Published in final edited form as:

J Cardiovasc Transl Res. 2017 February ; 10(1): 69–81. doi:10.1007/s12265-017-9732-6.

Impaired Tissue Oxygenation in Metabolic Syndrome Requires Increased Microvascular Perfusion Heterogeneity

P. Mason McClatchey^{1,2,3}, Fan Wu⁴, I. Mark Olfert⁵, Christopher G. Ellis⁶, Daniel Goldman⁶, Jane E. B. Reusch^{2,4}, and Jefferson C. Frisbee⁶

¹Division of Endocrinology, University of Colorado Anschutz, Medical Campus, Aurora, CO, USA

²Department of Bioengineering, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

³Department of Medicine, Denver Veterans Affairs Medical Center, Denver, CO, USA

⁴Drug Metabolism and Pharmacokinetics, Novartis Institutes for BioMedical Research, East Hanover, NJ, USA

⁵Division of Exercise Physiology, West Virginia University Health Sciences Center, Morgantown, WV, USA

⁶Department of Medical Biophysics, Schulich School of Medicine and Dentistry, University of Western Ontario, Medical Sciences Building, M407, 1151 Richmond Street North, London, ON N6A 5C1, Canada

Abstract

Metabolic syndrome (MS) in obese Zucker rats (OZR) is associated with impaired skeletal muscle performance and blunted hyperemia. Studies suggest that reduced O₂ diffusion capacity is required to explain compromised muscle performance and that heterogeneous microvascular perfusion distribution is critical. We modeled tissue oxygenation during muscle contraction in control and OZR skeletal muscle using physiologically realistic relationships. Using a network model of Krogh cylinders with increasing perfusion asymmetry and increased plasma skimming, we predict increased perfusion heterogeneity and decreased muscle oxygenation in OZR, with partial recovery following therapy. Notably, increasing O₂ delivery had less impact on VO₂ than equivalent decreases in O₂ delivery, providing a mechanism for previous empirical work associating perfusion heterogeneity and impaired O₂ extraction. We demonstrate that increased skeletal muscle perfusion asymmetry is a defining characteristic of MS and must be considered to effectively model and understand blood-tissue O₂ exchange in this model of human disease.

Correspondence to: Jefferson C. Frisbee.

Author Contributions PMM, FW, and JCF researched data. PMM, FW, and JCF wrote manuscript. All reviewed/edited manuscript. All contributed to discussion.

Compliance with Ethical Standards

Conflict of Interest JCF has received research grants from American Heart Association (US) and National Institutes of Health (US). JEHR has received research grants from the National Institutes of Health (US) and the Veterans Affairs Administration (US). PMM, IMO, FW, CGE, and DG declare that they have no conflict of interest.

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Keywords

Microcirculation; Oxygenation; Rodent models of obesity; Blood flow control; Simulation

Introduction

The growing incidence and prevalence of the metabolic syndrome and associated type 2 diabetes mellitus represent one of the greatest challenges to public health facing developed economies worldwide [1, 2]. The metabolic syndrome is generally defined as the combined presentation of obesity, impaired glycemic control, atherogenic dyslipidemia, and moderate hypertension, with the additional contributing conditions of pro-oxidant, pro-thrombotic, and pro-inflammatory phenotypes [3–5]. This clinical condition is present in an enormous and growing number of afflicted people worldwide, it has a powerful influence on reducing patient quality of life and life expectancy, and it causes enormous increases to the direct and indirect economic costs that must be borne by society as a result of its negative effects on health outcomes [1, 2]. These considerations mandate detailed investigation into this multipathology state.

In the general population [6] and in specific elements of the metabolic syndrome such as type 2 diabetes mellitus (T2DM; ref. 7), aerobic exercise capacity as measured by maximal oxygen consumption (VO_{2max}) is the strongest clinical predictor of mortality. Further, in both T2DM and metabolic syndrome, exercise capacity has been demonstrated to be reduced [8–10]. While the precise mechanisms underlying impaired exercise capacity in these states remain unclear, there are distinctive mechanistic hints in the existing literature. In both human and animal studies of these states, hyperemic responses to exercising muscle are reduced with manifestation of the metabolic syndrome [11, 12], yet oxygen extraction not only can fail to increase to compensate for the ischemia but can be reduced itself [11, 13]. Given that mitochondrial capacity exceeds the oxygen delivering capacity of the vasculature [14], the coincidence of impaired blood flow and impaired oxygen extraction cannot be explained through reduced mitochondrial demand or function. Understanding these apparent phenotypic contradictions requires detailed mechanistic study of O_2 transport and consumption comparing theoretical predictions to empirical data.

The obese Zucker rat (OZR; *fa/fa*) represents an excellent animal model of the metabolic syndrome with high utility in terms of comparing cardiovascular (dys)function to pathology in human subjects. OZR develops the metabolic syndrome due to chronic hyperphagia secondary to profound leptin resistance and rapidly develops the systemic phenotypes listed above to comparable levels of severity with those identified in human subjects. Also, similar to health outcomes in humans, OZR exhibiting the metabolic syndrome suffers from a progressive vasculopathy that ultimately develops into overt peripheral vascular disease (PVD; ref. 15), albeit one without the development of significant atherosclerotic lesions within macrovessels.

Recent experimental studies have revealed significant impairments to the fatigue resistance (i.e., the ability to maintain contractile performance over time) of in situ skeletal muscle of OZR as compared to that in lean Zucker rats (LZR) [11, 16]. The extent of these

impairments has been generally correlated with reductions in bulk blood flow and hyperemic responses to the elevated metabolic demand [11]. However, the relationship between fatigue and reduced blood flow is not particularly robust, and there is potential that factors beyond simple bulk perfusion could be responsible for the functional manifestation of PVD in metabolic syndrome.

