundary conditions known.

In this method, the role of the target shear stress and pressure is to ensure that these parameters are in physiologically realistic ranges throughout the network, not to force shear stresses and pressures in each vessel to match the target values. The computed flows imply wide distributions of shear stress and pressure in the network.

It must be recognized that this method has significant limitations. It represents an attempt to solve a system that is mathematically underdetermined when constrained by the available data. Therefore, the results are inevitably approximate. Despite good overall agreement between results from this approach and the “true” values, significant errors are obtained for a subset of segments in the network. These errors decline as the number of known boundary conditions is increased. The method depends on the availability of independent information on distributions of pressures and wall shear stresses in the type of network under consideration. In the examples considered here, such information is available because of the suitability of mesenteric networks for study by intravital microscopy. In other tissues, such data are more difficult to obtain. While data from one tissue, such as mesentery, may be applicable to other tissues, this approach may neglect systematic differences between the microcirculation of different tissues. For example, tumors exhibit systematic differences from normal tissues, as already mentioned. Finally, the algorithm is computationally
intensive: hundreds of solutions to the network flow problem must be computed as part of the optimization procedure. When applied to networks containing hundreds of segments, the algorithm takes a few minutes to run on a standard personal computer.

The algorithm does not require \textit{a priori} information about whether vessels are arterioles, capillaries, or venules. If vessel types are known, such that flow directions can be specified in advance in some vessels, then the method can readily be modified to take this into account, by fixing the sign of the target shear stress in such vessels.

In conclusion, the method presented here enables the estimation of blood flows in all segments of a microvascular network, given incomplete information about flow or pressure in the boundary segments, together with independent estimates or correlations describing distributions of pressure and wall shear stress in the tissue under consideration. Structural information in combination with flow information allows detailed simulations of transport of oxygen and other solutes [86]. Therefore, we anticipate that this method will assist in the interpretation of data obtained from three-dimensional imaging of microvascular networks containing large numbers of segments, and will potentially lead to new insights into functional properties of the microcirculation in various tissues under a range of conditions.
Chapter 3

Vascular recruitment in a theoretical model for blood flow regulation in heterogeneous microvascular networks

3.1 Introduction

Proper distribution of blood within organs is essential for the matching of blood supply to tissue demand. In the microcirculation, local regulation of blood flow is achieved by contraction and relaxation of the vascular smooth muscle (VSM) surrounding the arterioles and small arteries. Changes in VSM tone are elicited by responses to changes in several factors, including levels of oxygen and other metabolites, intraluminal pressure, and luminal wall shear stress [20]. These vasoactive stimuli not only act locally but also induce responses that are propagated upstream, causing vessels feeding the site of the stimulus to constrict or dilate. The resulting coordinated control of VSM tone in vessels proximal to the affected site contributes to blood flow regulation in response to changes in the metabolic demand of the tissue [5, 15, 88].

Regulation of blood flow is especially important in skeletal muscle, which can exhibit wide ranges of metabolic conditions and blood flow rates [83, 87]. How this matching of perfusion to demand is achieved is a question that has not been fully resolved. Starting with the work of Krogh [48, 49], it has been proposed that one of the contributing mechanisms is capillary recruitment, whereby vessels that are not flowing at rest commence flowing in response to increased metabolic demand [5, 46]. The mechanisms of recruitment, however, remain incompletely understood, and even the occurrence of recruitment is not universally accepted [13].

Previous theoretical models have addressed the effects of varying the number and location of perfused capillaries on tissue oxygenation in skeletal muscle, using
oxygen transport simulations [32, 57]. These models did not address the mechanisms
determining the distributions of flowing vessels. Previous models of flow regulation
[1, 10, 16, 45, 90] have generally considered vascular networks as sets of compartments
connected in series, where each compartment contains multiple identical vessels of a
given type, connected in parallel. Such models do not lend themselves to studying
recruitment, which necessarily involves differences in behavior among capillaries, with
some ceasing to flow while others continue flowing.

In microvascular networks with heterogeneous structures, experiments have demon-
strated unequal partition of hematocrit at diverging bifurcations, such that the daugh-
ter vessel with the larger flow rate tends to receive a larger hematocrit than the other
daughter vessel [84]. This phenomenon, also referred to as phase separation, creates a
non-uniform distribution of hematocrit throughout the network that depends on the
flow rates in the individual vessels. A recent study [82] examined metabolic flow regu-
lation in a heterogeneous network structure derived from the rat mesentery, assuming
that the metabolic signal was derived from red blood cells (RBCs). Including phase
separation in the model led to significantly different behavior than was predicted
if uniform hematocrit was assumed. This shows the importance of considering the
effects of phase separation when analyzing flow regulation in heterogeneous networks.

The objective of this study is to test the hypothesis that capillary recruitment
occurs in heterogeneous microvascular networks as a consequence of local blood flow
regulation by changes of arteriolar VSM tone, and to analyze the contribution of
unequal hematocrit partition at vessel bifurcations to this phenomenon. Theoretical
models are used to simulate blood flow, oxygen transport, and local flow regulation
in these networks. The microvascular network used in the simulations is based on
previously published experimental results in the hamster cremaster muscle [4]. The
simulation of blood flow includes the effects of phase separation on the distribution
of hematocrit. Oxygen transport is simulated using a method that takes into account
the effects of all surrounding vessels on the oxygen level at each point in the tissue
The theoretical model for metabolic flow regulation is based on modulation of arteriolar diameters according to the length-tension characteristics of VSM [1, 10, 11, 82]. Responses of VSM tone to myogenic, shear-dependent, and metabolic stimuli are included.

3.2 Methods

3.2.1 Network

Most previous models of blood flow regulation have described the microcirculation in terms of representative blood vessel segments [1, 10, 16, 90]. In such models, the vasculature is divided into several classes of vessels, e.g., artery, large arteriole, small arteriole, capillary, small venule, large venule, and vein. The vessels in a particular vessel class are assumed to be equivalent and arranged in parallel, with the different classes arranged in series. This assumption simplifies computations, but does not take into account structural heterogeneities present in actual microvasculature. In the study of Roy et al. [82], the heterogeneity of network structure was represented by replacing the small arterioles, capillaries, and small venules in the representative segment model with a realistic network structure derived from experimental observations in the rat mesentery. A similar approach is used in the present study, as shown in Figure 3.1. The network structure is derived from observations of the hamster cremaster muscle [4, 5], in which a microvessel network was imaged and mapped in a control state and in a pharmacologically-induced maximally dilated state, and RBC fluxes were measured in the arterioles and capillaries. Some vessels had no observable RBC flux in the control state but observable RBC flux in the maximally dilated state, indicating the occurrence of vessel recruitment. Only the vessels with observable RBC fluxes were included in the network maps for the two states [4].

From the experimentally obtained maps of network structure in the two states, a single network structure was derived that contained all vessels included in either
Figure 3.1. Schematic of the network model. A complete flow pathway through the circulation is formed by an experimentally observed microvascular network, with the addition of upstream and downstream segments representing arteries, large arterioles, large venules, and veins. The distribution of intravascular pressures is shown for the reference state of the network model.

of the observed maps. This network, which contains 125 vessels, is used as the basis for the present model. The observed maps included arterioles and capillaries up to the points at which they converged to form venules, but did not include information about the location of the venules. In order to complete the network structure, 12 venules were inserted, so as to connect all disconnected capillaries, while minimizing the length of venules added. This procedure resulted in a 137-segment microvessel network. The pressure drop in the venules is typically a small fraction of the overall pressure drop in the network. Moreover, the venules make a minor contribution to the overall oxygen exchange in the network. Therefore, the distributions of blood flow and oxygen transport in the network are relatively insensitive to the assumed geometry of this added venular network.

Vessels in the 137-segment microvessel network are classified as arterioles, capillaries, or venules. Arterioles, which are capable of active regulation, are defined based on the branching pattern: if a vessel is the parent vessel at a diverging bifurcation and has a diameter in the dilated state of at least 8 μm, it is classified as an arteriole.
All other vessels (except the added venules) are classified as capillaries. This results in 32 arterioles, 93 capillaries, and 12 venules (Figure 3.2). To form a complete flow pathway through the systemic circulation, two upstream vessels (artery A, and large arteriole LA) and four downstream vessels (large venules LV1, LV2, and veins V1, V2) are added, one of which (LA) is capable of active regulation, bringing the total number of vessels in the simulated network to 143.

