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Model analysis of the relationship between intracellular \( \text{PO}_{2} \) and energy demand in skeletal muscle

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Spires J, Gladden LB, Grassi B, Saied GM, Lai N. Model analysis of the relationship between intracellular \( \text{PO}_{2} \) and energy demand in skeletal muscle. Am J Physiol Regul Integr Comp Physiol 303: R1110–R1126, 2012. First published September 12, 2012; doi:10.1152/ajpregu.00106.2012.—On the basis of experimental studies, the intracellular \( \text{O}_{2} \) (\( \text{iPO}_{2} \))-work rate (WR) relationship in skeletal muscle is not unique. One study found that \( \text{iPO}_{2} \) reached a plateau at 60% of maximal WR, while another found that \( \text{iPO}_{2} \) decreased linearly at higher WR, inferring capillary permeability-surface area (PS) and blood-tissue \( \text{O}_{2} \) gradient, respectively, as alternative dominant factors for determining \( \text{O}_{2} \) diffusion changes during exercise. This relationship is affected by several factors, including \( \text{O}_{2} \) delivery and oxidative and glycolytic capacities of the muscle. In this study, these factors are examined using a mechanistic, mathematical model to analyze experimental data from contracting skeletal muscle and predict the effects of muscle contraction on \( \text{O}_{2} \) transport, glycolysis, and \( \text{iPO}_{2} \). The model describes convection, \( \text{O}_{2} \) diffusion, and cellular metabolism, including anaerobic glycolysis. Consequently, the model simulates \( \text{iPO}_{2} \) in response to muscle contraction under a variety of experimental conditions. The model was validated by comparison of simulations of \( \text{O}_{2} \) uptake with corresponding experimental responses of electrically stimulated canine muscle under different \( \text{O}_{2} \) content, blood flow, and contraction intensities. The model allows hypothetical variation of PS, \( \text{O}_{2} \) glycolytic capacity, and \( \text{O}_{2} \) blood flow and predictions of the distinctive effects of these factors on the \( \text{iPO}_{2} \)-contraction intensity relationship in canine muscle. Although PS is the main factor regulating \( \text{O}_{2} \) diffusion rate, model simulations indicate that PS and \( \text{O}_{2} \) gradient have essential roles, depending on the specific conditions. Furthermore, the model predicts that different convection and diffusion patterns and metabolic factors may be responsible for different \( \text{iPO}_{2} \)-WR relationships in humans.

THE SKELETAL MUSCLE INTRACELLULAR \( \text{PO}_{2} \) (\( \text{iPO}_{2} \))-exercise intensity relationship was previously investigated to study the regulatory mechanisms of \( \text{O}_{2} \) consumption (\( \text{VO}_{2} \)) in working skeletal muscle (49, 55, 57). In particular, from these NMR studies in humans, skeletal muscle \( \text{iPO}_{2} \) was inferred from measurements of deoxyhemoglobin concentration during muscle contraction. The relationships between \( \text{iPO}_{2} \) and metabolic intensity [i.e., work rates (WR)], however, were dissimilar. Molé et al. (49) reported that \( \text{iPO}_{2} \) decreased linearly as WR increased above 50–60% of peak \( \text{VO}_{2} \) (\( \text{VO}_{2} \text{peak} \)), whereas Richardson et al. (55, 57) found that \( \text{iPO}_{2} \) was low and constant above 60% of \( \text{VO}_{2} \text{peak} \) during knee extensor exercise. In addition, Molé et al. inferred that the blood-tissue \( \text{O}_{2} \) gradient, rather than capillary permeability-surface area (PS), as proposed by Richardson et al., was the dominant factor determining \( \text{O}_{2} \) diffusion. PS is an overall transport coefficient equal to the rate of \( \text{O}_{2} \) transport between capillary blood and myocytes divided by the corresponding \( \text{O}_{2} \) concentration difference. It is directly related to diffusion conductance or diffusing capacity. Previous mathematical models suggested that \( \text{O}_{2} \) diffusion primarily depends on the spacing and clearance of RBCs (18, 26), two factors that contribute to PS. Therefore, the role of the \( \text{O}_{2} \) gradient and PS on the regulation of \( \text{O}_{2} \) diffusion at different muscle contraction intensities is still not conclusive.

Because these studies were done on different muscles undergoing different exercise protocols, they are difficult to compare directly. \( \text{VO}_{2} \) and blood flow (Q) distribution in skeletal muscle likely differ in the two experimental protocols. In particular, single-legged knee extensor exercise results in much higher Q and \( \text{VO}_{2} \) per unit muscle than does plantar flexion or two-legged cycle ergometry (6, 49, 56). Confounding the interpretation of these results further is the heterogeneity of \( \text{PO}_{2} \) within the muscle, which affects NMR measurements and subsequent estimates of \( \text{iPO}_{2} \).

\( \text{O}_{2} \) delivery and cellular metabolism are the main processes that can limit the dynamic response of skeletal muscle \( \text{VO}_{2} \) during contraction (63). Therefore, besides \( \text{O}_{2} \) convection and diffusion effects on \( \text{iPO}_{2} \), the mechanisms regulating the utilization of aerobic and anaerobic energy sources must be taken into account in quantifying the \( \text{iPO}_{2} \)-WR relationship during higher metabolic demand. Previous studies using the canine gastrocnemius model have provided insightful information on the factors limiting muscle oxidative metabolism during contraction (23–25). At submaximal metabolic intensity (23, 24) (e.g., 60% of \( \text{VO}_{2} \text{peak} \)), \( \text{VO}_{2} \) kinetics responses appear to be limited primarily by cellular metabolism; at higher metabolic intensity (100% of \( \text{VO}_{2} \text{peak} \)), \( \text{O}_{2} \) delivery (i.e., diffusion and convection) and metabolic processes appear to play a role (25).
However, these studies of skeletal muscle contraction did not evaluate $iPO_2$ or the rate of $O_2$ diffusion.

In spontaneously perfused muscle, $Q$ increases with $V_O_2$ and energy demands of the muscle. Imposing an elevated $Q$ would not be expected to alter $V_O_2$ but might affect $iPO_2$ or other key metabolites. Although the effects of $Q$ on the kinetics of $V_O_2$ have been determined experimentally, the effects of elevated $Q$ and $PS$ on $iPO_2$ in canine muscle are not known.

A quantitative approach proposed by Wagner (64) was successfully applied to understand the interaction between $O_2$ convection and diffusion and their roles in determining maximal $V_O_2$. However, this approach is limited to steady-state analysis, and the interaction between $O_2$ delivery and aerobic and anaerobic systems is not considered. In response to muscle contraction, the metabolic fluxes associated with the glycolytic and oxidative systems are regulated by compensatory mechanisms involving ATP/ADP and NADH/NAD changes (7). Therefore, glycolytic and oxidative systems are indirectly related through the metabolic fluxes that share common substrates. Thus anaerobic glycolysis can indirectly affect $iPO_2$ in skeletal muscle at higher exercise intensity.

To quantify the relationships between $iPO_2$, $O_2$ utilization, and WR in skeletal muscle at higher exercise intensity, a mechanistic, mathematical model of $O_2$ transport and metabolism can be used. For this purpose, a model of anaerobic glycolysis (44) was combined with a model of $O_2$ transport and utilization (43). The resulting model delivers $O_2$ by convection and diffusion and supplies energy by oxidative and glycolyglycosynthetic mechanisms. Accordingly, it can simulate skeletal muscle responses to submaximal and maximal metabolic demands. Model outputs compared with experimental data obtained under different experimental conditions provide model validation. Model simulations of canine skeletal muscle during contraction show how the $iPO_2$-energy demand relationship is affected by $PS$, blood perfusion patterns, and oxidative and glycolyglycosynthetic characteristics. Differences in $O_2$ delivery and energy utilization patterns associated with various experimental conditions can explain and reconcile apparent differences in the $iPO_2$-WR relationship at different levels of metabolic demand. Since simultaneous measurements of tissue metabolites and $iPO_2$ with contracting muscle are difficult to obtain, a mechanistic, mathematical model is used to simulate the metabolic response to higher metabolic rates. These simulations can provide a reliable basis for evaluating 1) the importance of $O_2$ gradient and $PS$ in the control of $O_2$ diffusion and 2) the conditions under which $iPO_2$ decreases linearly or remains constant in response to increasing muscle contraction intensity.