We have previously published observations of reduced blood flow (Fig. 1a), reduced oxygen extraction (Fig. 1b), increased muscle fatigue associated with reduced diffusion capacity (Fig. 1c), and increased perfusion heterogeneity (Fig. 1e) in the OZR [11, 17–19]. Perfusion heterogeneity was quantified using the parameter γ (Fig. 1d), defined as the fraction of total blood flow diverted to the higher flow daughter vessel at a microvascular bifurcation. Pharmacological intervention (e.g., TEMPOL; 1-oxyl-2,2,6,6-tetramethyl-4-hydroxypiperidine, a cell permeable superoxide dismutase mimetic which acts as a powerful antioxidant) correcting this perfusion heterogeneity in the smallest arterioles (3a-5a) normalizes oxygen extraction, and intervention correcting both bulk blood flow and perfusion heterogeneity (using a combined treatment of TEMPOL, the α_1/α_2 adrenoceptor antagonist phentolamine and the $\text{TxA}_2/\text{PGH}_2$ receptor blocker SQ-29548) fully restores oxygen uptake in the OZR to LZR levels [11, 17, 18]. Therefore, it is of interest to be able to quantitatively test the hypothesis that oxygen supply from blood to skeletal muscle is a major limiting factor for muscle contractile performance under conditions of elevated metabolic demand within OZR and, if observed, to define factors limiting oxygen delivery.

In this work, a computational model of oxygen transport within skeletal muscle was constructed based on the classical Krogh cylinder-type model [20], which has been further extended to account for capillary wall transport barrier, myoglobin-facilitated diffusion, and Michaelis-Menten kinetics of oxygen consumption [21]. The constructed computational model was then applied to analyze oxygenation levels and metabolic rates using experimental data on bulk tissue blood flow and blood oxygen tensions measured from in situ blood-perfused contracting skeletal muscle of LZR and OZR and reported in a previous publication [11]. The predictions of this model were compared to previously published empirical observations to determine the effects of perfusion heterogeneity in an idealized microvascular network taking into account phase separation (i.e., erythrocytes from plasma) effects at bifurcations [22].

Materials and Methods

Microvascular Network Model

We have previously reported increased microvascular blood flow heterogeneity in OZR compared with that in LZR [17–19]. To test the hypothesis that the associated mismatch between oxygen supply and working capacity [11] is caused by the flow heterogeneity, an idealized microvascular network (shown in Fig. 2a) was applied to examine impacts of flow heterogeneity on oxygen transport on the microvascular level. Empirical data from a previous publication [11] was used for this analysis.

The idealized microvascular network employed in this analysis was based on Murray's cube law [23] and assumed a fully symmetric vascular tree (i.e., all vessels of the same generation

are the same length and diameter). The resulting vascular tree is consistent with the L-system fractal formalism of Zamir [24], has recently been applied to assess the impact of the endothelial glycocalyx on microvascular blood flow distribution [25], and has been extensively validated as a reasonable approximation of microvascular branching architecture in vivo [26]. The idealized microvascular network included in this analysis branched from a 192- μm parent vessel to 6 μm diameter capillaries over the course of 15 vessel generations.

At each microvascular bifurcation, the uneven distribution of blood flow into the daughter branches results in heterogeneity of downstream discharge hematocrits as described by the following equation [22]

$$\gamma_E = \frac{1}{1 + \exp(0.018 + 1.22 \cdot \text{logit}(0.5 - 1.042 \cdot (0.5 - \gamma_B)))}, \quad (1)$$

where logit function is defined as

$$\text{logit}(x) = \ln\left(\frac{x}{1-x}\right), \quad (2)$$

The ratios of bulk blood volume flow and erythrocyte volume flow are defined as

$$\gamma_B = \frac{F_{B,1}}{F_B}, \quad (3)$$

$$\gamma_E = \frac{\gamma_{B,1} \cdot \text{Hct}_1}{\gamma_B \cdot \text{Hct}}, \quad (4)$$

respectively, with subscript 1 denoting daughter branch 1.

During the simulations, the ratio of bulk blood volume flow (γ_B) was varied from 0.5 to 0.7 (using notation consistent with our previously published perfusion heterogeneity analyses), and the discharge hematocrits in the daughter branches were calculated using Eqs. 2–4 for all vessel generations. In this work, arteriolar oxygen delivery is neglected; thus, inlet PO_2 to each capillary is assumed to be equal to arterial PO_2 , a reasonable assumption under conditions of high flow and high oxygen demand. Then, the oxygen transport was calculated for each capillary using lookup table of C_{TV} values simulated from a range of representative discharge Hct and F values for LZR or OZR (see Fig. 2b). Mitochondrial demand (V_{max}) in all cases was adjusted to recreate whole-muscle VO_2 for LZR at each level of stimulation without perfusion heterogeneity, consistent with observations of minimal perfusion heterogeneity in the LZR [17–19].

Krogh Cylinder-Type Model

In the Krogh cylinder-type model, the tissue is assumed to consist of uniformly spaced cylinders, and each tissue cylinder is supplied by a capillary with perfusing blood flow within its volume. The oxygen carried by blood supports local metabolic demand through diffusion across the capillary wall and into the skeletal muscle cell. The geometry of the Krogh cylinder-type model is shown in Fig. 2b.

Governing Equations

The Krogh cylinder-type model is described by two ordinary differential equations for radial and axial oxygen transport, respectively [1, 25].