### 3.2.2 Network hemodynamics

The microvessel network is represented as a set of interconnected segments, each with a defined diameter and length. Pressure-driven flow in each segment and conservation of mass at each node are assumed. Phase separation of RBCs at diverging bifurcations is implemented based on previously derived experimental relationships \[77\]. Flow in each segment is governed by Poiseuille’s law, with an apparent viscosity that depends on vessel diameter and includes effects of an endothelial surface layer \[74\]. Using an iterative technique \[93\], the resulting nonlinear system of equations is solved at each time step with fixed diameters to obtain the apparent viscosity, flow rate, hematocrit, and wall shear stress in each segment.

In the experimental observations \[4\], vessels were considered to be flowing if they had observable RBC fluxes. Since RBC flux was reported in steps of 25 cells/s, the threshold for observable RBC flux is assumed to be \( F_{\text{threshold}} = 12.5 \) cells/s. In the following, vessels with RBC flux above this threshold are referred to as flowing and others as non-flowing.

### 3.2.3 Flow regulation model

The model for flow regulation is based on the modulation of arteriolar diameters according to the passive and active length-tension characteristics of VSM, and includes myogenic, shear-dependent, and metabolic responses \[1, 10\]. Steady-state tension in
Figure 3.2. Network used in simulations. Arterioles (red) and capillaries (green) are derived from observations of hamster cremaster muscle [4]. Venules (blue) are added to form connections with downstream ends of capillaries.
the vessel wall ($T_{total}$) is represented as the sum of a passive component and an active component generated by the VSM [11]:

$$T_{total} = T_{pass} + AT_{act}^{max}. \quad (3.2.1)$$

Here $A$ represents the local activation level of the muscle in each vessel, and has a range from 0 to 1, where $A = 0$ represents no vascular tone and $A = 1$ represents maximal vasoconstriction. The passive tension in the wall of an arteriole with diameter $D$ is given by

$$T_{pass} = C_{pass} \exp[C_{pass}'(D/D_0 − 1)], \quad (3.2.2)$$

where $C_{pass}$ and $C_{pass}'$ are constants representing the magnitude and diameter dependence of passive tension, and $D_0$ is the passive diameter at an intraluminal pressure of 100 mmHg. The maximal active wall tension is described by

$$T_{act}^{max} = C_{act} \exp \left[ - \left( \frac{D/D_0 - C_{act}''}{C_{act}'} \right)^2 \right], \quad (3.2.3)$$

where $C_{act}$, $C_{act}'$, and $C_{act}''$ are constants representing maximally active VSM peak tension, length dependence, and tension range, respectively. Equations (3.2.2) and (3.2.3) were well-validated against steady-state myogenic response experimental data in various tissues in [11]. A target activation level is introduced, which is assumed to be a saturating function of the total vasoactive signal, $S_{tone}$:

$$A_{total} = \frac{1}{1 + \exp(-S_{tone})}. \quad (3.2.4)$$

The parameter $S_{tone}$ represents the combined effects of the myogenic, shear-dependent, and metabolic input stimuli [1, 10]:

$$S_{tone} = C_{myo}T - C_{shear} \tau_{wall} - S_{meta} + C_{tone}'', \quad (3.2.5)$$

where $C_{myo}$, $C_{shear}$, and $C_{tone}''$ are constants representing the sensitivity of $S_{tone}$ to the various stimuli. The wall tension is given by $T = PD/2$, where $P$ is the average
segment intraluminal pressure, $\tau_{wall}$ is the wall shear stress, and $S_{meta}$ is the conducted metabolic response signal, computed as described below. The form of Equation (3.2.5) was also well-validated against experimental myogenic response data in [11], and the addition of the shear-stress-dependent term was validated against experimental data for a physiological range of flow rates and pressures in [10].

Parameter values in the model for VSM tone were chosen to match those used previously [1, 82]. For the parameter $C_{act}$, the maximally active VSM peak tension, values were needed for smaller diameter vessels ($< 30 \mu m$ in the maximally dilated state) than those considered in the previous models. Therefore, $C_{act}$ was estimated by fitting a power-law relationship to six data sets in [11] with the smallest average value of $D_0$, including four data sets for hamster microvessels (Figure 3.3). Values of parameters used in the model are given in Table 3.1, and model variables are given in Table 3.2.

### 3.2.4 Oxygen transport

Previous models for metabolic flow regulation considered oxygen exchange between each vessel and an associated local tissue region (or “tissue sleeve”) of fixed width surrounding the vessel [1, 82]. A limitation of this type of oxygen transport model is that as blood flow rate approaches zero in an individual vessel, the $PO_2$ must go to zero in that vessel. In reality, tissue cells may receive oxygen from any vessel that is sufficiently close. A decrease in flow in a particular vessel does not necessarily cause hypoxia if other well-oxygenated vessels are nearby. Therefore, models based on a fixed oxygen consumption rate per vessel length tend to overestimate the flow-dependent changes in intravascular oxygen levels and exaggerate the ability of metabolic responses to maintain flow in all vessels. Such models are therefore not suitable for investigating vascular recruitment.

Here, a more realistic oxygen transport model is used. The model is capable of
Figure 3.3. Power-law fit of $C_{act}$ versus $D_0$, for six data sets in [11]. The points used in the fit are weighted by the numbers of vessels in each data set. (●) Experimental data [11]. (−) Power-law fit to data: $C_{act} = 1.30 D_0^{1.48}$.

Simulating a spatially-varying oxygen field, including effects of diffusive interaction between all vessels in the network and the entire tissue region. It uses a Green’s function method [86], in which each vessel is represented as a set of discrete oxygen sources, and the oxygen field in the tissue is represented as a superposition of the fields resulting from the sources. This allows for more efficient computation, relative to other computational approaches, as it reduces the number of unknowns needed to represent the oxygen field. In the tissue region, Michaelis-Menten oxygen utilization kinetics are assumed, and a Michaelis constant of 1 mmHg is used [86]. Oxygen demand ($M_0$) is varied between 0.5 and 8 cm$^3$ O$_2$/100 cm$^3$/min, corresponding to a range of metabolic conditions.
The geometric locations of the added upstream and downstream vessels are not specified. For the purpose of modeling oxygen transport, it is assumed that the arterial vessels (A and LA) are surrounded by a tissue sleeve of width 18.8 µm in which oxygen is consumed at a fixed rate. The sleeve width is chosen to correspond to a typical measured value of skeletal muscle capillary density of 500/mm² [1]. Oxygen exchange by the added venous vessels (LV1, LV2, V1, and V2) is neglected. The Hill equation is used to calculate oxyhemoglobin saturation as a function of vessel PO₂, with parameters of \( n = 2.55 \) and \( P_{50} = 26 \) mmHg, based on estimates in hamster cremaster muscle [47]. Oxygen content of all inflowing vessels to the network is prescribed in terms of the PO₂. At the arterial inflow to the network, a PO₂ of 100 mmHg is assumed. At venular inflows to the network, a PO₂ of 20 mmHg is assumed, based on estimates of venular saturation [25].

### 3.2.5 Conducted metabolic signal

The origins of the metabolic signals involved in local flow regulation are not definitely established. Roy et al. [82] analyzed flow regulation in heterogeneous microvascular networks, assuming either wall-derived or RBC-derived metabolic signals, and showed that a wall-derived signal was more effective in matching perfusion to local oxygen demand. In reality, several mechanisms likely contribute to metabolic flow regulation, involving wall-derived, tissue-derived, and/or RBC-derived signals. In the present study, the metabolic signals in each vessel segment are assumed to be generated by signals from the vessel walls, since this type of signaling was found to be effective for local flow regulation in heterogeneous networks [82]. The signals are assumed to be conducted upstream to the arterioles, resulting in metabolic control of arteriolar tone [88]. For each vessel segment, the local wall-dependent signal generated, \( S_{loc} \), is a decreasing function of the local PO₂, as it is assumed that the release of vasodilators
from the vessel wall is most sensitive to the presence of hypoxia:

\[ S_{\text{loc}} = C_{\text{meta}} \frac{P_0}{P_0 + P_{O_2}}, \]  

(3.2.6)

where \( C_{\text{meta}} \) is a parameter describing the strength of the signal, and \( P_0 \) is the partial pressure of oxygen at which the signal is half-maximal. It is assumed that the wall-dependent signaling mechanism is defined in terms of the deficit between oxygen demand and consumption, so that \( S_{\text{loc}} \) takes the form of Equation (3.2.6) according to Michaelis-Menten kinetics for oxygen consumption. Here, \( P_0 \) is set to 1 mmHg, corresponding to the Michaelis constant for tissue oxygen consumption. The signal is propagated upstream with exponential decay according to a characteristic length constant. As in [82], at bifurcations diverging in the upstream direction, the conducted signal is partitioned in proportion to the vessel diameters; at upstream converging bifurcations, the signal is summed. The total metabolic signal in each vessel, \( S_{\text{meta}} \), is the sum of the propagated signal from downstream vessels and the signal generated locally (\( S_{\text{loc}} \)). Virtual segments are added distal to draining vessels to generate conducted signals are the network outflows.