### Glossary

- $C_{ATP}$: Total concentration of ATP, ADP, and AMP
- $C_{PC}$: Total concentration of phosphocreatine and creatine
- $C_{Mbg}$: Concentration of Hb in blood
- $C_{Na}$: Total concentration of NAD and NADH
- $C_{Mb}$: Concentration of myoglobin in myocytes
- $C_{Hb}$: Concentration of Hb in RBCs
- $D_{O_2,b}$: Effective dispersion coefficient of $O_2$ in blood
- $D_{O_2,c}$: Effective dispersion coefficient of $O_2$ in tissue
- $I_b$: Blood capillary volume fraction in muscle
- $Hct$: Hematocrit
- $k_{ATPase}$: ATPase rate constant
- $K_i$: Rate coefficient for a reaction involving metabolite $i$
- $K_{Hb}$: Hill constant at which Hb is 50% saturated by $O_2$
- $K_{Mb}$: Hill constant at which myoglobin is 50% saturated by $O_2$
- $M_{lac}$: Michaelis-Menten transport constant of lactate
- $M_{pyr}$: Michaelis-Menten transport constant of pyruvate
- $n_H$: Hill coefficient
- $OxP$: Oxidative phosphorylation
- $PO_2^R$: Resting $PO_2$ in the vein
- $P_{S_{rest}}$: Permeability-surface area at rest
- $P_{S_{max}}$: Maximal value of permeability-surface area
- $T_{lac}$: Maximal transport flux of lactate
- $V_{max}$: Maximal flux rate
- $V_{mus}$: Total muscle volume
- $W_{mc}$: Volume fraction of myoglobin in myocytes
- $\alpha_{gly}$: Glycogenolytic capacity
- $\alpha_{O_2}$: $O_2$ solubility in blood
- $\gamma_{O_2,b}$: Coefficient that accounts for the equilibrium between free and bound $O_2$ in blood
- $\gamma_{O_2,c}$: Coefficient that accounts for the equilibrium between free and bound $O_2$ in tissue

### Subscripts and Superscripts

- A-V: Arteriovenous difference
- art: Artery
- b: Blood
- c: Cell
- cap: Capillary
- exp: Experimental data
- f: Forward reaction
- mod: Model simulation
- mus: Muscle
- R: Resting condition
- r: Reverse reaction
- S: Contraction condition
- SS: Steady-state condition
- T: Total $O_2$ concentration
- tis: Tissue
- ven: Venous

### Enzymes

- ADK: Adenylate kinase
- ALD: Fructose bisphosphate aldolase
- ATPase: Myosin ATPase
- CK: Creatine kinase
- ENOL: Enolase
- GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
- GP: Glycogen phosphorylase
- LDH: l-Lactate dehydrogenase
- PFK: 6-Phosphofructokinase
- PGI: Phosphoglucone isomerase
METHODS

A mathematical model is developed to simulate and predict dynamic metabolic responses of skeletal muscle in vivo to a change in energy demand and experimental conditions. This model (Fig. 1) is based on two earlier models: 1) O₂ transport and metabolism in blood and tissue (43) and 2) anaerobic glycogenolysis with key metabolites (including lactate and pyruvate) (44). The glycogenolytic pathway in the model specifically refers to glycogenolytic metabolism that results in lactate accumulation, i.e., an increase in intramuscular lactate concentration and lactate release into the blood. This model assumes that chemical species change continuously in space between the arterial input and venous output in blood and in tissue cells. The total muscle volume consists primarily of capillary blood and extravascular muscle tissue: \( V_{\text{mus}} = V_{\text{cap}} + V_{\text{tis}} \).

**Spatially Distributed, Metabolite Dynamics**

**Concentration dynamics in blood.** Free O₂ concentrations in the capillary blood \( [C_{O_2,b}(v,t)] \) and in muscle cells \( [C_{O_2,c}(v,t)] \) depend on time \( (t) \) and tissue location, as indicated by the cumulative muscle volume \( (v) \) from the arterial input \( (v = 0) \) to the venous output \( (v = V_{\text{mus}}) \). On the basis of a dynamic O₂ balance in blood (43)

\[
\frac{\partial C_{O_2,b}}{\partial t} = -Q \frac{\partial C_{O_2,b}}{\partial v} + D_{O_2,b} \frac{\partial^2 C_{O_2,b}}{\partial v^2} - \frac{f_{O_2,b}^c}{\gamma_{O_2,b}} \quad 0 < v < V_{\text{mus}}
\]

where \( f_{O_2,b} = V_{\text{cap}}/V_{\text{mus}}, D_{O_2,b} \) is effective axial dispersion coefficient in blood, \( f_{O_2,b}^c \) is effective axial dispersion coefficient in muscle cells, and \( \gamma_{O_2,b} \) is a coefficient that accounts for the equilibrium between free and bound O₂ in blood (see APPENDIX b). A similar dynamic mass balance describes lactate and pyruvate concentrations

\[
\frac{\partial C_{\text{lac},b}}{\partial t} = -Q \frac{\partial C_{\text{lac},b}}{\partial v} - f_{\text{lac},b,c} \quad (i = \text{lac, pyr})
\]

**Concentration dynamics in extravascular tissue.** The dynamics of free O₂ concentration in muscle cells of tissue can be described as

\[
\frac{\partial C_{O_2,c}}{\partial t} = -Q \frac{\partial C_{O_2,c}}{\partial v} + D_{O_2,c} \frac{\partial^2 C_{O_2,c}}{\partial v^2} - f_{O_2,c} \phi_{O_2,c} \quad (i = \text{lac, pyr})
\]

where \( f_{\text{lac}} = V_{\text{tis}}/V_{\text{mus}} = 1 - f_{\text{lac}} \) is the effective axial dispersion coefficient in blood, and \( \gamma_{O_2,c} \) is a coefficient that accounts for the equilibrium between free and bound O₂ in muscle cells. For \( j \) species other than O₂ (see APPENDIX a), the dynamic mass balance in tissue has the following general form

\[
\frac{\partial C_{i,b}}{\partial t} = -Q \frac{\partial C_{i,b}}{\partial v} - f_{i,b,c} \quad (i = \text{lac, pyr})
\]
Initially, the concentrations are distributed in a resting, steady state, when described by ordered bi-bi reactions (see APPENDIX B) (44). ATPase, creatine kinase, and adenylate kinase reactions, as well as substrate (S) or product (P) and their free O2, lactate, and pyruvate concentrations (C0) are related by mass conservation of adenosine (A), Cr (C), and NAD (N) within the cell. For constant total concentrations and volumes of the tissue C_A = C_AMP + C_AD + C_ATP, \[ C_T = C_{PC} + C_{Cr}, \quad C_T = C_{NAD} + C_{NADH} \]

Another constraint associated with the application of this model is the assumption of constant glycogen concentration.

**Blood-cell transport fluxes.** The capillary-tissue transport of O2 between blood and muscle cells depends on the PS and their free O2 concentrations \[ J_{bc} = PS(C_{O2,b} - C_{O2,c}) \]

When a change occurs in muscle Q, PS is assumed to change with the same dynamic pattern (43). For a step-up change of Q with characteristic time constant \( \tau_Q \), we assume \[ PS(t) = PS_{rest} + (PS_{max} - PS_{rest})[1 - \exp(-t/\tau_Q)] \]

where \( PS_{max} \) is the maximal value and \( PS_{rest} \) is the value at resting, steady state, when \( t = t_0 \). When Q is pump-perfused (PP), PS is set at a constant \( PS_{max} \).

For lactate and pyruvate \( j = lac, pyr \), the net transport fluxes \( J_{bc} \) between blood and tissue depend on their concentrations \( (C_{j,b}, C_{j,c}) \) according to carrier-mediated transport (see Table 7) (14).

\[ J_{bc} = T \left( \frac{C_{j,b}}{M_j + C_{j,b}} - \frac{C_{j,c}}{M_j + C_{j,c}} \right) \]

**Metabolic fluxes.** The mathematical model describes the metabolic reactions within the cells (Fig. 1), including the glycogenolytic pathway, and ATPase, creatine kinase, and adenylate kinase reactions, as well as oxidative phosphorylation. Most of the reaction fluxes are described by ordered bi-bi reactions (see APPENDIX B) (44).

\[ \frac{V_{max,i}C_A C_b}{K_{max,i}K_c} - \frac{V_{max,i}C_p C_q}{K_{max,i}K_p} \]

where \( V_{max,i} \) is the maximal metabolic flux reverse and forward for each \( i \) reaction are calculated using the Haldane relation (44). These \( V_{max,i} \) values do not change with contraction intensity.