Oxygen movement in the radial direction—Based on Fick's law of diffusion and mass transport, the radial oxygen transport is described by

$$D \cdot \frac{1}{r} \frac{d}{dr} \left(r \frac{dC^*}{dr} \right) = VO_2, \quad (5)$$

where D denotes the molecular diffusivity of oxygen, C^* denotes the modified tissue oxygen concentration accounting for myoglobin-facilitated diffusion, and VO_2 denotes oxygen consumption rate in the tissue.

The modified tissue oxygen concentration C^* is defined as

$$C^* = C + \frac{D_{Mb}}{D} \cdot C_{Mb} \cdot V_m \cdot S_{Mb}, \quad (6)$$

where D_{Mb} denotes the molecular diffusivity of myoglobin, C_{Mb} denotes the tissue myoglobin concentration, V_m denotes the molecular volume of oxygen, and S_{Mb} denotes the myoglobin saturation level.

The myoglobin saturation level is calculated from

$$S_{Mb} = \frac{C}{C + C_{50,Mb}}, \quad (7)$$

where $C_{50,Mb}$ denotes half-maximal myoglobin saturation O_2 concentration.

The oxygen consumption rate (M) is assumed to follow Michaelis-Menten kinetics

$$M(r, z) = \frac{V_{\max} \cdot C}{C + K_M}, \quad (8)$$

where $V_{\max}$ denotes the maximal mitochondrial oxygen consumption rate and K_M denotes the Michaelis-Menten constant of oxygen consumption in the skeletal muscle.

Assuming that no oxygen exchange occurs across the outer boundary of the tissue cylinder (i.e., the outer boundary of the cylinder represents a local minimum of PO_2), a no-flux boundary condition is obtained

$$\left. \frac{dC^*}{dr} \right|_{r=R_2} = 0. \quad (9)$$

At the interface between capillary and tissue, an oxygen permeability barrier is assumed

$$D \cdot \left. \frac{dC^*}{dr} \right|_{r=R_2} = p_w \cdot \left(C_f - C(r) \right) \Big|_{r=R_1}, \quad (10)$$

where D denotes the molecular diffusivity of oxygen in tissue, p_w denotes cross-capillary wall permeability of oxygen, and R_2 denotes the outer radius of the tissue cylinder.

Oxygen movement in the axial direction—Since the axial oxygen gradient is much less than the radial oxygen gradient, the axial oxygen transport is assumed to be dominated by convection and is described as follows

$$\frac{dC_T(z)}{dz} = - \frac{VO_{2\text{avg}}(z)}{F \cdot \rho_{\text{tissue}} \cdot L}, \quad (11)$$

where C_T denotes the total oxygen concentration in blood, $VO_{2\text{avg}}$ denotes the oxygen consumption rate averaged along the radial direction, F denotes the flow volumetric rate normalized to tissue mass, ρ_{tissue} denotes the tissue mass density, and L denotes the capillary length.

The average oxygen consumption rate ($VO_{2\text{avg}}$) is defined as

$$\begin{aligned} VO_{2\text{avg}}(z) &= \frac{\int_{r=R_1}^{r=R_2} 2\pi r \cdot VO_2(r, z) \cdot dr}{\int_{r=R_1}^{r=R_2} 2\pi r \cdot dr} \\ &= \frac{\int_{r=R_1}^{r=R_2} r \cdot VO_2(r, z) \cdot dr}{(R_2^2 - R_1^2)/2} \end{aligned} \quad (12)$$

The total oxygen concentration (C_T) is defined as

$$C_T = C_f \cdot (1 - \text{Hct}) + C_{\text{RBC}} \cdot \text{Hct} + \text{Hct} \cdot C_{\text{Hb}} \cdot S_{\text{Hb}}, \quad (13)$$

where C_f denotes free oxygen concentration in plasma, C_{RBC} denotes free oxygen concentration in red blood cells (RBCs), Hct denotes discharge hematocrit, C_{Hb} denotes concentrations of oxygen binding sites in RBCs, and S_{Hb} denotes hemoglobinoxygen saturation level.

The hemoglobin saturation level is calculated from

$$S_{\text{Hb}} = \frac{\text{PO}_2^{n_H}}{\text{PO}_2^{n_H} + P_{50, \text{Hb}}^{n_H}}, \quad (14)$$

where $P_{50, \text{Hb}}$ denotes half-maximal hemoglobin saturation O_2 tension and n_H denotes the Hill coefficient for hemoglobinoxygen binding.

The free oxygen concentration in plasma and RBCs can be related to oxygen tension (PO_2) as

$$C_f = \text{PO}_2 \cdot \alpha_{\text{plasma}} \quad (15)$$

$$C_{\text{RBC}} = \text{PO}_2 \cdot \alpha_{\text{RBC}} \quad (16)$$

where α_{plasma} and α_{RBC} are oxygen solubility in plasma and RBCs, respectively.

Lookup Tables of Venous and Muscle Oxygenation

The Krogh cylinder-type single-capillary model discussed in the previous section was applied recursively at each level of muscle stimulation (changes *mitochondrial demand* and blood flow parameters), for each animal model (LZR vs OZR, changes capillary density and blood flow parameters), and for blood flow values ranging from zero to 200× average. These values were then compiled into lookup tables (LUTs) of venous and muscle oxygenation (see Figs. 3 and 4). These LUTs were combined with the network perfusion model discussed above to rapidly obtain an array of single-capillary values for venous oxygen content and mean muscle oxygenation. The oxygen concentration of the mixed venous effluent was then calculated as

$$C_{TV} = \frac{\sum Q_i \cdot C_{ti}}{\sum Q_i}, \quad (17)$$

Finally, mean muscle PmO_2 was calculated from the mean of all single-capillary PmO_2 values.