### 3.2.6 Dynamics of arterioles

The dynamic behavior of each arteriole is described by differential equations for the variation with time of diameter and activation [1, 10, 82]:

\[ \frac{dD}{dt} = \frac{1}{\tau_d} \frac{D_c}{T_c} (T - T_{\text{total}}), \]  

(3.2.7)

\[ \frac{dA}{dt} = \frac{1}{\tau_a} (A_{\text{total}} - A), \]  

(3.2.8)

where \( D_c \) and \( T_c \) are the diameter and wall tension in the reference state (described below), and \( \tau_d \) and \( \tau_a \) are time constants. Equations (3.2.7) and (3.2.8) are integrated from \( t = 0 \) to 200 s using an explicit Euler method. After 200 s, the system is
found to either reach a steady state or to show stable oscillations, consistent with the occurrence of vasomotion [2]. For purposes of analysis, the final values of system variables are defined by averaging over the interval from 100 to 200 s. The simulation of oxygen transport is the most computationally demanding part of the calculation. The iterative method used in the Green’s function method at each time step involves solutions of large linear systems, which can be implemented with parallel algorithms. Simulation of 200 s of real time requires 800 computational time steps, with each time step taking 10 to 60 s of computer time using a graphical-processing-unit-based parallel processing system.

3.2.7 Reference state

A reference state is defined for the network with a moderate level of arteriolar tone, corresponding to a relatively low level of oxygen consumption in the skeletal muscle. The distribution of pressure drops in the network model is chosen to correspond where possible to the previous model [1], with pressure drops in the A, LV, and V segments of 10 mmHg, 1.49 mmHg, and 1 mmHg. The pressure drop across the microvessel network is chosen to minimize the total squared deviation between vessel RBC fluxes in the reference state and those observed experimentally in the control state [4], resulting in a pressure of 66 mmHg at the arterial side of the microvessel network. This was achieved by adjusting the length of the LA segment. The arterial inflow and venous outflow pressures are set to 100 and 12.91 mmHg, respectively [82]. The resulting distribution of pressures is indicated in Figure 3.1.

To establish the distribution of tone in the reference state, values of $S_{\text{tone}}$ in each arteriole are determined as follows. If an arteriole had observable RBC flux in the maximally dilated experimental network [4], then the passive vessel diameter at a pressure of 100 mmHg ($D_0$) is set as the measured diameter in the maximally dilated state. If an arteriole had observable RBC flux also in the control experimental
network [4], then the diameter in the model reference state \((D_c)\) is set as the measured diameter in the control state. The reference state is assumed to be at equilibrium, so that \(T = T_{total}\) and \(A = A_{total}\), which allows for an explicit calculation of \(S_{tone}\) in each arteriole based on Equations (3.2.1) and (3.2.4):

\[
S_{tone} = \ln \left( \frac{PD_c - 2T_{pass}}{2(T_{pass} + T_{act}^{max}) - PD_c} \right). \tag{3.2.9}
\]

For some vessels in the experimental data set, the diameter in the maximally dilated state was less than or equal to the diameter in the control state. Such behavior might result from a decrease in pressure in some arterioles due to overall vasodilation. In these cases, it is assumed that the vessels are close to maximal dilation in the reference state; i.e., \(D_c \approx D_0\), and \(S_{tone}\) is set to a large negative value (-1000) so that \(A \approx 0\). Some arterioles had observable RBC fluxes only in the dilated state of the network. In the reference state, these arterioles are assigned small diameters \(D_c\) in the range 2.9 to 3.8 \(\mu m\), such that all have RBC fluxes below \(F_{threshold}\).

Of the capillaries, 32 were observed as flowing only in the dilated state, implying that they should have RBC fluxes less than \(F_{threshold}\) in the reference state, but greater than \(F_{threshold}\) when arteriolar diameters are set to their dilated values. When arteriolar diameters were initially set to their \(D_c\) values as described above, only 11 of the 32 capillaries had RBC fluxes less than \(F_{threshold}\) in the simulated reference state. The absolute RMS error in individual diameter measurements in microvessel networks was estimated as ±1.2 \(\mu m\), based on imaging limitations [80]. Therefore, small adjustments (≤ 1.2 \(\mu m\) increase or decrease) in capillary diameter were made where this led to an increase in the number of non-flowing capillaries in the reference state. After these changes were made, 19 of the 32 capillaries flowing only in the dilated state are non-flowing in the reference state, and all 32 are flowing when arteriolar diameters are set to their dilated values. In total, of the 125 capillaries and arterioles in the microvessel network, 27 (21.6%) are non-flowing in the reference state, and therefore have the capacity for recruitment.
As is evident from the above description, the procedure for defining the control state involves a number of assumptions. The resulting reference state may not accurately represent, at the level of individual vessels, the conditions that existed in the experimental preparation. However, the procedure used was designed to ensure that distributions of geometric and hemodynamic parameters and their degree of heterogeneity in the reference configuration are similar to those existing in the in vivo preparation. The purpose of the reference state is to provide an internally consistent network with realistic properties as a reference configuration, which serves as a control for further simulations in which hemodynamic or metabolic parameters are varied.

3.3 Results

The numbers of flowing arterioles and capillaries observed experimentally and predicted by the model are shown in Figure 3.4. In the experimental observations, the total number of flowing vessels increased by 26% (from 85 to 107) between the control and vasodilated states. The combined network, consisting of the union of the networks in the control and vasodilated states, contains more vessels (125) than either one individually, because a number of vessels (18) were observed to be flowing in the control state but not in the dilated state. Such behavior may result from hemodynamic effects including redistribution of hematocrit with vasodilation, or it may reflect incomplete visualization of all flowing vessels in the network in the experiments. The combined network is used as the basis for the model simulations.

As shown in Figure 3.4, the model predicts that the numbers of flowing arterioles and capillaries increase progressively with increasing oxygen demand. This supports the hypothesis that vessel recruitment can occur as a consequence of local metabolic regulation of blood flow in a network with heterogeneous structure. Overall, the number of flowing arterioles and capillaries increases by about 16% (from 98 to 114) as
Figure 3.4. Numbers of vessels in experimentally observed networks and numbers of flowing vessels in model simulations.

demand ($M_0$) is raised from 1 to 8 cm$^3$ O$_2$/100 cm$^3$/min. The spatial distributions of flowing and non-flowing vessels are shown in Figure 3.5, for oxygen demand $M_0$ of 1, 2, 4, and 8 cm$^3$ O$_2$/100 cm$^3$/min. In general, increasing oxygen demand causes transitions from non-flowing to flowing states, but a few vessels stop flow with increasing demand (Figure 3.5A).

The effects on predicted tissue oxygenation of including flow regulation and hematocrit partition in the model are illustrated in Figure 3.6, as spatial distributions (upper panels) and frequency distributions (lower panels). For the reference state, with oxygen demand $M_0 = 1$ cm$^3$ O$_2$/100 cm$^3$/min, virtually no hypoxic tissue is predicted (Figure 3.6A). When oxygen demand is increased to $M_0 = 4$ cm$^3$ O$_2$/100 cm$^3$/min, without metabolic flow regulation (i.e., $S_{meta} = 0$ in each vessel) or phase separation included in the model, the distribution of tissue PO$_2$ is shifted towards lower PO$_2$ levels (Figure 3.6B). When metabolic flow regulation is included (Figure 3.6C), the
Figure 3.5. Spatial distributions of non-flowing vessels and flowing arterioles, capillaries, and venules when (A) $M_0 = 1$; (B) $M_0 = 2$; (C) $M_0 = 4$; (D) $M_0 = 8$ cm$^3$ O$_2$/100 cm$^3$/min. Black arrows indicate vessels that start flow with an increase in oxygen demand. Purple arrows in panel A indicate vessels that stop flow with an increase in oxygen demand.

tissue PO$_2$ distribution shifts toward higher levels, compared to the case without metabolic flow regulation. Figure 3.6D shows the distribution with both metabolic flow regulation and phase separation included. The inclusion of non-uniform hematocrit distribution further shifts the tissue PO$_2$ distribution to higher levels.