**Energy supply and demand.** Fluxes related to energy supply and demand change depending on experimental conditions. The energy demand is associated with the ATPase reaction described by mass action (44).

\[ \phi_{ATPase} = K_{ATPase}C_{ATP} \]

The fluxes of energy supply are related to glycolysis (\( \phi_{gly} = \phi_{PPF}, \phi_{PK}, \phi_{PK,C} \)), oxidative phosphorylation (\( \phi_{Ox}, \phi_{OxP} \)), creatine kinase (\( \phi_{CK} \)), and adenylate kinase (\( \phi_{ADK} \)). At steady state, flux of energy demand is balanced by the fluxes of energy supply.

\[ \phi_{ATPase} = \phi_{gly} + \phi_{OxP} - \phi_{CK} - \phi_{ADK} \]

The previous metabolic flux expression used for oxidative phosphorylation (43) was modified to account for \( P \), according to the previous model developed (5) to simulate ATP production in mitochondria of cardiac muscle

\[ \phi_{OxP} = V_{max,OX} \left( \frac{C_{O2,c}}{C_{O2,c} + K_{O2}} \right) \left( \frac{C_{ADP}}{C_{ADP} + K_{ADP}} \right) \left( \frac{C_{P_i}}{C_{P_i} + K_{P_i}} \right) \]

\[ V_{O2} \] For comparison with minimally invasive measurements, model outputs include \( V_{O2} \) and O2 saturation in blood and tissue. The rate of muscle \( V_{O2} \) per volume of muscle is

\[ V_{O2}(t) = \frac{Q(t)C_{A,V}(t)}{\mu} \]

\[ C_{A,V}(t) = C_{O2,art} - C_{O2,rest}(t) \]

\[ C_{A,V}(t) = C_{O2,art} - C_{O2,rest}(t) \]

where \( C_{A,V} \) is the arteriovenous oxygen concentration difference of total (free and bound) O2. The equilibrium relationship between total and free O2, e.g., arterial O2 \( C_{O2,art} \), is presented in APPENDIX B (43).

**Simulation Strategy.**

This model is intended to simulate and predict the spatially distributed concentrations of O2, lactate, pyruvate, Cr, PCr, AMP, and metabolites of the glycogenolytic pathway. Specifically, the model is applied to analyze underlying metabolic and transport processes based on responses of the canine gastrocnemius muscle in situ to tetanic stimuli (23–25) under a variety of experimental conditions: 1) normoxia or hyperoxia, 2) spontaneous self-perfused (SP) or controlled (PP) Q patterns, and 3) O2 utilization rate equivalent to 60% or 100% of peak, steady-state \( V_{O2} \) (O2 uptake) (Table 1). Canine muscle was used for validation because of a readily available database containing direct measures of metabolic rate and many of its related components under different conditions: Q, muscle contraction frequency, and arterial O2 content (23–25).

In model simulations, the measured arteriovenous concentration of free O2 \( (C_{O2,art} - C_{O2,rest}) \) corresponding to normoxia or hyperoxia was applied. The simulations also incorporated the measured Q(t). Simulations of...
the corresponding O2 utilization rate are based on equivalent estimation of $k_{\text{ATPase}}$ (Table 2).

There are uncertainties about the glycogenolytic enzyme capacity used in this model. In particular, $V_{\text{max,f},r}$ reported in the computational study (44) to simulate the whole glycogenolytic process in skeletal muscle, was adapted to describe only the anaerobic glycogenolysis in our model. To lower the anaerobic glycogenolytic capacity to physiological levels, a glycogenolytic capacity fraction $0 < \alpha_{\text{gly}} < 1$ was introduced. The parameter $\alpha_{\text{gly}}$ modifies the maximal flux rates (44) of key glycogenolytic reactions (Table 3). This fraction does not apply to the reactions of oxidative phosphorylation, creatine kinase, and adenylate kinase.

Model validation is based on the simulations of CTA-V(t) and $V_{\text{O2}}(t)$ under all conditions with the same basic model parameter values. The model can also predict the dynamics of spatially averaged concentrations and metabolic fluxes in tissue. A general expression for any spatially averaged variable in this model with respect to tissue volume is as follows:

$$\langle y(t) \rangle = \frac{\int_0^V y(v, t) dv}{V_{\text{tis}}} \quad (19)$$

Of special interest are the averaged concentrations of ATP and O2 ([C_{\text{ATP}}] and [C_{\text{O2}}]) and fluxes of oxidative phosphorylation and ATPase ($i_{\text{ATPase}}$ and $i_{\text{ATPase}}$). Under steady-state conditions, the potential metabolic and transport limitations become apparent from several spatially averaged outputs. The average iPO2, which is directly proportional to the average free O2 concentration in cells ([C_{\text{O2,c}}]), reflects the O2 available in the tissue through transport from the blood. The O2 gradient, the difference between O2 concentration in capillaries and tissue ([C_{\text{O2,b}}] - [C_{\text{O2,c}}]), contributes to the O2 transport between blood and tissue. Average extracellular PO2 (ePO2) and iPO2 by O2 solubility in blood and tissue, respectively. A constant average C_{\text{ATP}} (C_{\text{ATP}}) indicates that energy supply is meeting demand energy. The maximal flux fraction $\alpha_{\text{gly}}$ was increased to explore the effects of glycogenolytic capacity on (iPO2) at higher ATP demand. Although there is no evidence that the full glycogenolytic capacity is used during the canine muscle contractions that were modeled, this condition may be relevant for intense human exercise. Also, to examine the effects of O2 transport limitations on the (iPO2)-WR relationship, physiologically normal and elevated values of blood perfusion and PS were simulated.

### Parameter Estimation and Numerical Solution

Values of most parameters in this model (Table 7) are available from previous studies: transport parameters of pyruvate and lactate (14), concentrations of arterial lactate and pyruvate (50), and parameters of the metabolic fluxes (Tables 3–6) (44, 63a). These parameter values apply to all experiments and are independent of the input conditions. The value of $T_{\text{lac}}$ was chosen so that simulation of lactate output was the same as that measured by Grassi et al. (23) at submaximal metabolic intensity under constant $Q$. $T_{\text{pyr}}$ was similarly determined from pyruvate uptake data (20).

In these simulations, most model parameter values are independent of the experimental conditions; therefore, their values are the same. Other parameter values for simulation depend on the experimental conditions (Tables 1 and 2). Values of muscle volume ($V_{\text{mus}}$) were obtained from experimental measurements of muscle weight and typical muscle density (23–25). Values of $k_{\text{ATPase}}$ for resting and working muscle and for $V_{\text{max,OxP}}$ were estimated by least-squares optimization (43) using measurements of arteriovenous O2 difference obtained under hypoxic conditions. These experiments were conducted with constant PP $Q$ and injection of a drug (RSR-13) that

### Table 2. Parameter values associated with experimental conditions

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<tr>
<th>Parameter</th>
<th>Hyperoxia</th>
<th>Normoxia</th>
<th>Hyperoxia</th>
<th>Normoxia</th>
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### Table 3. Maximal metabolic fluxes

<table>
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<tr>
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<td>$V_{\text{max,OxP}}$</td>
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</table>

Values are mM/min.
causes a rightward shift in the O2-Hb dissociation curve (24). In simulating the muscle response to contraction, values of k\textsubscript{ATPase} for resting and working muscle were estimated to fit steady-state V\textsubscript{O2} data for each experimental condition (23–25). The value of V\textsubscript{max,OxP} was unchanged for simulations under all experimental conditions.

The initial model concentrations depend on the experimental conditions: arterial metabolite concentrations and Q\textsubscript{˙} (Tables 1 and 2). These are obtained by numerical solution of the model equations using parameter values of Tables 3–7. The steady-state concentration values are the initial conditions at which the stimulus is applied.

For numerical solution of the model equations, the spatial derivatives are discretized to convert partial differential equations to ordinary differential equations (method of lines) using a well-developed code (DSS/2) (61). Consequently, the model consists of a set of ordinary differential-difference equations that constitute an initial-value problem solved using a robust algorithm for stiff systems (DLSODE) (27). The parameter estimation was obtained by minimizing the objective function represented by the difference between model simulation and experimental data. The objective function is minimized by numerical optimization using an adaptive, nonlinear algorithm (DN2FB; http://www.netlib.org) (16). Depending on the operating point in parameter space, changes of just 10% in the parameters of interest can produce significant differences in the simulated outputs.