Sensitivity Analysis Methods

Microvascular perfusion heterogeneity results from a complex combination of microfluidic mechanical changes (e.g., glycocalyx degradation, capillary plugging by adherent leukocytes) and changes in vessel tone/diameter. Vessel diameter influences distribution of RBCs independently of flow distribution [22], and it is plausible that these effects, which were not considered in our muscle oxygenation simulations, are significant in vivo. To assess this possibility, we performed a sensitivity analysis on the simulated effects of diameter randomization independent of perfusion heterogeneity. The maximum degree of flow asymmetry considered in this manuscript ($\gamma = 0.7$) can be produced by a ~9% difference in downstream vessel diameters, assuming laminar flow. Accordingly, we simulated CvO_2 , PmO_2 , and VO_2 with up to 10% randomization of vessel diameter in each vessel segment. One hundred simulations at each degree of diameter randomization were performed, and the coefficient of variance ($\text{CV} = \text{SD}/\text{mean}$) of simulation results was used as a metric of the effect size of diameter randomization.

Model Parameters

The parameters used in this model are taken from either the present experimental results or from validated literature values and are summarized in Table 1.

The measured blood flow (F) and arterial oxygen tensions (P_aO_2) were used as inputs to the computational model to simulate venous oxygen tensions (P_vO_2). The model parameters were set up using values listed in Table 1, except that the oxygen consumption demand (VO_2) at rest and at 5 Hz were generated via model optimization by minimized differences between model-predicted and experimentally measured PvO_2 .

The Krogh cylinder-type model was implemented on a standard desktop personal computer using MATLAB (Version R2013b, MathWorks, Natick, MA). The optimization was performed using the `fmincon` function included in MATLAB Optimization Toolbox.

Results

Effects of Perfusion Heterogeneity on Oxygen Transport in Microvascular Networks

An idealized microvascular network (Fig. 2a) was used to examine the effects of microvascular flow heterogeneity on oxygen transport. The simulations were conducted by assuming that the mitochondrial demand (V_{max}) in LZR and OZR are equal and sufficient to produce measured values of VO_2 with homogeneous perfusion in the LZR. Empirical data from a previous publication [11] was used for this analysis. The results of this simulation are shown in Fig. 6. Model simulations of PvO_2 using varying flow distributions reveal that simulated PvO_2 matches measured PvO_2 at values of $\gamma = 0.6164$ for untreated OZR, $\gamma = 0.5562$ for TEMPOL-treated OZR, and at $\gamma = 0.5121$ for LZR at 5 Hz (Fig. 6a), indicating heterogeneous perfusion in the OZR that can be partially corrected by TEMPOL treatment. Using the model-predicted values of $\gamma = 0.5121$, 0.5562, and 0.6164 for LZR, TEMPOL-

treated OZR, and untreated OZR, respectively, our model predicts a mean muscle PmO_2 of 30.4 mmHg in the LZR, 18.9 mmHg in untreated OZR, and 23.48 mmHg in TEMPOL-treated OZR (Fig. 6b) at 5 Hz, indicating muscle hypoxia during exercise in the OZR that can be partially corrected by TEMPOL treatment. Our simulations also predict that the contributions of perfusion heterogeneity account for more than half of the total decrease in VO_2 in the OZR relative to LZR (Fig. 6c), a majority of which is due to perfusion heterogeneity in the small 3a-5a arterioles where TEMPOL most profoundly influences perfusion heterogeneity [17].

Results from Sensitivity Analysis—The results of our sensitivity analysis are shown in Fig. 5. Results were largely consistent among the results for CvO_2 (Fig. 5a), PmO_2 (Fig. 5b), and VO_2 (Fig. 5c). In all cases, the CV of the oxygenation metric of interest increases quadratically with increasing diameter randomization. The influence of diameter randomization independent of perfusion heterogeneity is several orders of magnitude smaller than that of perfusion heterogeneity independent of diameter randomization. These results suggest that our muscle oxygenation analysis with varying degrees of perfusion heterogeneity was negligibly influenced by our choice to neglect possible effects from vessel tone independent of blood flow and its distribution.

Mechanisms Underlying the Effects of Perfusion Heterogeneity

To determine the mechanism(s) by which microvascular perfusion heterogeneity would theoretically reduce oxygen diffusion capacity, we compared the effects of equivalent increases or decreases in flow or hematocrit on oxygen uptake in single capillaries (Fig. 7), using LZR 5 Hz values [11] in a single-cylinder version of our model for illustration purposes. Increasing capillary blood flow increases oxygen uptake, but not to the same degree that an equivalent decrease in capillary blood flow decreases oxygen uptake. The mean oxygen uptake of two capillaries with a fixed total blood flow supply thus decreases with increasing flow disparity between the two capillaries (Fig. 7a). Similarly, increasing capillary hematocrit increases oxygen uptake, but not to the same degree that an equivalent decrease in capillary hematocrit decreases oxygen uptake. The mean oxygen uptake of two capillaries with a fixed total RBC flux thus decreases with increasing hematocrit disparity between the two capillaries (Fig. 7b). Due to the plasma-skimming effect at microvascular bifurcations [22], capillary flow and discharge hematocrit are positively correlated (see Fig. 7c, uses simulated $\gamma = 0.7$ distribution for illustration purposes). The effects of flow and hematocrit disparities thus synergize to produce the cumulative effects of perfusion heterogeneity.