In Figure 3.7, variables reflecting blood flow and tissue oxygenation are plotted as functions of oxygen demand from $M_0 = 0.5$ to $8$ cm$^3$ O$_2$/100 cm$^3$/min, for three cases: constant hematocrit without metabolic regulation, constant hematocrit with metabolic regulation, and variable hematocrit with metabolic regulation. Figure 3.7A shows total blood flow to the network. With metabolic regulation, inflow rate increases as oxygen demand is increased. Introducing phase separation has little effect on total flow. Figure 3.7B shows the number of flowing arterioles and capillaries. The number of vessels with RBC flux above $F_{\text{threshold}}$ is lower when phase separation is included. The number increases 13% between the lowest and highest oxygen demand states with constant hematocrit, whereas the increase is 25% with phase separation.
Figure 3.6. Contour plots (top panels) and histograms (bottom panels) of tissue PO$_2$ distribution at hundreds of tissue points. Oxygen levels in the vessels and in the surrounding tissue are color-coded according to the scale at left (in mmHg). (A) $M_0 = 1$ cm$^3$ O$_2$/100 cm$^3$/min. (B) $M_0 = 4$ cm$^3$ O$_2$/100 cm$^3$/min with constant hematocrit and without metabolic flow regulation. (C) $M_0 = 4$ cm$^3$ O$_2$/100 cm$^3$/min with constant hematocrit and with metabolic flow regulation. (D) $M_0 = 4$ cm$^3$ O$_2$/100 cm$^3$/min with non-uniform hematocrit and with metabolic flow regulation.

Thus, phase separation increases the extent of vessel recruitment as oxygen demand is increased. As expected, the network inflow rate and the number of flowing vessels are independent of oxygen demand when metabolic flow regulation is not included (Figures 3.7A and 3.7B).

Median tissue PO$_2$ declines with increasing oxygen demand (Figure 3.7C). Inclusion of metabolic flow regulation leads to an increase of about 10 mmHg in median tissue PO$_2$. The increase is larger when phase separation is included, relative to the
uniform hematocrit case. These differences are present for the entire range of oxygen demand considered. Figure 3.7D shows the hypoxic fraction (fraction of tissue with \( \text{PO}_2 < 1 \text{ mmHg} \)). For oxygen demand up to \( M_0 = 2 \text{ cm}^3 \text{O}_2/100 \text{ cm}^3/\text{min} \), there is less than 1% hypoxia in all cases. However, for \( M_0 = 4 \), with no metabolic flow regulation, the hypoxic fractions are 0.031 and 0.028 for variable \( H_D \) (not shown) and constant \( H_D \), respectively. When metabolic flow regulation is included, the hypoxic fraction is much lower, with values of 0.006 and 0.005, respectively. Similarly, for \( M_0 = 8 \), the hypoxic fractions without metabolic flow regulation increase to 0.14 (for both variable and constant \( H_D \)), compared to 0.06 (variable \( H_D \)) and 0.061 (constant \( H_D \)) with metabolic regulation included. Thus, for \( M_0 > 2 \), tissue hypoxic fraction is much lower when metabolic flow regulation is included.

### 3.4 Discussion

Metabolic regulation is necessary for matching blood flow to oxygen demand in skeletal muscle, but the mechanisms by which it occurs have not been fully determined. It has been proposed that the ability to meet widely varying metabolic demands in the tissue is partially achieved via capillary recruitment. This study uses a theoretical model to test the hypothesis that vessel recruitment can occur as a consequence of local changes in arteriolar tone, and to investigate the role of RBC phase separation at vessel bifurcations in this phenomenon.

The results of this model support the above hypothesis. When applied to a heterogeneous network structure in skeletal muscle derived from a hamster cremaster preparation [4], the model predicts that metabolic vasodilation not only increases overall flow rate (Figure 3.7A), but also increases the number of flowing vessels (Figures 3.4, 3.5, and 3.7B), defined in terms of RBC flux above a threshold value. With increasing oxygen demand, vessel recruitment is predicted at both the capillary and arteriolar levels. This recruitment occurs as a consequence of metabolic flow regula-
Figure 3.7. Variables describing network blood flow and tissue oxygenation, as functions of oxygen demand. (A) Relative arteriolar inflow rate. (B) Relative number of flowing vessels. (C) Median tissue PO\(_2\). (D) Hypoxic tissue fraction (defined as PO\(_2\) < 1 mmHg). (●) Variable H\(_D\) (with phase separation) with metabolic flow regulation. (○) Constant H\(_D\) (no phase separation) with metabolic flow regulation. (▼) Constant H\(_D\) without metabolic flow regulation.

...tion, based on modulation of arteriolar diameters, acting in conjunction with unequal hematocrit partition (phase separation) at diverging microvascular bifurcations.

The concept that capillary recruitment plays a role in controlling the delivery of
oxygen to tissue dates back to the work of August Krogh [48, 49], who hypothesized that flow is regulated at the level of individual capillaries by pre-capillary sphincters. These sphincters, however, have not been observed experimentally in skeletal muscle [55, 87]. Rather, control of flow in capillaries of skeletal muscle has been observed to occur at the level of terminal arterioles [4, 18]. Such observations led to the concept that the smallest unit for control of blood flow in skeletal muscle is the microvascular unit, consisting of a terminal arteriole and group of capillaries fed by it (numbering about 20) [18, 26, 57]. According to this concept, recruitment might be expected to occur at the level of microvascular units, not individual capillaries. However, Lindbom and Arfors [55] observed a sequential increase in the number of RBC-perfused capillaries fed by a single terminal arteriole during vasodilation elicited by a decrease in ambient oxygen levels. Their observations imply that recruitment of individual capillaries can occur in response to dilation of terminal arterioles. The mechanism for such recruitment has remained unclear. A previous theoretical study [85] suggested that nonlinear rheological effects associated with RBC motion in non-uniform capillaries could lead to qualitatively similar behavior, but only at flow velocities much lower than those seen in the experiments [55].

The present study provides a potential resolution to these apparently conflicting concepts of capillary recruitment in skeletal muscle. In the simulations, recruitment and de-recruitment of individual capillaries are predicted to occur as a consequence of arteriolar dilation and constriction. Here, as in experimental studies, capillary perfusion is defined in terms of RBC flux above a minimum observable threshold. In a heterogeneous network structure, the unequal partition of hematocrit at diverging bifurcations causes vessel hematocrits to vary depending on the flow distribution. As a consequence, recruitment of capillaries within a single microvascular unit can occur sequentially, rather than synchronously, during gradual vasodilation of the feeding arteriole. According to this concept, modulation of flow and vascular recruitment are two aspects of the same physiological response, and it is not meaningful to quantify
their individual contributions to the metabolic regulation of flow.

The effects of metabolic flow regulation, with and without unequal hematocrit partition at vessel bifurcations, on predicted hemodynamic and oxygen transport characteristics are illustrated in Figure 3.7. Including phase separation has little effect on the total flow to the network (Figure 3.7A), as variations in individual vessel flow rates tend to cancel when aggregated. However, phase separation has a substantial effect on the number of flowing vessels (Figure 3.7B). With phase separation, a larger number of vessels have low hematocrit and low RBC fluxes, and are classified as non-flowing. The extent of recruitment and de-recruitment is greater with phase separation, because decreases in either flow rate or vessel hematocrit may cause the RBC flux to decrease below the threshold level.

These dual mechanisms contributing to the distribution of RBC flux in microvascular networks and the recruitment and de-recruitment of capillaries are illustrated schematically in Figure 3.8. Regardless of whether phase separation is included in the model, low arteriolar flows may result in capillaries with RBC fluxes below $F_{\text{threshold}}$ that can be recruited when arteriolar flow increases in response to increased oxygen demand. A second mechanism becomes evident itself only when phase separation is included. Capillary RBC fluxes below $F_{\text{threshold}}$ may occur because a parent arteriole receives a low hematocrit. With vasodilation, the arteriole may receive a higher hematocrit, leading to recruitment of the capillaries that it feeds. Capillary recruitment is thereby augmented as a result of unequal hematocrit partition at vessel bifurcations.

An unexpected model prediction is that inclusion of phase separation in the model with metabolic flow regulation leads to improved tissue oxygenation, relative to the behavior without phase separation (i.e., with constant hematocrit), as shown by the shift to higher values in the distribution of $\text{PO}_2$ (Figures 3.4C and 3.4D) and the median tissue $\text{PO}_2$ (Figure 3.7C). This improvement in oxygenation occurs despite the fact that fewer vessels are flowing when phase separation is included (Figure 3.7B). Phase separation is thus seen to contribute to metabolic flow regulation. By
Figure 3.8. Schematic diagram showing two mechanisms for capillary recruitment and de-recruitment in response to changes in arteriolar diameter. Black arrows indicate magnitude of RBC flux. Gray arrows indicate arteriolar vasoconstriction. Gray areas within vessels represent regions containing RBCs. (A) Initial configuration with dilated arteriole. (B) Effect of vasoconstriction without phase separation. De-recruitment may occur because RBC flux drops below threshold level. (C) Effect of vasoconstriction with phase separation. De-recruitment may occur because hematocrit entering the arteriole drops to a low level or zero.