### Table 4. Michaelis-Menten constants and other reaction parameters for aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase

<table>
<thead>
<tr>
<th>Notation</th>
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<th>Reference</th>
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<tbody>
<tr>
<td><strong>Glycogen phosphorylase form A</strong></td>
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<tr>
<td>$K_{gly}$</td>
<td>1.7 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{Ps}$</td>
<td>4.0 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>2.0 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>4.7 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>0.15 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>2.7 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ADP}$</td>
<td>10.1 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>0.42 mM</td>
<td>44</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.4</td>
<td>44</td>
</tr>
<tr>
<td><strong>Glycogen phosphorylase form B</strong></td>
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<tr>
<td>$K_{Ps}$</td>
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</tr>
<tr>
<td>$K_{gly}$</td>
<td>15 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>1.5 mM</td>
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</tr>
<tr>
<td>$K_{gly}$</td>
<td>7.4 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>4.4 mM</td>
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</tr>
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<td>$K_{AMP}$</td>
<td>0.097 mM</td>
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<td>pH</td>
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<tr>
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<td>$\alpha$</td>
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<td>$K_{AMP}$</td>
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<tr>
<td>$K_{GP}$</td>
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<tr>
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<td>$K_{G6P}$</td>
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<td>$K_{eq}$</td>
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<tr>
<td><strong>Phosphofructokinase</strong></td>
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</tr>
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<td>$K_{F6P}$</td>
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</tr>
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<td>$K_{F6P}$</td>
<td>20 mM</td>
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</tr>
<tr>
<td>$K_{ATP}$</td>
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</tr>
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<td>$K_{TP}$</td>
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</tr>
<tr>
<td>$K_{TP}$</td>
<td>4.02 mM</td>
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</tr>
<tr>
<td>$K_{F6P}$</td>
<td>4.02 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>2.7 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ADP}$</td>
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<td>44</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
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<td>$K_{AMP}$</td>
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<tr>
<td>$d$</td>
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<td>$e$</td>
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<tr>
<td>$L_o$</td>
<td>13</td>
<td>44</td>
</tr>
</tbody>
</table>

### Table 5. Michaelis-Menten constants and other reaction parameters for aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase, enolase, pyruvate kinase, and lactate dehydrogenase

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aldolase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{gly}$</td>
<td>0.05 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{GP}$</td>
<td>2.0 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>0.5 $\times 10^{-6}$ mM</td>
<td>Present study</td>
</tr>
<tr>
<td><strong>Triose phosphate isomerase</strong></td>
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<td></td>
</tr>
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<td>$K_{GP}$</td>
<td>0.32 mM</td>
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</tr>
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<td>$K_{GP}$</td>
<td>0.61 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>0.052</td>
<td>44</td>
</tr>
<tr>
<td><strong>Glyceraldehyde-3-phosphate dehydrogenase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{GP}$</td>
<td>0.0025 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{NAD}$</td>
<td>0.09 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{Ps}$</td>
<td>0.29 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{3P}gpg$</td>
<td>8.0 $\times 10^{-4}$ mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{NADH}$</td>
<td>0.0033 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>8.9 $\times 10^{-5}$ mM$^{-1}$</td>
<td>44</td>
</tr>
<tr>
<td><strong>Phosphoglycerate kinase</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{3P}gpg$</td>
<td>0.0022 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ADP}$</td>
<td>0.008 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{3P}gpg$</td>
<td>1.2 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>0.35 mM</td>
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</tr>
<tr>
<td>$K_{eq}$</td>
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<td>44</td>
</tr>
<tr>
<td><strong>Phosphoglyceromutase</strong></td>
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<td></td>
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<tr>
<td>$K_{3P}gpg$</td>
<td>0.2 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{2P}gpg$</td>
<td>0.014 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
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<tr>
<td><strong>Enolase</strong></td>
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<tr>
<td>$K_{2P}gpg$</td>
<td>0.1 mM</td>
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</tr>
<tr>
<td>$K_{2P}gpg$</td>
<td>0.37 mM</td>
<td>44</td>
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<td>$K_{eq}$</td>
<td>0.49</td>
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<tr>
<td><strong>Pyruvate kinase</strong></td>
<td></td>
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</tr>
<tr>
<td>$K_{2P}gpg$</td>
<td>0.08 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ADP}$</td>
<td>0.3 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{pyr}$</td>
<td>7.05 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>1.13 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>10,304</td>
<td>44</td>
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<tr>
<td><strong>Lactate dehydrogenase</strong></td>
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<td>$K_{pyr}$</td>
<td>0.335 mM</td>
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<td>$K_{NADH}$</td>
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<tr>
<td>$K_{lac}$</td>
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</tr>
<tr>
<td>$K_{NAD}$</td>
<td>0.849 mM</td>
<td>44</td>
</tr>
<tr>
<td>$K_{eq}$</td>
<td>16,198</td>
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</table>

R1115

SKELETAL MUSCLE INTRACELLULAR PO2 AT HIGHER ATP DEMAND

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To simulate the metabolic response to contraction under blood SP conditions, Q˙ and V˙O2 data of canine muscle under different contraction frequencies (22–25, 28, 31, 39, 66), were used (see Fig. 8A).

RESULTS

Parameter Estimation and Model Validation

By fitting simulated arteriovenous O2 differences to experimental data obtained under the conditions reported in Table 1 (hyperoxia + RSR-13, PP), optimal estimates were obtained for oxidative phosphorylation maximal flux rate (Vmax,OxP; Table 3) as well as kATPase in resting (kATPase,R) and working (kATPase,S) muscle (Table 2). The model simulations produced dynamic outputs comparable to experimental data (Fig. 2) of Q, arteriovenous O2 concentration difference (CA-V), and muscle uptake (V˙O2 = QC - CA-V). The validity of the mathematical model was tested by comparison of simulated muscle responses under various conditions (Tables 1 and 2) with corresponding experimental data (23–25). Values of Vmax,OxP and other common parameters, estimated from previous studies, were assumed to be the same under all experimental conditions (Tables 3–7).

In response to energy demand corresponding to muscle contraction, the model simulations produced dynamic outputs comparable to experimental data (Figs. 3–5) for Q, CA-V, and V˙O2. Simulated responses of other variables are predictions for which corresponding experimental data are not available. Model simulation of V˙O2, obtained during normoxia with spontaneous (SP) and PP Q, shows that the dynamic and steady-state responses are not noticeably affected by the muscle Q pattern at 60% of V˙O2peak (Fig. 3). Apparently, differences in Q are compensated by changes in CA-V. With constant perfusion and hyperoxia, CA-V shows a delayed dynamic response with a larger slope (Fig. 4). The delayed dynamic response with a larger slope is still evident in the V˙O2 dynam-

### Table 6. Michaelis-Menten constants and other reaction parameters for creatine kinase, adenosine kinase, and oxidative phosphorylation

<table>
<thead>
<tr>
<th>Notation</th>
<th>Value</th>
<th>Reference</th>
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<tr>
<td>KPCr</td>
<td>1.11 mM</td>
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</tr>
<tr>
<td>KATP</td>
<td>3.5 mM</td>
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</tr>
<tr>
<td>KADP</td>
<td>0.135 mM</td>
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</tr>
<tr>
<td>KPCr</td>
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<td>KATP</td>
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<td>KATPase,R</td>
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### Table 7. Miscellaneous physiological parameter values used to simulate all experimental conditions