Discussion

It has been clearly demonstrated by our group [11, 16] and by others [32] that the development of the metabolic syndrome is associated with alterations to skeletal muscle arterial and arteriolar function. These changes are correlated with impaired hyperemic responses to elevated metabolic demand, constrained oxygen uptake (VO_2) across skeletal muscle, and impaired fatigue resistance of in situ blood perfused skeletal muscle. However, beyond a restricted number of studies, there has been much less effort dedicated to

determining how these key parameters relate to one another. The purpose of this study was to take the first steps into developing a computational model for microvascular perfusion under the conditions of the metabolic syndrome and to model, based on established literature values and original data from the OZR model of the metabolic syndrome, how microvascular function might be altered based on the underlying principles of mass transport and exchange. This is intended to be a first step in a larger effort to develop predictive models and biosimulations of sufficient veracity as to be useful for hypothesis development and informing experimental design. These models will be refined with the ultimate goal of application to clinical settings to inform a better understanding of conditions affecting microvascular function, cardiorespiratory fitness, and response to clinical intervention.

A major initial interpretation of the results from the present simulations is that the striking similarity determined in the heatmaps (Figs. 3 and 4) for oxygen transport and exchange between LZR and OZR is conceptually in contrast to all of the functional (i.e., muscle performance) data collected to date. If oxygen transport is minimally affected in between LZR and OZR using flow characteristics, then differences in perfusion and/or distribution are required to explain the discrepancy in outcomes. This highlights the importance of introducing perfusion heterogeneity into our simulations of microvascular perfusion in the OZR manifesting metabolic syndrome in order to more accurately understanding the system function in this challenged state.

Our present simulations show that perfusion heterogeneity is mathematically sufficient to account for our previously published observations of increased venous oxygenation (Fig. 6a) in the OZR and sufficient to explain why venous oxygenation is partially corrected by TEMPOL treatment [11]. Perfusion heterogeneity is also mathematically sufficient to account for literature reports of muscle hypoxia (Fig. 6b) in the OZR [33]. Our model predicts that this muscle hypoxia is partially relieved by TEMPOL treatment. Although this prediction has not yet been directly tested, it does provide a plausible explanation for partial recovery of oxygen uptake with TEMPOL treatment [11]. Finally, our model predicts that perfusion heterogeneity accounts for a majority of the oxygen uptake (Fig. 6c) defect in during 5 Hz contractions the OZR, consistent with experimental recovery of a majority of the oxygen uptake defect during 5 Hz contractions when treated with a drug cocktail that fully reverses microvascular perfusion heterogeneity [11, 17, 18]. The degree of perfusion heterogeneity required to mathematically account for oxygen transport differences between OZR and LZR in our simulation is not only qualitatively but also quantitatively similar to experimentally determined values of γ in LZR and OZR [17, 18]. Finally, our simulations provide a mechanism by which perfusion heterogeneity results in impaired oxygen transport (Fig. 7). Namely, an individual capillary's oxygen uptake is more sensitive to decreases in oxygen supply than to increases of similar magnitude. Similar to ventilation-perfusion mismatch in the lung, over-perfused vessels cannot fully compensate for under-perfused vessels.

Our simulations show that microvascular perfusion heterogeneity is mathematically *sufficient* to account for oxygen transport defects in the OZR not caused by reduced bulk blood flow. We have also previously published experiments showing that perfusion heterogeneity is empirically *necessary* to account for these same defects [17–19]. Given the

theoretical sufficiency and empirical necessity of perfusion heterogeneity to account for the OZR phenotype, it is very likely that (1) our model describes tissue-level oxygen transport to a reasonable degree of accuracy, and (2) perfusion heterogeneity is a key pathological feature of the metabolic syndrome.

In contrast with this manuscript, the bulk of the scientific literature discussing exercise capacity in the metabolic syndrome and T2DM focuses on the total mount blood flow delivered to exercising muscle. Given the previously demonstrated impairments to vascular/arteriolar function in the skeletal muscle of OZR [16], the progressive rarefaction of the microvascular networks [34] and the reduction to bulk perfusion to the skeletal muscle across metabolic intensities [11], impaired oxygen transport is not particularly surprising. However, combining our present model results with our previous findings of heterogeneous microvascular blood flow distribution using both direct microvascular visualization [16–18] and tracer washout kinetics [19], an alternate interpretation of literature data suggests that increased muscle fatigue in OZR may also reflect an increasingly heterogeneous distribution of perfusion within microvascular networks. Microvascular perfusion heterogeneity contributes to impaired muscle function by causing reduced O_2 extraction and an elevated PvO_2 . Our simulations and previously published experiments support the hypothesis that perfusion heterogeneity, along with the resulting phase separation of plasma from RBCs [22], results in failure to compensate for reduced blood flow in OZR with increased oxygen extraction.

Collectively, these echoes of previously published data in our simulations lend credibility to the notion that microvascular perfusion heterogeneity is necessary and sufficient to account for experimentally observed barriers to oxygen transport in OZR. Although there are assumptions within our model that require further refinement (e.g., neglecting axial O_2 diffusion, neglecting heterogeneous non-Kroghian capillary morphology, neglecting microscopic variances in blood O_2 content), the broad consistency between our computational results, our previously published experimental results, and the broader published literature suggests that our simulations were sufficient to predict observed oxygen transport phenotypes from first principles. These findings suggest that clinical strategies aiming to improve bulk blood flow without also improving perfusion may have limited impact in the metabolic syndrome. Microvascular perfusion heterogeneity represents an unaddressed therapeutic target for improving tissue oxygenation and muscle function.

Acknowledgments

Funding This work was supported by the American Heart Association (IRG 14330015, EIA 0740129N) and the National Institutes of Health (RR 2865AR; R01 DK64668), VA Merit Review BX002046 (JEBR), CU SOM Center for Women's Health Research, Bioengineering T32 (5T32HL072738).