Directing increased hematocrit preferentially to vessels with increased flow rates, it provides a mechanism to amplify the changes in RBC flux resulting from modulation of arteriolar diameters. In the present simulations, the inclusion of phase separation has only slight effects on the fraction of hypoxic tissue (Figure 3.7D). However, the hypoxic regions are small and lie mainly near the boundaries of the network (see Figure 3.6), and so this result may not be representative of the behavior that would occur with more widespread hypoxia.

In the present model, the signal for metabolic regulation in response to changes in oxygen levels is assumed to be generated in the vessel wall, according to Equation (3.2.6). A previous theoretical study [82] examined the distribution of blood flow and
tissue oxygenation in a heterogeneous network, assuming that the signal for metabolic flow regulation originated within RBCs. In that case, inclusion in the model of phase separation resulted in poorer oxygenation, because a reduction in RBC flux to a given vessel generally caused a reduction in the RBC-derived metabolic signal, thereby exacerbating the reduction in flow. Taken together, these models show that phase separation can have a substantial effect on local metabolic flow regulation, but its effect depends on the metabolic signaling mechanisms involved.

Estimates of the capacity for capillary recruitment in skeletal muscle vary widely [13]. In some experiments, it has been found to be at least 100% and possibly as high as 200%, relative to the number of flowing capillaries at rest [39, 54]. In the present simulations, almost 80% of the arterioles and capillaries in the network are flowing in the reference state, and so the capacity for recruitment is limited to about 25%. Furthermore, the range of flow regulation in the present model study is restricted according to the observed changes in diameter between the two states of the cremaster preparation [4]. In reality, a wider range of flow rates is observed in many skeletal muscles [87]. The ranges of flow modulation and capillary recruitment that could be generated by the hypothesized mechanisms have no inherent restrictions. Given a network with more vasoconstriction and fewer flowing capillaries in the resting state, the present model would predict a wider range of flow regulation and vascular recruitment.

In conclusion, the results of the simulations imply that recruitment of arterioles and capillaries can occur in heterogeneous microvascular networks as a consequence of local arteriolar vasodilation in response to increased metabolic demand. The unequal partition of hematocrit at diverging microvascular bifurcations contributes significantly to this phenomenon.
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<td>Passive reference vessel diameter</td>
<td>$F_{threshold}$</td>
<td>12.5</td>
<td>cells/s</td>
<td>[4]</td>
</tr>
<tr>
<td>Threshold for observable RBC flux</td>
<td>$P_0$</td>
<td>1</td>
<td>mmHg</td>
<td>[86]</td>
</tr>
</tbody>
</table>

**Table 3.1.** Parameter values used in the model.
<table>
<thead>
<tr>
<th>Description</th>
<th>Variable</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vessel wall tension</td>
<td>$T_{\text{total}}$</td>
<td>dyn/cm</td>
</tr>
<tr>
<td>Passive tension</td>
<td>$T_{\text{pass}}$</td>
<td>dyn/cm</td>
</tr>
<tr>
<td>Maximally active VSM tension</td>
<td>$T_{\text{act max}}$</td>
<td>dyn/cm</td>
</tr>
<tr>
<td>VSM activation</td>
<td>$A$</td>
<td>μm</td>
</tr>
<tr>
<td>Vessel diameter</td>
<td>$D$</td>
<td>μm</td>
</tr>
<tr>
<td>Vessel wall shear stress</td>
<td>$\tau_{\text{wall}}$</td>
<td>dyn/cm²</td>
</tr>
<tr>
<td>VSM activation stimulus</td>
<td>$S_{\text{tone}}$</td>
<td>dyn/cm²</td>
</tr>
<tr>
<td>Conducted metabolic response signal</td>
<td>$S_{\text{meta}}$</td>
<td>μM·cm</td>
</tr>
<tr>
<td>Target VSM activation</td>
<td>$A_{\text{total}}$</td>
<td>dyn/cm</td>
</tr>
<tr>
<td>Current vessel wall tension</td>
<td>$T$</td>
<td>dyn/cm</td>
</tr>
<tr>
<td>Average segment intraluminal pressure</td>
<td>$P$</td>
<td>mmHg</td>
</tr>
<tr>
<td>Local wall-dependent metabolic signal</td>
<td>$S_{\text{loc}}$</td>
<td></td>
</tr>
<tr>
<td>Control diameter</td>
<td>$D_c$</td>
<td>μm</td>
</tr>
<tr>
<td>Control tension</td>
<td>$T_c$</td>
<td>dyn/cm</td>
</tr>
</tbody>
</table>

Table 3.2. Model variables.
Chapter 4

Distinct roles of red-blood-cell-derived and wall-derived mechanisms in metabolic regulation of blood flow

4.1 Introduction

In order to properly match the wide range of oxygen demand in working skeletal muscle, microvessel networks must be able to acutely regulate blood flow. In the microcirculation, this regulation is achieved via the local control of arteriolar smooth muscle tone [87]. Vascular smooth muscle (VSM) cells surrounding the arterioles contract and relax in response to various hemodynamic and metabolic stimuli. Increases in intraluminal blood pressure result in the constriction of arterioles (myogenic response), whereas increases in luminal wall shear stress result in arteriolar dilation (shear-dependent response) [20]. Low levels of oxygen in microvascular networks cause arterioles to dilate both locally and upstream of the hypoxic site [5, 15, 88], a process denoted here as the metabolic response. The mechanisms for the metabolic response, however, have not yet been fully elucidated.

Multiple mechanisms have been proposed, both as the result of experiments and theoretical simulations. Ellsworth et al. [22, 23] have demonstrated that red blood cells (RBCs) release adenosine triphosphate (ATP) at a rate inversely related to their oxyhemoglobin saturation level. The ATP released diffuses to the vessel wall, and binds to P2Y purinergic receptors on the endothelial cells, initiating a conducted response that propagates upstream to the ascending arterioles. This results in the local and upstream release of nitric oxide (NO), causing the arterioles to dilate. This mechanisms is henceforth referred to as RBC signaling. It is also known that in response to a decrease in local PO2, the vessel wall itself can release vasoactive metabolites,
such as NO [17, 35, 67], adenosine [61], prostaglandins [9, 63], and endothelium-derived hyperpolarizing factor (EDHF) [21]. This mechanism will be denoted as wall signaling.

Previous theoretical models have addressed the effectiveness of RBC signaling for metabolic blood flow regulation in heterogeneous microvascular networks. On the longer timescale of structural adaptation, the models of Reglin et al. suggested that metabolic flow regulation via a RBC signal gave unrealistic distributions of blood flow velocities and shrinkage of vessels [81]. With regard to acute flow regulation, Roy et al. compared the effectiveness of RBC signaling with an RBC-independent metabolic signaling mechanism in a heterogeneous network [82]. Their results implied that metabolic flow regulation by solely a RBC-derived mechanism created an instability caused by the unequal hematocrit partitioning at vessel bifurcations (see Chapter 3). That is, low-flow channels which also received low hematocrit were unable to generate a sufficiently large signal to communicate to upstream arterioles the downstream tissue needs. These unrealistic behaviors demonstrated in the models imply that in a heterogeneous microvascular network, metabolic regulation by a solely RBC-derived signaling mechanism would not be effective.

All of these previous models have considered metabolic flow regulation by either RBC or wall signaling; however, none have assessed the roles of combinations of these two modes. As experiments have demonstrated the existence of the RBC signaling pathway, as well as the release of vasoactive substances from the vessel wall, it is apparent that multiple mechanisms are working together to acutely coordinate local changes in vascular tone. Different mechanisms may act in a complementary way, e.g., by responding to changes in oxygen level in different ranges, or acting at different structural levels of organization.

The goal of this study is to examine the effectiveness of combinations of metabolic regulation mechanisms in a heterogeneous microvascular network under a range of metabolic conditions, and to investigate whether a combination of mechanisms can
be more effective than each mechanism individually. Specifically, the objective is to test the hypothesis that even though the wall signal acting alone is effective in avoiding hypoxia within vessels and preventing the aforementioned signaling instability, a combination of RBC and wall signaling better maintains vessel PO$_2$ levels high enough to provide a sufficient gradient for diffusion of oxygen into tissue. First, RBC-dependent and wall-dependent mechanisms are tested individually in response to a decrease in oxygen delivery, simulated by systemic hemodilution throughout the network. Then, the effectiveness of combinations of the RBC and wall signals in responding to changes in oxygen demand is analyzed.