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<tr>
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<th>Value</th>
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<tr>
<td>Volume fraction and O2 axial dispersion</td>
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<tr>
<td>f5</td>
<td>7%</td>
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</tr>
<tr>
<td>Wmc</td>
<td>75%</td>
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<tr>
<td>D02,b</td>
<td>2 × 10⁻⁵ l/min</td>
<td>43</td>
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<tr>
<td>D02,c</td>
<td>1.2 × 10⁻⁵ l/min</td>
<td>43</td>
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<tr>
<td>Hb and Mb O2 relationships</td>
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<tr>
<td>αO2</td>
<td>1.338 × 10⁻³ mM/mmHg</td>
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<tr>
<td>Cinc,Mb</td>
<td>0.4 mM</td>
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<tr>
<td>Kf</td>
<td>308.642 mM⁻¹</td>
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<tr>
<td>Cinc,HHb</td>
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<tr>
<td>Kf</td>
<td>7210.9 mM⁻¹</td>
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<td>nH</td>
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<td>Constant concentrations</td>
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<td>CClc,art</td>
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<tr>
<td>Cpyr,art</td>
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<td>50</td>
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<td>C2</td>
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<td>CCA</td>
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<tr>
<td>CCl</td>
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<td>Blood-tissue transport parameters</td>
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<td>P0rest</td>
<td>4.1·1⁻¹·min⁻¹</td>
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</tr>
<tr>
<td>Pmax</td>
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<td>Tlac</td>
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<tr>
<td>Tpyr</td>
<td>0.004 mMol/min</td>
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<tr>
<td>Mlac</td>
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<td>14</td>
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<tr>
<td>Mpyr</td>
<td>1 mM</td>
<td>14</td>
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![Fig. 2. Blood flow (Q), arteriovenous O2 difference (CA-V), and O2 uptake (V˙O2) for pump-perfused (PP) canine gastrocnemius muscle stimulated at 60% of peak V˙O2 (V˙O2peak) under hyperoxia with RSR-13. Experimental data are from Ref. 24. Vertical line denotes transition between rest and contraction.](http://ajpregu.org/article-pdf/10.1152/ajpregu.00106.2012/001062012.pdf)
ics, but the overall \( \dot{V}O_2 \) kinetics remain unchanged. However, under more frequent muscle stimulation corresponding to 100% of \( \dot{V}O_2_{\text{peak}} \), elevated muscle PP \( Q \dot{\omega} \) results in a faster \( \dot{V}O_2 \) dynamic response (Fig. 5).

In addition to these outputs, the mathematical model predicts a myoglobin (Mb) saturation fraction of about 0.95 at rest. Also, during the first 30 s of muscle contraction, the Mb saturation fraction and the iPO\(_2\) decrease rapidly, which contributes to an increase of the capillary-tissue O\(_2\) gradient.

### Factors Affecting Metabolic Response to Higher Energy Demand

**Metabolic responses to contraction.** To simulate responses to higher rates of muscle contraction or ATP demand, equivalent higher values of \( k_{\text{ATPase}} \) were used. The model simulations reported in Figs. 6 and 7 were obtained using model parameters corresponding to 100% of \( \dot{V}O_2_{\text{peak}} \) with PP \( Q \dot{\omega} \) of 101 ml·100 g\(^{-1}\)·min\(^{-1}\) through the transition from rest to contractions. The \( k_{\text{ATPase}} \) value predicted at 100% of \( \dot{V}O_2_{\text{peak}} \) (i.e., maximal \( k_{\text{ATPase}} \) in the canine gastrocnemius muscle preparation) is labeled \( k_{\text{ATPase,max}} \) (Figs. 6–8). As ATP demand (i.e., \( k_{\text{ATPase}} \)) increases during submaximal muscle contractions, the average O\(_2\) (\((cPO_2)-(iPO_2)\)) gradient (Figs. 6C and 7C) and muscle \( \dot{V}O_2 \) (Figs. 6B and 7B) increase, while \( iPO_2 \) decreases (Figs. 6A and 7A). \( C_{\text{ATP}} \) is constant over a wide range of submaximal energy demand but decreases at higher energy demand (Figs. 6D and 7D).

**Effects of glycolytic capacity.** Changes in glycolytic capacity were simulated by changing the maximal metabolic flux rate parameters \( V_{\text{max,l}} \) associated with the glycolytic pathway as determined by \( \alpha_{\text{gly}} \). Increased \( \alpha_{\text{gly}} \) changes the steady-state relationships of O\(_2\) gradient, \( \langle iPO_2 \rangle \), and \( \dot{V}O_2 \) with contraction intensity (Fig. 6). For the same ATP demand (but before significant \( C_{\text{ATP}} \) decrease), an increase in \( \alpha_{\text{gly}} \) lowers \( \dot{V}O_2 \) (Fig. 6B) and O\(_2\) gradient (Fig. 6C) but increases \( iPO_2 \) (Fig. 6A).

**Effects of O\(_2\) diffusion.** Changes in the O\(_2\) diffusion rate from capillaries to myocytes were simulated by changing the capillary PS via its maximum value \( (PS)_{\text{max}} \). For different \( (PS)_{\text{max}} \), the steady-state relationships of \( iPO_2 \), \( \dot{V}O_2 \), O\(_2\) gradient, and \( C_{\text{ATP}} \) vary with energy demand (Fig. 7). At low or high ATP demand, lower values of \( (PS)_{\text{max}} \) result in lower \( iPO_2 \) and higher O\(_2\) gradient. At lower energy demand, \( \dot{V}O_2 \) and \( C_{\text{ATP}} \) are unaffected by \( (PS)_{\text{max}} \) (Fig. 7), while at higher energy demand, \( \dot{V}O_2 \) and \( C_{\text{ATP}} \) are reduced at lower \( (PS)_{\text{max}} \). The energy demand at which \( C_{\text{ATP}} \) begins to decrease is lower with lower \( (PS)_{\text{max}} \) (Fig. 7).

**Effects of convective transport.** Figures 6 and 7 show the effects of increased PP \( Q \dot{\omega} \) at all contraction intensities. In addition, \( iPO_2 \) was simulated for physiological values of SP Q\(_{\text{max}}\).

---

**Fig. 3.** \( Q \), arteriovenous O\(_2\) concentration difference, and \( \dot{V}O_2 \) for self-perfused (SP) and PP canine gastrocnemius muscle stimulated at 60% of \( \dot{V}O_2_{\text{peak}} \) under normoxia. Experimental data are from Ref. 23. Vertical line denotes transition between rest and contraction.

**Fig. 4.** \( Q \), arteriovenous O\(_2\) difference, and \( \dot{V}O_2 \) for PP canine gastrocnemius muscle stimulated at 60% of \( \dot{V}O_2_{\text{peak}} \) under normoxia and hyperoxia. Experimental data are from Refs. 23 and 24. Vertical line denotes transition between rest and contraction.
A variety of experimental data were used (22–25, 28, 31, 39, 66) to determine an empirical linear relationship between steady-state \( \bar{Q} \) and \( \bar{V}O_2 \) (Fig. 8A). The relationship was used in the model to estimate \( k_{\text{ATPase}} \) for several metabolic rates under physiological (spontaneous) perfused \( Q \) to examine the effects of \( Q \) on \( ipO_2 \), \( \langle ePO_2 \rangle - \langle iPO_2 \rangle \) gradient, \( VO_2 \), and ATP. Optimal estimates for \( k_{\text{ATPase}} \) were obtained for steady-state \( Q \) values, which are model inputs. Under spontaneous \( Q \), \( VO_2 \), \( O_2 \) gradient, and \( C_{\text{ATP}} \) are similar to values obtained with elevated (controlled) \( Q \) (not shown). However, the simulated \( ipO_2 \) is higher with elevated muscle perfusion than with normally perfused muscle at low contraction rates (Fig. 8B). At higher contraction rates, \( ipO_2 \) is similar under both flow conditions.

At higher energy demand, as represented by a large \( k_{\text{ATPase}} \), a \( VO_2 \) plateau and decline in \( C_{\text{ATP}} \) appear (Figs. 6B, 6D, 7B, and 7D). This occurs when \( ipO_2 \) decreases enough (Figs. 6A and 7A) to exert feedback control on the oxidative phosphorylation flux. In this situation, oxidative phosphorylation and anaerobic processes (e.g., glycolysis, creatine kinase, and adenylate kinase) cannot meet the energy demand without a reduction in \( C_{\text{ATP}} \). For the same muscle oxidative capacity (\( V_{\text{max,oxp}} \)), higher glycolytic capacity (i.e., \( \alpha_{\text{gly}} \)) or \( PS \) (i.e., \( P_{\text{Smax}} \)) allows the metabolic system to perform at higher energy demand without a decrease in \( C_{\text{ATP}} \) (Figs. 6 and 7).