References

1. Boudreau DM, Malone DC, Raebel MA, Fishman PA, Nichols GA, Feldstein AC, Boscoe AN, Ben-Joseph RH, Magid DJ, Okamoto LJ. Health care utilization and costs by metabolic syndrome risk factors. *Metabolic Syndrome and Related Disorders*. 2009; 7(4):305–314. [PubMed: 19558267]

2. Shamseddeen H, Getty JZ, Hamdallah IN, Ali MR. Epidemiology and economic impact of obesity and type 2 diabetes. *The Surgical Clinics of North America*. 2011; 91(6):1163–1172. [PubMed: 22054146]
3. Aleixandre de Artiñano A, Miguel Castro M. Experimental rat models to study the metabolic syndrome. *The British Journal of Nutrition*. 2009; 102(9):1246–1253. [PubMed: 19631025]
4. Fellmann L, Nascimento AR, Tibiriça E, Bousquet P. Murine models for pharmacological studies of the metabolic syndrome. *Pharmacology & Therapeutics*. 2013; 137(3):331–340. [PubMed: 23178510]
5. Tofovic SP, Jackson EK. Rat models of the metabolic syndrome. *Methods in Molecular Medicine*. 2003; 86:29–46. [PubMed: 12886760]
6. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. *The New England Journal of Medicine*. 2002; 346(11):793–801. [PubMed: 11893790]
7. Church TS, Cheng YJ, Earnest CP, Barlow CE, Gibbons LW, Priest EL, Blair SN. Exercise capacity and body composition as predictors of mortality among men with diabetes. *Diabetes Care*. 2004; 27(1):83–88. [PubMed: 14693971]
8. Fang ZY, Sharman J, Prins JB, Marwick TH. Determinants of exercise capacity in patients with type 2 diabetes. *Diabetes Care*. 2005; 28(7):1643–1648. [PubMed: 15983314]
9. Regensteiner JG, Sippel J, McFarling ET, Wolfel EE, Hiatt WR. Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Medicine and Science in Sports and Exercise*. 1995; 27(6):875–881. [PubMed: 7658949]
10. Wong CY, O'Moore-Sullivan T, Fang ZY, Haluska B, Leano R, Marwick TH. Myocardial and vascular dysfunction and exercise capacity in the metabolic syndrome. *American Journal of Cardiology*. 2005; 96(12):1686–1691. [PubMed: 16360358]
11. Frisbee JC, Goodwill AG, Butcher JT, Olfert IM. Divergence between arterial perfusion and fatigue resistance in skeletal muscle in the metabolic syndrome. *Experimental Physiology*. 2011; 96(3):369–383. [PubMed: 21123363]
12. Lalande S, Gusso S, Hofman PL, Baldi JC. Reduced leg blood flow during submaximal exercise in type 2 diabetes. *Medicine and Science in Sports and Exercise*. 2008; 40(4):612–617. [PubMed: 18317387]
13. Baldi JC, Aoina JL, Oxenham HC, Bagg W, Doughty RN. Reduced exercise arteriovenous O₂ difference in type 2 diabetes. *Journal of Applied Physiology*. 2003; 94(3):1033–1038. [PubMed: 12571134]
14. Boushel R, Gnaiger E, Calbet JA, Gonzalez-Alonso J, Wright-Paradis C, Sondergaard H, Ara I, Helge JW, Saltin B. Muscle mitochondrial capacity exceeds maximal oxygen delivery in humans. *Mitochondrion*. 2011; 11(2):303–307. [PubMed: 21147270]
15. Frisbee JC, Delp MD. Vascular function in the metabolic syndrome and the effects on skeletal muscle perfusion: lessons from the obese Zucker rat. *Essays in Biochemistry*. 2006; 42:145–161. [PubMed: 17144886]
16. Frisbee JC, Butcher JT, Frisbee SJ, Olfert IM, Chantler PD, Tabone LE, d'Audiffret AC, Shrader CD, Goodwill AG, Stapleton PA, Brooks SD, Brock RW, Lombard JH. Increased peripheral vascular disease risk progressively constrains perfusion adaptability in the skeletal muscle microcirculation. *American Journal of Physiology. Heart and Circulatory Physiology*. 2016; 310(4):H488–H504. [PubMed: 26702145]
17. Frisbee JC, Wu F, Goodwill AG, Butcher JT, Beard DA. Spatial heterogeneity in skeletal muscle microvascular blood flow distribution is increased in the metabolic syndrome. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*. 2011; 301(4):R975–R986.
18. Frisbee JC, Goodwill AG, Frisbee SJ, Butcher JT, Wu F, Chantler PD. Microvascular perfusion heterogeneity contributes to peripheral vascular disease in metabolic syndrome. *J Physiol*. 2014; 1–11. [PubMed: 24382918]
19. Wu F, Beard DA, Frisbee JC. Computational analyses of intravascular tracer washout reveal altered capillary-level flow distributions in obese Zucker rats. *The Journal of Physiology*. 2011; 589(Pt 18):4527–4543. [PubMed: 21788350]