4.2 Methods

4.2.1 Network

The network used in the first set of simulations (hemodilution) is derived from the rat mesentery [79], as in [82]. Similarly to the method from Chapter 3, the heterogeneity of network structure was represented by replacing the small arterioles, capillaries, and small venules in the representative segment model [1, 10] by a realistic network structure, shown in Figure 4.1. This mesenteric network contained 546 segments. As in Chapter 3, to complete the flow pathway through the network, segments were added upstream (A and LA) and downstream (V and LV) of the network, one of which was capable of active flow regulation, bringing the total number of segments to 550 [82] (Figure 4.2). The network used in the second set of simulations (combinations of RBC and wall signals) is the same as in Chapter 3, derived from the hamster cremaster muscle [4] (see Figures 3.1 and 3.2).
Figure 4.1. Network used in first set of simulations. Arterioles (red), capillaries (green), and venules (blue) are derived from observations of the rat mesentery [82].
4.2.2 Network hemodynamics

As in Chapter 3, the microvessel network is represented as a set of interconnected segments, each with a defined diameter and length. Assuming pressure-driven flow and conservation of mass at each node, the apparent viscosity, flow rate, hematocrit, and wall shear stress are calculated in each segment using an iterative scheme. In the mesenteric network, other inflow rates are specified based on experimental measurements [79].

In the second set of simulations, vessels are considered flowing if they have RBC flux above 12.5 cells/sec (see Chapter 3).

4.2.3 Flow regulation model

The model for flow regulation is as described in Chapter 3. Parameter values were again chosen to match those used previously [1, 10, 82]. The maximally active peak tension in each vessel, $C_{act}$, was chosen to be a linear function of $D_0$ (the passive diameter at an intraluminal pressure of 100 mmHg) [82] in the first set of simulations in the mesentery; in the second set of simulations in the cremaster muscle, $C_{act}$ was
fit to a power law function of $D_0$, as in Chapter 3.

### 4.2.4 Oxygen transport

Oxygen exchange between vessels and tissue is modeled using the Green’s function method [86], described previously (see Chapters 1 and 3). Michaelis-Menten oxygen utilization kinetics are assumed in the tissue region, with a Michaelis constant of 1 mmHg [86].

As in Chapter 3, the added artery (A) and large arteriole (LA) vessels are assumed to be surrounded by a fixed-width tissue sleeve, in which oxygen is consumed at a fixed rate [1]. In both sets of simulations, oxygen exchange by the added venous vessels is neglected. Oxyhemoglobin saturation is calculated as a function of vessel PO$_2$ using the Hill equation. In the first set of simulations, the parameters used are $n = 3$ and $P_{50} = 38$ mmHg, based on estimates in rat blood [34]; in the second set, $n = 2.55$ and $P_{50} = 26$ mmHg, based on estimates in the hamster cremaster muscle [47]. In both sets of simulations, a PO$_2$ of 100 mmHg is assumed at the arterial inflow to the network. In the skeletal muscle network, venular inflow PO$_2$ values of 20 mmHg are assumed, based on estimates of venular saturation in hamster cremaster [25].

In the first set of simulations, inflow hematocrit is altered from 0.4 to 0.2, simulating reduced oxygen delivery by systemic hemodilution. In the second set, oxygen demand ($M_0$) is varied between 1 and 8 cm$^3$ O$_2$/100 cm$^3$/min, corresponding to a range of metabolic conditions.

### 4.2.5 Conducted metabolic signals

In the present study, the metabolic signals involved in local blood flow regulation are assumed to be a combination of RBC-derived and wall-derived mechanisms, since these mechanisms have been previously implicated individually as contributors to metabolic regulation of vascular tone [23, 61, 82]. As in Chapter 3, the signals are
assumed to be conducted upstream to feeding arterioles, resulting in local and proximal control of arteriolar tone [88]. In each vessel segment, the local metabolic signal, $S_{loc}$, is defined as

$$S_{loc} = \lambda C_{meta}^{RBC} S_{meta}^{RBC} + (1 - \lambda) C_{meta}^{wall} S_{meta}^{wall}, \quad (4.2.1)$$

where $\lambda$ is the weight given to each mechanism, where $0 \leq \lambda \leq 1$, with $\lambda = 0$ corresponding to solely wall signal and $\lambda = 1$ corresponding to solely RBC signal. The parameters $C_{meta}^{RBC}$ and $C_{meta}^{wall}$ are scaling factors for each of the signals. The RBC-derived signal, $S_{meta}^{RBC}$, is the average ATP concentration in the vessel [1]. As in [82], the wall signal is defined as

$$S_{meta}^{wall} = C_{ATP}^{0} \frac{P_0}{P_0 + P}, \quad (4.2.2)$$

where $C_{ATP}^{0}$ represents the arterial inflow ATP concentration and is a scaling factor for the wall signal term, $P_0$ is the vessel PO$_2$ at which the the wall signal is half maximal (here, $P_0 = 1$ mmHg, based on the Michaelis constant for tissue oxygen consumption [86]), and $P$ is the vessel wall PO$_2$. In the first set of simulations, $\lambda$ is set to either 0 (all wall signal) or 1 (all RBC signal); in the second set, $\lambda$ is varied between 0 and 1.

As in Chapter 3, the signal is propagated upstream with exponential decay. As in [82], the signal behaves like a current, such that in the upstream direction, the conducted signal is partitioned in proportion to vessel circumference at diverging bifurcations and summed at converging bifurcations.
4.2.6 Arteriolar dynamics

The dynamics of arteriolar diameter and activation are as described in Chapter 3, with

\[
\frac{dD}{dt} = \frac{1}{\tau_d} \frac{D_c}{T_c} (T - T_{total}) \tag{4.2.3}
\]

\[
\frac{dA}{dt} = \frac{1}{\tau_a} (A_{total} - A) \tag{4.2.4}
\]

Simulations are carried out over \( t = 0 \) to 200 seconds, and results are reported as averages over \( t = 100 \) to 200 seconds.

4.2.7 Reference state

As in Chapter 3, a reference state is defined for the network with a moderate level of arteriolar tone, corresponding to a relatively low level of oxygen consumption. In the first set of simulations, this state is established by setting \( S_{tone} \) to 0 in each regulating vessels (so that activation level is set to 0.5), and calculating passive vessel diameters \( (D_0) \) so that \( T = T_{total} \) (and thus, \( \frac{dD}{dt} = 0 \)). The method for establishing the reference state in the cremaster network is described in Chapter 3. In both sets of simulations, the arterial inflow and venous outflow pressures are set to 100 and 12.91 mmHg, respectively [82].

4.3 Results

Figures 4.3 and 4.4 come from the first set of simulations in the mesentery microvessel network. Figure 4.5 comes from the second set of simulations in the cremaster muscle microvessel network.

The effects on predicted tissue oxygenation of metabolic flow regulation by solely RBC-derived and wall-derived signals are illustrated in Figure 4.3. PO\textsubscript{2} distributions
are shown in the control state, before (Figure 4.3A) and after (Figure 4.3B) hemodilution (where inflow hematocrit is lowered from 0.4 to 0.2), as well as after hemodilution and metabolic regulation by RBC-derived (Figure 4.3C) and wall-derived (Figure 4.3D) signals. After hemodilution, there is an initial decline in tissue PO$_2$ network-wide. After regulation by a RBC-derived metabolic signal, the distribution of PO$_2$ is shifted significantly toward low levels, with large regions of widespread hypoxia. After regulation by a wall-derived metabolic signal, PO$_2$ levels are significantly higher than with the RBC signal.

The effects on predicted inflow RBC flux and hypoxic tissue fraction of metabolic flow regulation by solely RBC-derived and wall-derived signals are illustrated in Figure 4.4. Figure 4.4A shows the RBC flux in the inflow arteriole to the network. With the wall-derived metabolic regulation mechanism, the RBC flux is about 110 nL/min, whereas it is only about 55 nL/min with the RBC-derived metabolic regulation mechanism. Figure 4.4B shows the time series of this RBC flux data. Although the inflow RBC fluxes initially start out at the same value, with the RBC metabolic regulation signal, over time the inflow RBC flux decreases to a level significantly lower than with the wall signal. Figure 4.4C shows the tissue hypoxic fraction (defined as PO$_2 < 1$ mmHg). With the wall-derived signal, it is less than 15%; with the RBC-derived signal, it is more than 40%. Figure 4.4D shows the time series of this tissue hypoxic fraction data. Although the tissue hypoxic fractions initially start out at the same value, with the RBC metabolic regulation signal, over time the tissue hypoxic fraction increases to a level significantly higher than with the wall signal.