Comparison with human experimental data. According to the proportionality between \( k_{\text{ATPase}} \) and ATP demand, it is assumed that \( k_{\text{ATPase}} \) is proportional to \( WR \) [i.e., \( (k_{\text{ATPase}} - k_{\text{ATPase,R}})/(k_{\text{ATPase,max}} - k_{\text{ATPase,R}}) = WR/WR_{\text{max}} \)]. Therefore, the simulated \( ipO_2 \) values in contracting canine muscle were compared with those obtained experimentally in humans (57, 49). At rest, simulated \( ipO_2 \) was similar to the NMR-measured \( ipO_2 \) in humans (58). Simulations were performed with a range of \( PS_{\text{max}} \) values, with \( WR \) increased until \( C_{\text{ATP}} \) decreased to

Fig. 5. \( Q \), arteriovenous \( O_2 \) difference, and \( VO_2 \) for SP and PP canine gastrocnemius muscle stimulated at 100% of \( VO_2\text{peak} \) under normoxia. Experimental data are from Ref. 25. Vertical line denotes transition between rest and contraction. Note that elevated \( Q (Q_m) \) at the onset of contractions at this higher metabolic rate resulted in faster \( VO_2 \) on-kinetics (bottom).

Fig. 6. Effect of glycolytic capacity (\( \alpha_{\text{gly}} \)) on steady-state relationships of intracellular \( P_{\text{O2}} \) (\( ipO_2 \)), \( VO_2 \), \( O_2 \) gradient [extracellular \( P_{\text{O2}} \) (\( ePO_2 \)) - \( ipO_2 \)], and averaged concentration of ATP (\( C_{\text{ATP}} \)) with work rate (\( k_{\text{ATPase}} \)). Solid, dashed, and dotted lines represent simulations obtained for \( \alpha_{\text{gly}} = 0.2, 0.4, \) and 0.6, respectively.
70% of its initial value. For each $PS_{\text{max}}$, $WR_{\text{max}}$ in the experimental canine model corresponds to specific $k_{\text{ATPase,max}}$ values at $Q = 100 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ according to the linear $V_{\text{O2}}$-$Q$ relationship (Fig. 8A). Under spontaneous $Q$, the effect of $PS_{\text{max}}$ on $i\text{PO}_2$ is similar to that observed with elevated blood perfusion (Fig. 9). Qualitatively, the model’s prediction of the $i\text{PO}_2$-$WR$ relationship is similar to that found by Molé et al. (49) and Richardson et al. (57). At low $WR$, the simulated $i\text{PO}_2$ is higher than that observed during knee extensor exercise (57).

DISCUSSION

Experimental studies with human skeletal muscles show apparently contradictory results with respect to the change of $i\text{PO}_2$ with increased energy demand ($WR$). One study (49) showed that $i\text{PO}_2$ decreased linearly as $WR$ increased, while others (55, 57) found that $i\text{PO}_2$ decreased with increased $WR$ to a plateau $\sim 60\%$ of $V_{\text{O2peak}}$. Because these studies examined different muscles during different types of exercise, their results are difficult to compare directly. Furthermore, reliable...
was modified to simulate experiments with perfused canine muscle in situ under different conditions using a scaling parameter \( \alpha_{\text{gly}} \) for the maximal glycogenolytic fluxes. When \( \alpha_{\text{gly}} = 0.2 \), the glycogenolytic fluxes involving ATP (\( \Phi_{\text{gly}}^{\text{ATP}} \)) contribute \( \sim 10\% \) of the ATP required to meet moderate ATP demand. This corresponds to the percentage found for a canine muscle preparation during moderate contraction intensity (11, 12). However, if \( \alpha_{\text{gly}} \) is set at \( >0.2 \), then the simulated tissue lactate concentration and glycolytic contributions to the energy demand are greater than those found under physiological conditions for canine muscle contraction (30).

Model simulations describe \( C_{\text{A-V}} \) and \( V_{\text{O}_2} \) responses to different \( Q \) and \( O_2 \) input concentrations to energy demand (Figs. 2–5). These mimic experimental responses from electrically stimulated canine gastrocnemius muscle (23–25). While steady-state ATP demand must be estimated for each condition, the model is able to successfully predict \( V_{\text{O}_2} \) and \( C_{\text{A-V}} \) dynamics without adjustment of any parameters. Simulations (Figs. 2–4) show that, in the canine gastrocnemius, convective transport does not limit oxidative metabolism at submaximal energy demand (23, 24) but could limit it at maximal energy demand (Fig. 5), e.g., \( 100\% V_{\text{O}_2\text{peak}} \) (25). At maximal contraction intensity, the rise time of \( V_{\text{O}_2} \) dynamics in response to a step increase of energy demand is shorter under elevated PP (controlled) \( Q \) than under SP (spontaneous) \( Q \). This indicates a convective limit to \( O_2 \) transport rate from blood to tissue, because controlled, elevated \( Q \) can meet \( O_2 \) demand more quickly. Nevertheless, factors such as oxidative and glycolytic capacity and \( O_2 \) convection (SP and PP) patterns, as well as diffusion, can affect the relationship between iPO2 and energy demand. These results provide the basis for model validity with respect to mechanisms of the \( V_{\text{O}_2} \) dynamics. Consequently, the model is sufficiently robust to predict underlying responses associated with \( O_2 \) transport and metabolism that have not been measured.

**Relationship Between iPO2 and Energy Demand**

**Effect of PS on intracellular \( O_2 \) and \( O_2 \) diffusion.** When the \( O_2 \) transport rate increases to match ATP demand, the \( O_2 \) gradient adjusts according to \( PS_{\text{max}} \). With increased energy demand from low to high contraction rate, the blood-tissue \( O_2 \) gradient increases linearly (Fig. 7C), which causes \( O_2 \) diffusion to increase linearly as well. At lower \( PS_{\text{max}} \), the \( O_2 \) gradient is increased for the same \( k_{\text{ATPase}} \) value (Fig. 7C) to provide enough muscle \( O_2 \) delivery and sustain the same muscle \( V_{\text{O}_2} \) and \( O_2 \) utilization at submaximal energy demand (Fig. 7B). Consequently, at every metabolic rate, the \( O_2 \) gradient is higher and the average \( i\text{PO}_2 \) or \( \langle i\text{PO}_2 \rangle \) is lower than the values obtained at higher \( PS_{\text{max}} \) (Figs. 7A and 9), although \( V_{\text{O}_2} \) is unchanged (Fig. 7C). Thus, under physiological conditions, \( PS_{\text{max}} \) can affect (\( e\text{PO}_2 \)) and \( \langle i\text{PO}_2 \rangle \) temporal profiles during muscle contraction. At higher energy demand and lower \( PS_{\text{max}} \), the \( O_2 \) gradient and, therefore, \( O_2 \) diffusion cannot increase further, because \( \langle i\text{PO}_2 \rangle \) is close to zero (Fig. 7A). As a result, oxidative phosphorylation is limited according to Eq. 17 and \( V_{\text{O}_2} \) reaches a plateau (Fig. 7B). Higher \( PS_{\text{max}} \) allows \( O_2 \) diffusion and, therefore, \( V_{\text{O}_2} \) to reach plateaus higher than those obtained at lower \( PS_{\text{max}} \). At \( PS_{\text{max}} = 200 \text{ l·l}^{-1} \cdot \text{min}^{-1} \), plateaus occur for \( V_{\text{O}_2} = 16 \text{ ml O}_2 \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1} \) and for \( \langle i\text{PO}_2 \rangle = 10 \text{ mmHg} \) (Fig. 7, A and B). Although \( \langle i\text{PO}_2 \rangle \) is greater than the critical estimates of iPO2 are very difficult to obtain because of heterogeneities within the investigated muscles. However, these different relationships can be explained by a quantitative analysis with our mechanistic, mathematical model that simulates \( O_2 \) transport and cellular metabolism in canine skeletal muscle. The canine muscle preparation was chosen to validate the mathematical model and to quantitatively relate the model-simulated (iPO2) changes to energy demand under conditions of normal (spontaneous) and elevated \( Q \), as well as different \( O_2 \) diffusion and metabolic characteristics, during contraction. In contrast to the experimental conditions in human studies, the canine preparation allows control of muscle \( Q \) to obtain key information on factors affecting muscle \( V_{\text{O}_2} \) kinetics. Model simulations show that the different experimental iPO2-WR relationships are valid for different experimental conditions. The response of (iPO2) to ATP demand depends on \( O_2 \) convection, \( O_2 \) diffusion, and metabolic capacity. In particular, the supply to meet the demand. The glycogenolytic model (44)
PO₂, iPO₂ is close to zero in some areas of the muscle tissue (simulations not reported), limiting O₂ diffusion and utilization. Besides this transport limitation, there is also metabolic limitation resulting in a VO₂ plateau.