20. Krogh A. The rate of diffusion of gases through animal tissue with some remarks on the coefficient of invasion. *The Journal of Physiology*. 1918; 52:391–409.
21. McGuire BJ, Secomb TW. Theoretical predictions of maximal oxygen consumption in hypoxia: effects of transport limitations. *Respiratory Physiology & Neurobiology*. 2004; 143(1):87–97. [PubMed: 15477175]
22. Pries AR, Ley K, Claassen M, Gaehtgens P. Red cell distribution at microvascular bifurcations. *Microvascular Research*. 1989; 38(1):81–101. [PubMed: 2761434]
23. Murray CD. The physiological principle of minimum work I. The vascular system and the cost of blood volume. *Proceedings of the National Academy of Sciences*. 1926; 12(3):207–214.
24. Zamir M. Arterial branching within the confines of fractal L-system formalism. *The Journal of General Physiology*. 2001; 118(3):267–276. [PubMed: 11524457]
25. McClatchey PM, Schafer M, Hunter KS, Reusch JE. The endothelial glycocalyx promotes homogeneous blood flow distribution within the microvasculature. *Am J Physiol Heart Circ Physiol*. 2016 ajpheart-00132.
26. Zamir M, Medeiros JA. Arterial branching in man and monkey. *The Journal of General Physiology*. 1982; 79(3):353–360. [PubMed: 7077288]
27. Kiwull-Schone H, Heidrun BG, Kiwull P. The effects of CO₂ and fixed acid on the O₂-Hb affinity of rabbit and cat blood. *Pflügers Archiv*. 1987; 408(5):451–457. [PubMed: 3110734]
28. Beard DA, Schenkman KA, Feigl EO. Myocardial oxygenation in isolated hearts predicted by an anatomically realistic microvascular transport model. *American Journal of Physiology. Heart and Circulatory Physiology*. 2003; 285(5):H1826–H1836. [PubMed: 12869375]
29. Goldman, D., Popel, AS., Computational modelling of oxygen transport from complex capillary networks. *Oxygen transport to tissue XXI*. Springer US; 1999. p. 555-563.
30. Skalak TC, Schmid-Schonbein GW. The microvasculature in skeletal muscle. IV. A model of the capillary network. *Microvascular Research*. 1986; 32(3):333–347. [PubMed: 3796306]
31. Honig CR, Feldstein ML, Frierson JL. Capillary lengths, anastomoses, and estimated capillary transit times in skeletal muscle. *American Journal of Physiology—Heart and Circulatory Physiology*. 1977; 233(1):H122–H129.
32. Prieto D, Contreras C, Sánchez A. Endothelial dysfunction, obesity and insulin resistance. *Current Vascular Pharmacology*. 2014; 12(3):412–426. [PubMed: 24846231]
33. Frisbee JC. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. *American Journal of Physiology. Heart and Circulatory Physiology*. 2001; 281(4):H1568–H1574. [PubMed: 11557545]
34. Frisbee JC, Goodwill AG, Frisbee SJ, Butcher JT, Brock RW, Olfert IM, DeVallance ER, Chantler PD. Distinct temporal phases of microvascular rarefaction in skeletal muscle of obese Zucker rats. *American Journal of Physiology. Heart and Circulatory Physiology*. 2014; 307(12):H1714–H1728. [PubMed: 25305181]

Fig. 1.

Previous studies establish impaired oxygen diffusion and microvascular perfusion heterogeneity in the OZR. **a** Femoral artery blood flow is significantly reduced in the OZR during 5 Hz contractions. This defect is not affected by treatment with TEMPOL. **b** Oxygen extraction is significantly reduced in the OZR during 5 Hz contractions. This defect is reversed by treatment with TEMPOL. **c** Oxygen diffusion capacity, a determinant of oxygen extraction, is a determinant of muscle fatigue for both LZR and OZR across multiple pharmacological treatments. **d** Schematic illustrating the parameter γ used to quantify microvascular perfusion heterogeneity. **e** Microvascular perfusion heterogeneity in 4a and 5a arterials is significantly increased in the OZR. This defect is reversed by treatment with TEMPOL. **a–c** Recreated with permissions from Frisbee et al. Exp Physiol 2011 [11]. **d, e** Recreated with permissions from Frisbee et al. Am J Physiol 2011 [17]

Fig. 2.

Visualizations of simulations performed. **a** A schematic illustration of the idealized microvascular network used in the present study. Note that 4 vessel generations are visualized, whereas 15 vessel generations were used in the simulation. Please see text for details. **b** A schematic illustration of the Krogh cylinder-type model used for simulation of single-capillary oxygen transport in this study

Fig. 3.

Heatmaps visualizing lookup tables (LUTs) of venous oxygenation as a function of capillary hematocrit and relative blood flow. **a** LUT of simulated venous oxygenation in the LZR at rest. **b** LUT of simulated venous oxygenation in the LZR during 5 Hz contractions. Note that venous oxygenation is substantially decreased (i.e., oxygen extraction is increased) relative to rest given similar flow and hematocrit. **c** LUT of simulated venous oxygenation in the OZR at rest. **d** LUT of simulated venous oxygenation in the OZR during 5 Hz contractions. Note that venous oxygenation is substantially less than at rest and marginally less than in the OZR given similar flow and hematocrit

Fig. 4.

Heatmaps visualizing lookup tables (LUTs) of skeletal muscle oxygenation as a function of capillary hematocrit and relative blood flow. **a** LUT of simulated muscle oxygenation in the LZR at rest. **b** LUT of simulated muscle oxygenation in the LZR during 5 Hz contractions. Note that muscle oxygenation is substantially decreased relative to rest given similar flow and hematocrit. **c** LUT of simulated muscle oxygenation in the OZR at rest. **d** LUT of simulated muscle oxygenation in the OZR during 5 Hz contractions. Note that muscle oxygenation is substantially less than at rest and marginally less than in the OZR given similar flow and hematocrit

Fig. 5.