In Figure 4.5, variables reflecting blood flow and tissue oxygenation are plotted as functions of $\lambda$, the fraction of the metabolic signal that is RBC-derived, for three cases of tissue oxygen demand: $M_0 = 2, 4,$ and $8$ cm$^3$ O$_2$/100 cm$^3$/min. Results in this figure come from simulations using the hamster cremaster muscle network, as in Chapter 3. Figure 4.5A shows total blood flow to the network. In all three oxygen demand cases, the total inflow rate decreases as $\lambda$ is increased. A metabolic signal
Figure 4.3. Contour plots of tissue PO\textsubscript{2} distribution with $M_0 = 1$ cm\textsuperscript{3} O\textsubscript{2}/100 cm\textsuperscript{3}/min. Oxygen levels in the vessels and in the surrounding tissue are color-coded according to the scale at right (in mmHg). (A) Inflow $H_D = 0.4$, $t = 0$ s. (B) Inflow $H_D = 0.2$, $t = 0$ s. (C) Inflow $H_D = 0.2$, $t = 200$ s, with RBC-derived metabolic regulation signal. (D) Inflow $H_D = 0.2$, $t = 200$ s, with wall-derived metabolic regulation signal.
Figure 4.4. Histograms (left panels) and time series (right panels) of inflow arteriole RBC flux and tissue hypoxic fraction (defined as $\text{PO}_2 < 1 \text{ mmHg}$). (A) Inflow RBC flux initially, and after hemodilution and regulation by RBC and wall signals. (B) Time series of inflow RBC flux from $t = 0$ to $t = 200$ s, with regulation by RBC and wall signals. (C) Tissue hypoxic fraction initially, and after hemodilution and regulation by RBC and wall signals. (D) Time series of tissue hypoxic fraction from $t = 0$ to $t = 200$ s, with regulation by RBC and wall signals.
derived completely from the vessel wall is predicted to give the largest total inflow to the network, whereas a metabolic signal derived completely from RBCs is predicted to give the smallest total inflow, independent of tissue oxygen demand. The largest difference in inflow rate between RBC-derived and wall-derived metabolic signals occurs with $M_0 = 2$; the smallest difference occurs with the highest oxygen demand, $M_0 = 8$. Figure 4.5B shows the number of flowing arterioles and capillaries. In all three oxygen demand cases, the number of flowing vessels declines as the fraction of the metabolic signal derived from RBCs increases. Increasing oxygen demand from 2 to 4 cm$^3$ O$_2$/100 cm$^3$/min has little effect on the number of flowing vessels; however, increasing $M_0$ to 8 cm$^3$ O$_2$/100 cm$^3$/min results in a significantly larger number of flowing vessels, for all values of $\lambda$.

Figure 4.5C shows the hypoxic fraction (fraction of tissue with PO$_2$ < 1 mmHg). For oxygen demand of $M_0 = 2$ cm$^3$ O$_2$/100 cm$^3$/min, there is less than 1% hypoxia for all $\lambda$. However, for $M_0 = 4$, the hypoxic fraction increases monotonically from 0.64% with solely a wall-derived metabolic signal to 4.9% with solely a RBC-derived signal. For $M_0 = 8$, the hypoxic fraction increases monotonically from 6.0% to 12.1% as $\lambda$ increases from 0 to 1. Thus, for $M_0 > 2$, tissue hypoxic fraction is significantly affected by the combination of RBC- and wall-derived metabolic signals. Median tissue PO$_2$ increases with decreasing oxygen demand, for all values of $\lambda$ (Figure 4.5D). Unlike Figures 4.5A and 4.5B, the results are not monotonically decreasing in $\lambda$. Median tissue PO$_2$ initially increases with increasing fraction of the metabolic signal that is RBC-derived, with the maximum value occurring at a nonzero value of $\lambda$. This supports the hypothesis that a combination of RBC- and wall-derived metabolic flow regulation mechanisms provides better tissue oxygenation than solely a wall-derived mechanism.
Figure 4.5. Variables describing network blood flow and tissue oxygenation, as functions of $\lambda$, the fraction of the metabolic signals that is RBC-derived. (A) Relative arteriolar inflow rate. (B) Relative number of flowing vessels. (C) Hypoxic tissue fraction (defined as $PO_2 < 1$ mmHg). (D) Median tissue $PO_2$. (•) Oxygen demand of $M_0 = 2$ cm$^3$ O$_2$/100 cm$^3$/min. (○) Oxygen demand of $M_0 = 4$ cm$^3$ O$_2$/100 cm$^3$/min. (▼) Oxygen demand of $M_0 = 8$ cm$^3$ O$_2$/100 cm$^3$/min.
4.4 Discussion

In most tissues, to properly match the wide range of oxygen demand with supply, metabolic blood flow regulation is required at the microvascular level. However, the mechanisms by which metabolic regulation is achieved remain incompletely understood. Experiments have demonstrated the presence of RBC-derived and wall-derived metabolic mechanisms in helping to match oxygen supply and demand. This study uses a theoretical model to compare the effectiveness of combinations of RBC- and wall-derived mechanisms in a heterogeneous microvascular network in response to changes in oxygen delivery and demand.

The results of the model support the conclusion of a previous study [82] that metabolic regulation by solely a RBC-derived signal does not result in adequate tissue oxygen supply. Using a heterogeneous network structure derived from the rat mesentery [79] and a more realistic oxygen transport model [86], the model in the present study predicts that a decrease in oxygen delivery (simulated by hemodilution) results in poor tissue oxygenation and a large hypoxic tissue fraction, when the metabolic signal is assumed to originate from RBCs (Figures 4.3C, 4.4C, and 4.4D). This results from an instability of flow regulation by this mechanism. First, the decrease in hematocrit (hemodilution) causes reduced ATP release and a reduced metabolic signal. Second, the heterogeneity of the network results in unequal RBC partitioning at vessel bifurcations, which causes a RBC flux decline in smaller vessels, further reducing the metabolic signal. As a result of these metabolic signal reductions, the upstream arteriole constricts, further reducing flow. Thus, as time goes on, RBC flux is reduced (Figures 4.4A and 4.4B), until many vessels have zero RBC flux. This ineffective behavior is avoided with the use of an RBC-independent metabolic signal. Metabolic regulation via a wall signal leads to better tissue oxygenation (Figure 4.3D), lowered hypoxia (Figures 4.4C and 4.4D), and increased RBC flux (Figures 4.4A and 4.4B), due to the dependence on the local PO$_2$ level – not RBCs. This
suggests that some mechanism not dependent on RBCs must contribute to metabolic flow regulation, as implied by recent experimental results [65].

The results of the model also support the hypothesis that although the wall signal performs best in avoiding hypoxia and the signaling instability, a combination of RBC- and wall-derived signaling better maintains higher tissue PO$_2$ levels. For all oxygen demands considered, the model predicts that increases in the fraction of the metabolic signal derived from RBCs (λ) decreases both the overall flow rate (Figure 4.5A) and the number of flowing vessels (Figure 4.5B), due to the instability generated by the RBC signaling mechanism. For oxygen demands greater than 2 cm$^3$ O$_2$/100 cm$^3$/min, hypoxic tissue fraction is predicted to increase with increasing λ (Figure 4.5C). By these measures, any contribution from RBC signaling is hindering metabolic flow regulation. However, median tissue PO$_2$ is predicted to be maximal for λ ≠ 0 (Figure 4.5D), indicating that a nonzero RBC signal maintains median tissue PO$_2$ at a higher level than with solely a wall-derived signal.

This behavior may be explained as follows. The steepest part of the oxyhemoglobin saturation curve (Figure 4.6) occurs at moderate PO$_2$ levels, where small changes in blood PO$_2$ can have a large effect on the loading and offloading of oxygen to the RBCs. At these PO$_2$ levels, hypoxia is rare, so the sensitivity of the wall signal is low; however, blood flow is still tightly regulated. Since the amount of ATP released with the RBC signal is a decreasing function of the oxyhemoglobin saturation, the RBC metabolic signaling mechanism is most sensitive to changes in PO$_2$ at moderate levels, near the P$_{50}$ value (the PO$_2$ at which the oxyhemoglobin is 50% saturated). Thus, it is reasonable that metabolic blood flow regulation can be positively affected by a nonzero RBC signal fraction in the absence of low PO$_2$. In this way, different combinations of metabolic signaling mechanisms may be playing a role in flow regulation under different metabolic conditions. Specifically, RBC signaling may be most sensitive at moderate PO$_2$ levels, and RBC-independent (wall) signaling may be most sensitive in the presence of hypoxia.
Figure 4.6. Oxyhemoglobin saturation curve, from [7].

In the present model, the signaling mechanism for metabolic regulation in response to changing oxygen levels is assumed to be generated in the RBCs and in the vessel wall, according to Equation (4.2.1). A third possible mechanism is the release of vasodilators, such as adenosine [6] and carbon dioxide [8], from the tissue cells at a rate that depends on surrounding \( \text{PO}_2 \) levels; these metabolites then diffuse to nearby vessels and affect VSM tone. A previous theoretical study [81] investigating structural adaptation concluded that a tissue-derived signal may play a small part in long-term diameter adjustment, but is likely more relevant in avoiding short-term hypoxia. Further simulations using the model framework in the present study are needed to assess the impact of including tissue-derived signaling as another mechanism for metabolic flow regulation.