These simulation results predict that while ⟨iPO₂⟩ can decrease at higher WR [consistent with some data (49)], PSₘₐₓ is the major regulator of O₂ diffusion between blood and tissue in skeletal muscle [consistent with other data (57)]. For the same energy demand, PSₘₐₓ and the O₂ gradient are not alternative factors regulating O₂ diffusion. Rather, PSₘₐₓ is the controlling factor that determines ⟨iPO₂⟩ and the O₂ gradient. Even though PSₘₐₓ regulates O₂ diffusion, the O₂ gradient is important under various physiological conditions. NMR studies (10, 49, 55, 57) report a rapid decline in Mb saturation fraction at the onset of contraction, which reflects a rapid decrease in iPO₂. This dynamic response is also evident in model simulations. As a result, the simulated O₂ gradient from blood capillary to tissue increases, which enhances O₂ diffusion during muscle contraction, as indicated by other modeling (43) and experimental (62) studies.

PS can also affect the relationship between ⟨iPO₂⟩ and WR (%WRₘₐₓ - Fig. 9). Simulations show that ⟨iPO₂⟩ decreases significantly as %WRₘₐₓ increases in the lower range of WR. The rate of decrease is lower at higher WR, especially for lower values of PSₘₐₓ. These results indicate that the experimental ϕO₂-%WRₘₐₓ relationships reported in the plantar flexion (49) and knee extensor (57) studies, which appear to be different, may be produced by different muscle PS, which depends on the experimental conditions.

Resolution of apparent experimental discrepancies. These simulations explain quantitatively how O₂ diffusion may limit the capacity experimentally determined by other groups (59). Other specific models and analysis are required to study the ison between simulated and measured PO₂ in blood and tissue domains, as well as between simulated PS and O₂ diffusion capacity experimentally determined by other groups (59). Changes in PS may be related to physical factors such as vasodilation, capillary recruitment, and RBC spacing in capillaries (18, 32, 52, 53), which have been proposed to explain an increase in O₂ delivery during contraction. A mechanistic mathematical model is required to investigate the relationship between PS and these factors (18, 26) and their effects on O₂ transport at the microvascular level. This study does not incorporate such mechanisms that determine PS changes but, rather, deals with the phenomenological role of PS and blood-tissue O₂ gradient in determining the O₂ flux. Regardless of the underlying mechanisms, PS increases at the onset of muscle contraction. The conclusions reached from this study are based on simulated PS changes during exercise, which are consistent with previous studies (18, 26, 55). The mathematical model accounts for systemic Hct, but not capillary microvascular Hct. Capillary Hct, as measured in the microvasculature, is generally lower than systemic Hct due to differences in the velocity between plasma and RBCs (40).

Effects of glycogenolytic capacity. Greater glycolytic capacity (αₐ₅₆) is associated with higher ⟨iPO₂⟩, but lower VO₂ (Fig. 6, A–B) and oxidative phosphorylation. Under this condition, glycolgenesis (through ϕₐ₅₆ ATP) consumes more ADP and produces more ATP for the same energy demand. This lower ADP concentration reduces oxidative phosphorylation by feedback mechanisms; therefore, the oxidative contribution to the energy demand is smaller in muscle with higher glycolytic capacity. When αₐ₅₆ is higher, ⟨iPO₂⟩ reaches a plateau at slightly higher energy demand due to the smaller oxidative contribution. In our mathematical model, ⟨iPO₂⟩ is indirectly related to the glycolgenolytic capacity through the effect of ATP/ADP changes on the aerobic and anaerobic metabolic fluxes. These regulation mechanisms are in agreement with those proposed in a previous in silico study (7).

Model Achievements, Limitations, and Future Developments

Factors affecting O₂ transport. Our model not only simulated an iPO₂ plateau similar to the results of Richardson et al. (55), but it also predicted a VO₂ plateau not yet observed. Model simulations show that, at the same energy demand and VO₂, iPO₂ can be drastically different because of the capillary PS (Fig. 7). Corresponding to the experimental data (55), these simulations suggest that PSₘₐₓ may increase with energy demand. In the mathematical model, PS is represented by an empirical time function associated with a step change in Q, which can be justified by experimental studies (8, 65). Although this model assumes that PSₘₐₓ is constant and does not vary with energy demand, previous canine studies have reported an increase of PS with Q and energy demand (19). Even when Q is held constant, PS may increase with energy demand (17).

Changes in PS may be related to physical factors such as vasodilation, capillary recruitment, and RBC spacing in capillaries (18, 32, 52, 53), which have been proposed to explain an increase in O₂ delivery during contraction. A mechanistic mathematical model is required to investigate the relationship between PS and these factors (18, 26) and their effects on O₂ transport at the microvascular level. This study does not incorporate such mechanisms that determine PS changes but, rather, deals with the phenomenological role of PS and blood-tissue O₂ gradient in determining the O₂ flux. Regardless of the underlying mechanisms, PS increases at the onset of muscle contraction. The conclusions reached from this study are based on simulated PS changes during exercise, which are consistent with previous studies (18, 26, 55).

The mathematical model accounts for systemic Hct, but not capillary microvascular Hct. Capillary Hct, as measured in the microvasculature, is generally lower than systemic Hct due to differences in the velocity between plasma and RBCs (40). Our model was developed to provide a sufficiently detailed description of the overall O₂ transport in the whole muscle without describing the microvascular effects. It allows direct comparison between simulated and measured PO₂ in blood and tissue domains, as well as between simulated PS and O₂ diffusion capacity experimentally determined by other groups (59). Other specific models and analysis are required to study the effect of Hct differences at the microvascular level (4).
within different blood and tissue domains. Consequently, model simulations are not able to distinguish, for example, cytosolic gradients of \( \text{O}_2 \). Such distinctions require a more detailed model (26). However, these distinctions are not expected to affect the overall relationships of \( PS \) and \( \text{O}_2 \) gradient to \( i\text{PO}_2 \).

**Factors affecting the \( i\text{PO}_2\)-WR relationship.** With our model, we were able to analyze factors affecting the \( i\text{PO}_2\)-ATP demand relationship in contracting canine muscle by simulating \( i\text{PO}_2 \) in canine muscle at different WR. Comparisons are made to \( i\text{PO}_2 \) measured in human muscle by NMR under knee extensor and plantar flexion (Fig. 9). This comparison, however, is limited by differences between humans and dogs, as well as differences in protocols, muscle fiber recruitment, maximal Q, and metabolic rate. Under the assumption that ATP demand changes proportionally with WR, the simulated results predict an \( i\text{PO}_2\)-WR relationship qualitatively similar to that observed in humans.

\( PS \) and the \( \text{O}_2 \) gradient are not the only factors that can affect \( i\text{PO}_2 \). Other factors might include \( \text{O}_2 \) transport between myocytes (34), \( \text{Mb} \) buffering within myocytes (33), and the critical \( \text{PO}_2 \) of \( \text{Mb} \) buffering within myocytes (33), and the critical \( \text{PO}_2 \) of oxidative phosphorylation (45). The sensitivity of \( i\text{PO}_2 \) to these factors may be relatively small, but important, under some conditions. Quantification of the significance of the model parameters \( PS_{\text{max}} \) and \( \alpha_{\text{toy}} \) on \( i\text{PO}_2 \) requires more experimental data under a wide range of physiological conditions.

Fiber recruitment may also affect the \( i\text{PO}_2\)-WR relationship in exercising human muscle (3, 47). The model applied in this work describes metabolic processes in electrically stimulated canine gastrocnemius muscle, where the predominant fibers are highly oxidative and all are recruited at the onset of muscle contraction. The simulated (\( i\text{PO}_2 \)) reported in Fig. 9 is based on a mathematical model of canine muscle that does not take into account fiber-type composition. When type I and II fibers are recruited, muscle \( \text{V}_\text{O}_2 \) and metabolic control may differ from the case when only type I fibers are recruited. This is a consequence of differences in glycolytic and oxidative capacity and perhaps the P/O ratio of fiber types.

**Interpretation of \( i\text{PO}_2 \) changes with energy demand.** Our model simulations show that the \( i\text{PO}_2 \) data of Richardson et al. (57) and Molé et al. (49) (Fig. 9) are not necessarily inconsistent when examined together. The extent to which differences exist between electrically stimulated canine gastrocnemius, human plantar flexion, and human knee extensor exercise may be attributed to experimental conditions that affect the maximal metabolic rate, which differs between muscles and species. The maximal power output is \(~6.6\) times higher for knee extensor (54) than plantar flexion (49) exercise. This difference in power output seems to reflect differences in the metabolic state of each muscle at its \( WR_{\text{max}} \); at 100% \( WR_{\text{max}} \), ATP concentration remains at its resting value in plantar flexion (49) but is reduced by 30% in knee extension (2).