Sensitivity analysis reveals that the effects of varying vessel diameters are negligible relative to the effects of varying flow distributions. **a** Variance in simulated CvO_2 increases quadratically with increasing variance of vessel diameters. At the level of diameter variance (10%) required to explain the maximum degree of perfusion heterogeneity considered in this manuscript ($\gamma = 0.7$), the effects of diameter variance without flow heterogeneity are still several orders of magnitude less than the effects of flow heterogeneity without diameter variance. **b** Variance in simulated PmO_2 increases quadratically with increasing variance of vessel diameters. This effect is several orders of magnitude smaller than the effects of flow heterogeneity. **c** Variance in simulated VO_2 increases quadratically with increasing variance of vessel diameters. This effect is several orders of magnitude smaller than the effects of flow heterogeneity.

NOTE: Horizontal and vertical lines represent single-variable measures (e.g. VO_2 and γ were not simultaneously measured). They do NOT represent independence of axes.

Fig. 6.

Differences in venous oxygenation, muscle oxygenation, and perfusion heterogeneity can be predicted using a simulation of microvascular blood flow and oxygen transport. **a** Venous oxygenation increases with increasing perfusion heterogeneity in both the LZR and the OZR. The intersection between simulated venous oxygenation (light, dashed lines) and empirically measured venous oxygenation (bold lines) can be used to predict the degree of perfusion heterogeneity. Our model correctly predicts greater perfusion heterogeneity in the OZR and partial correction of perfusion heterogeneity with TEMPOL treatment. **b** The intersection between simulated muscle oxygenation (light, dashed lines) and model-predicted perfusion heterogeneity (bold lines) can be used to predict muscle oxygenation. Our model correctly predicts reduced muscle oxygenation in the OZR and also predicts partial correction of muscle oxygenation with TEMPOL treatment. **c** The intersections of simulated oxygen uptake (light, dashed lines) with empirically measured oxygen uptake (bold lines) and with model-predicted oxygen uptake without perfusion heterogeneity in the OZR (light, dotted line) can be used to predict the relative contributions of various oxygen transport parameters to the observed oxygen uptake defect. Our model predicts that perfusion heterogeneity in small (3a-5a) arterioles plays a major role in reduced oxygen uptake in the OZR.

Fig. 7.

Analysis of perfusion heterogeneity effects at a single bifurcation reveals the mechanisms by which perfusion heterogeneity interferes with oxygen transport. **a** Increasing flow through one capillary while subtracting the same amount of flow from another capillary results in a greater decrease in oxygen uptake by the under-perfused capillary than the corresponding increase in oxygen uptake by the over-perfused capillary. This effect occurs independently of hematocrit effects. **b** Increasing hematocrit in one capillary while subtracting the same number of RBCs from another capillary results in a greater decrease in oxygen uptake by the low-hematocrit capillary than the corresponding increase in oxygen uptake by the high-hematocrit capillary. This effect occurs independently of flow effects. **c** Capillary flow and hematocrit are correlated at the single-capillary level, and flow heterogeneity results in hematocrit heterogeneity. These consequences of the plasma-skimming effect cause the effects of flow heterogeneity and hematocrit heterogeneity to synergize in reducing oxygen uptake under conditions of microvascular perfusion heterogeneity.

Table 1

Summary of model parameters used in the present study

Parameter	Description	Value	Reference
Blood parameters			
P_{in}	Oxygen tension in inlet capillary flow	91 to 95 mmHg	Experimental data
P_{out}	Oxygen tension in outlet capillary flow	–	Model outputs
Flow (F)	Blood flow per tissue mass	$7.1\text{e-}3$ to $3.55\text{e-}2$ mL s ⁻¹ (mL tissue) ⁻¹	Experimental data
Hct	Hematocrit	0.45	Default value
n_H	Hill coefficient for Hb-oxygen binding	2.7	McGuire & Secomb [21]
$P_{O_2,Hb}$	Half-maximal Hb saturation O ₂ tension	38.8 mmHg	Kiwull-Schone et al. [27]
S_{Hb}	Hemoglobin saturation level	–	–
α_{plasma}	Oxygen solubility in plasma	$1.3\text{e-}6$ M mmHg ⁻¹	Beard et al. [28]
α_{RBC}	Oxygen solubility in red blood cells	$1.53\text{e-}6$ M mmHg ⁻¹	Beard et al. [28]
p_w	Permeability for O ₂ across the capillary wall	250 $\mu\text{m s}^{-1}$	Beard et al. [28]
Tissue parameters			
D	Oxygen diffusivity in tissue	2410 $\mu\text{m}^2 \text{s}^{-1}$	Goldman & Popel [29]
D_{Mb}	Myoglobin diffusivity in tissue	17.3 $\mu\text{m}^2 \text{s}^{-1}$	Goldman & Popel [29]
$C_{50,Mb}$	Half-maximal Mb saturation O ₂ concentration	$9.22\text{e-}6$ M	Goldman & Popel [29]
α_{tissue}	Oxygen solubility in muscle tissue	$1.74\text{e-}6$ M mmHg ⁻¹	Beard et al. [28]
K_M	Michaelis-Menten constant for O ₂ consumption	$1.74\text{e-}6$ M	McGuire & Secomb [21]
V_{max}	Mitochondrial oxygen demand (rest)	0.039 ml g ⁻¹ min ⁻¹	Model optimization
	Mitochondrial oxygen demand (5 Hz)	0.185 ml g ⁻¹ min ⁻¹	Model optimization
Geometric parameters			
R_1	Capillary radius	2.98 μm (LZR) 3.07 μm (OZR)	Skalak et al. [30]
R_2	Tissue cylinder outer radius	19.9 μm (LZR) 23.0 μm (OZR)	Experimental data
L	Capillary length	1012 μm	Honig et al. [31]