In conclusion, the results of the simulations imply that even though metabolic flow regulation by solely a wall-derived signal better avoids hypoxia and signaling instabilities, the addition of a RBC-derived metabolic flow regulation mechanism better maintains high median tissue \( \text{PO}_2 \) levels in a heterogeneous microvascular
network structure.
Chapter 5

Conclusions

Proper distribution of blood flow in the microcirculation is necessary to match changing oxygen demands in various tissues. This regulation of flow is achieved locally via changes in vascular resistance through alterations in tone of the vascular smooth muscle surrounding the arterioles and small arteries. How this coordination of perfusion and consumption occurs remains incompletely understood. Experiments have provided evidence of hemodynamic and metabolic stimuli that elicit changes in arteriolar diameter, but how these work together in large networks of microvessels is an unanswered question. Theoretical models can help bridge this knowledge gap by simulating a range of conditions that is difficult to obtain experimentally. Previous models have addressed blood flow regulation at the level of the microcirculation, but none have considered combinations of metabolic regulation mechanisms, nor have any explicitly taken into account the structural heterogeneity of an actual skeletal muscle microvessel network. In this study, theoretical models were used to answer the following questions concerning the regulation of blood flow at the microvascular level:

5.1 Questions addressed

5.1.1 How can blood flow rates be estimated in large microvascular networks when only a small fraction of the boundary flows or pressures are known?

Recent methods for imaging microvascular structures provide geometrical data on networks containing thousands of segments \([3, 12, 30, 31, 37, 43, 62, 89]\). Prediction of functional properties, such as solute transport, requires information on blood flow rates also, but experimental measurement of many individual flows is difficult. In
Chapter 2, a method was presented for estimating flow rates in a microvascular network based on incomplete information on the flows in the boundary segments that feed and drain the network [27]. With incomplete boundary data, the equations governing blood flow form an underdetermined linear system. An algorithm was developed that uses independent information about the distribution of wall shear stresses and pressures in microvessels to resolve this indeterminacy, by minimizing the deviation of pressures and wall shear stresses from target values. The algorithm was tested using previously obtained experimental flow data from four microvascular networks in the rat mesentery. With two or three prescribed boundary conditions, predicted flows showed relatively small errors in most segments and fewer than 10\% incorrect flow directions on average. The proposed method can be used to estimate flow rates in microvascular networks, based on incomplete boundary data, and provides a basis for deducing functional properties of microvessel networks.

5.1.2 What is the mechanism of capillary recruitment?

The theoretical model for microvascular blood flow regulation in Chapter 3 is based on the modulation of arteriolar diameters in response to metabolic and hemodynamic stimuli. A heterogenous microvessel network structure was derived from a hamster cremaster muscle preparation [4], in which the numbers of flowing vessels (based on RBC flux) were recorded in a control and in a maximally dilated state. From this experimental data, a network was created that had non-flowing vessels in a reference state with a low level of tissue oxygen demand. Model simulations predicted an increase in the number of flowing vessels (i.e., capillary recruitment) as oxygen demand was increased from the reference state. These results suggest that capillary recruitment can occur as a consequence of local changes in arteriolar tone. Further, simulation results predict that the unequal partitioning of RBCs at vessel bifurcations can enhance the capacity of a microvascular network for recruitment.
5.1.3 What combinations of wall-derived and RBC-derived mechanisms are most effective in the metabolic regulation of microvascular blood flow?

Different combinations of RBC-dependent and wall-dependent (RBC-independent) mechanisms were compared in Chapter 4 to determine the roles of each the metabolic regulation of blood flow. The RBC-derived signal is assumed to be dependent on the amount of ATP released by the RBCs into the bloodstream in response to changes in oxyhemoglobin saturation. The wall-derived signal is assumed to be generated at the vessel wall, inversely related to the local vessel PO$_2$ level. Both signals are assumed to be generated locally, as well as propagated upstream with exponential decay. Model results confirm previous experimental [65] and theoretical [82] studies that solely a RBC-derived signal is insufficient to adequate regulation blood flow, due to an instability in the mechanism caused by the unequal partitioning of RBCs at vessel bifurcations. The model predicts that, for a range of tissue oxygen demands, metabolic regulation by solely a wall-derived mechanism results in a larger total blood flow rate to the network, a larger number of flowing vessels, and a smaller hypoxic tissue fraction, in comparison with the cases when a nonzero fraction of the metabolic signal is RBC-derived. However, simulations predict that a nonzero fraction of RBC-derived signal results in a larger median tissue PO$_2$, for all oxygen demands considered. This suggests that there may be distinct roles for RBC-derived and wall-derived metabolic regulation mechanisms. Namely, the wall-derived mechanism may be most effective in preventing hypoxia, whereas the RBC-derived mechanism may play an important role in maintaining PO$_2$ levels high enough for an adequate gradient for diffusion for moderate levels of blood oxygen.
5.2 Future directions

The modeling framework discussed in the present study can be adapted to answer other questions regarding the regulation of microvascular blood flow.

5.2.1 Estimation of blood flow rates in coronary networks

The method outlined in Chapter 2 can be used to estimate blood flow rates in any microvascular network. It is particularly suited to large networks with high resolution, where many small vessels have been structurally imaged. There has been recent work imaging these types of microvessel networks in the heart [52], ranging in size from one thousand to one hundred thousand vessel segments. Prediction of coronary microvascular perfusion at the individual vessel level using this method could help with understanding and developing treatments for cardiac ischemia, a condition in which the heart receives insufficient blood flow.

5.2.2 Tissue signaling

The mechanisms for metabolic blood flow regulation considered in Chapter 4 are assumed to be generated either by the RBCs or by the vessel wall. A third possible mechanism is the release of vasodilators, such as adenosine [6] and carbon dioxide [8], from the tissue cells in relation to surrounding PO\textsubscript{2} levels; these metabolites then diffuse to nearby vessels and affect VSM tone. A previous theoretical study [81] investigating structural adaptation concluded that a tissue-derived signal may play a small part in long-term diameter adjustment, but is likely more relevant in avoiding short-term hypoxia. Further simulations using the model framework in the present study are needed to assess the impact of including tissue-derived signaling as another mechanism for metabolic flow regulation.
5.2.3 Renal blood flow regulation

It is especially important to have appropriately distributed flow in the kidneys, which filter the majority of the circulating blood and regulate the body’s electrolyte and acid-base balance. Many previous studies have focused on the kidney’s ability to maintain relatively constant blood flow over a wide range of pressures [58, 60] – a process called autoregulation; however, a more integrative model incorporating many known mechanisms of renal blood flow regulation is lacking. The model framework in the present study, which accounts for the roles of shear-dependent, myogenic, and metabolic mechanisms in the regulation of microvascular blood flow, could be modified to include the kidney-specific mechanism called tubulo-glomerular feedback (TGF). TGF is involved in the mediation of autoregulation, and is a negative feedback loop in which changes in tubular fluid electrolyte concentrations induce a signal that acts upon the feeding arteriole. Vessel diameters in the network would be affected by the TGF response, which would in turn affect pressure drops and thus flow and filtration rates. As in [51], the TGF feedback loop could be represented by a delay equation that is a saturating function of the tubular chloride concentration along the macula densa. Regulation of renal microvascular blood flow and solute transport is crucial for the body to maintain homeostasis. This extension of the current model has implications for how various forms of progressive kidney disease and septic shock are treated, as the (mal)distribution of flow at the microcirculatory level can impact the effectiveness of autoregulation, despite normal or increased larger-scale blood flow rates [38]. As the vast majority of cardiac output is filtered by the kidneys, it is essential to understand the regulatory mechanisms of this vital organ.

The theoretical model framework outlined in the present study can be used to help understand processes governing the regulation of blood flow in various tissues, including the impact of structural and functional heterogeneities present in microvascular networks.
Appendices
Capillary (vessel) recruitment - The increase in the number of vessels which were not flowing at rest that become flowing in response to an increase in metabolic demand

$H_D$ - Hematocrit, the fraction of the blood volume occupied by red blood cells

Oxyhemoglobin saturation - The fraction of hemoglobin binding sites occupied by an oxygen molecule

$PO_2$ - Partial pressure of oxygen, which is proportional to the concentration of dissolved oxygen

$RBC$ - Red blood cell

VSM - Vascular smooth muscle, which surrounds the arterioles
REFERENCES