In isometrically stimulated canine gastrocnemius muscle, there is no power output; however, the maximal \( \text{V}_\text{O}_2 \) [16 ml·100 g·1·min\(^{-1}\) (25)] is comparable to that in human plantar flexion [15 ml·100 g·1·min\(^{-1}\) (49)] and lower than that in human knee extension [60 ml·100 g·1·min\(^{-1}\) (54)]. Maximal muscle perfusion also differs between the human knee extensor [385 ml·100 g·1·min\(^{-1}\) (54)] and the isolated canine gastrocnemius in the present study [100 ml·100 g·1·min\(^{-1}\) (25)]. However, the slope of the linear \( Q\)-\( \text{V}_\text{O}_2 \) relationship is similar for canine muscle (5.4 ml/ml; Fig. 8A), knee extensor [5.6 ml/ml (54)], and plantar flexion [6.1 ml/ml (6)]. Although there are protocol differences in maximal metabolic rate and \( Q \), higher \( \text{O}_2 \) delivery is balanced by higher \( \text{V}_\text{O}_2 \). Therefore, these differences between experimental protocols are not expected to affect the \( i\text{PO}_2\)-WR relationship (Fig. 9). We note that \( \text{V}_\text{O}_2\text{peak} \) and \( Q_{\text{max}} \) in canine perfused muscle (25) used to determine the maximal \( k_{\text{ATPase}} \) (Fig. 9; i.e., \( WR_{\text{max}} \)) are lower than those obtained for the same canine gastrocnemius model (\( \text{V}_\text{O}_2\text{max} = 27 \text{ ml·100 g}^{-1}\text{·min}^{-1} \) and \( Q_{\text{max}} = 2.1 \text{ l}\text{·min}^{-1} \)) (38) with a different protocol. The \( \text{V}_\text{O}_2\text{peak} \) commonly reported for canine gastrocnemius studies may not be the true \( \text{V}_\text{O}_2\text{peak} \) achievable in this canine muscle preparation. However, addressing the limitations in reaching \( \text{V}_\text{O}_2\text{peak} \) in a canine muscle preparation is beyond the scope of this report. If the maximal \( \text{V}_\text{O}_2 \) and \( Q \) obtained by Kelley et al. (38) are used to determine \%\( WR_{\text{max}} \) in the canine gastrocnemius, the model simulations reported in Fig. 9 would be leftward-shifted. However, these higher values do not alter the \( Q\)-\( \text{V}_\text{O}_2 \) relationship.

NMR-measured \( i\text{PO}_2 \) (49, 55, 57) was compared with model-simulated (\( i\text{PO}_2 \)) (Fig. 9). The correct interpretation of this comparison of simulated and measured \( i\text{PO}_2 \) requires consideration of the NMR measurement limitations. In particular, \( i\text{PO}_2 \) measurement is affected by \( \text{P}_50 \) (i.e., \( \text{P}_50 \) at which 50% of \( \text{Mb} \) is bound to \( \text{O}_2 \)). The \( \text{P}_50 \) of skeletal muscle in vivo is still uncertain. Experimental values range from 1.5 to 5.5 mmHg (57), which can significantly change the quantitative measurement of \( i\text{PO}_2 \) obtained by NMR studies. For comparison with the model simulations, the data of Richardson et al. (57) assumed \( \text{P}_50 = 3.2 \text{ mmHg} \), while the data of Molé et al. (49) assumed \( \text{P}_50 = 2.9 \text{ mmHg} \). For the model simulations, we assumed \( \text{P}_50 = 2.42 \text{ mmHg} \). Therefore, quantitative comparison between simulated and measured \( i\text{PO}_2 \) may be affected, because \( \text{P}_50 \) may contribute to the different values of measured and simulated \( i\text{PO}_2 \). Although \( i\text{PO}_2 \) calculation is affected by \( \text{P}_50 \), the \( i\text{PO}_2\)-WR relationship will have the hyperbolic trend reported in Fig. 9, regardless of the exact \( \text{P}_50 \).

**Modeling oxidative and glycolytic systems.** The minimal mathematical model used in this study is sufficient for evaluating key factors related to \( \text{O}_2 \) transport and metabolic limitations with respect to increased energy demand. The model components representing skeletal muscle glycogenolysis were adequate to analyze \( \text{O}_2 \) diffusion limitation at higher energy demand. If sufficient experimental data, such as dynamic changes of glycolytic metabolite concentrations and/or fluxes with contraction intensity, were available, better estimates of model parameters could be obtained to further quantify mechanistic processes. Such data are needed to quantitatively analyze the potential mechanisms by which glycolysis is activated at higher WR (13, 21) and how glycogen limitations might affect \( \text{V}_\text{O}_2 \) (51). To account for pH-dependent enzyme kinetics, another model of glycogenolysis could also be incorporated (63a).

The simulated \( \text{V}_\text{O}_2 \) was obtained under the assumption that oxidative phosphorylation is controlled by ADP, \( P_i \), and \( i\text{PO}_2 \) according to previous experimental and modeling studies (5, 9, 43). In contrast, an experimental study (10) reported that \( \text{V}_\text{O}_2 \) is independent of ADP at the onset of contraction. For accurate prediction of key metabolite (e.g., ADP, NAD, and NADH) concentrations associated with regulation of phosphorylation and redox states in response to contraction, a model that distinguishes metabolic processes in cytosol and mitochondria is needed (46). A more detailed model of cellular respiration that includes oxidative phosphorylation, tricarboxylic acid cycle, and \( \beta \)-oxidation (5, 41, 46) would be worth incorporating.
if additional data were available to investigate regulatory mechanisms of energy metabolism in vivo (37). With this more detailed model, which would more accurately simulate NAD-/NADH transport and metabolism, the relationship between iPO2 and the accumulation of anaerobic metabolic markers above the anaerobic threshold could also be investigated.

Experimental studies have observed a linear VO2-WR relationship in skeletal muscle across a wide range of exercise intensities (1, 54, 60). Computational studies have attributed this linear relationship at higher exercise intensity to a parallel activation mechanism affecting oxidative phosphorylation (48). Our model is based on feedback control of ADP and Pi, without the inclusion of detailed activation mechanisms previously proposed (48). Even so, it is able to successfully simulate VO2 kinetics in the canine gastrocnemius at 60% and 100% of VO2peak. At submaximal (60% of VO2peak; i.e., below kATPase,max) and maximal (100% of VO2peak) contraction intensity (i.e., at kATPase,max), the model predicts a linear VO2-ATP demand relationship (Figs. 6B and 7B). Above kATPase,max, this relationship reaches a plateau where VO2 is no longer linearly related to ATP demand (Figs. 6B and 7B). Human studies (15, 35) reported a plateau in the VO2-WR relationship near maximal exercise intensity. However, uncertainties remain with regard to the maximal VO2 attainable in the canine muscle preparation (38). Because maximal VO2 at lower PSmax is primarily limited by O2 transport, inclusion of a parallel activation mechanism in our model would not significantly alter the simulated results and conclusion. Furthermore, in electrically (as opposed to neurally) stimulated muscle at the contraction intensities considered in this study, parallel activation may not occur (36, 42).

**Perspectives and Significance**

Model simulations demonstrate that, depending on experimental conditions, the interaction of O2 diffusion with oxidative and glycolytic processes can lead to different relationships between iPO2, VO2, and ATP demand (49, 57) in the canine gastrocnemius. The results of model simulations indicate that 1) PS is the main factor that regulates O2 diffusion and determines the O2 gradient during muscle contraction and 2) iPO2 may decrease linearly or remain constant at high-intensity exercise according to different muscle O2 transport and metabolic responses to contraction. Consequently, apparently contradictory experimental results (49, 57) relating iPO2 and WR or metabolic rate in humans can be quantitatively reconciled.

Model simulations can predict quantitatively how O2 transport from capillaries to myocytes affects VO2 under different physiological and pathophysiological conditions. These possible variations can change not only steady-state VO2, but also VO2 kinetics. In conjunction with experimental studies, model simulations provide a framework in which to distinguish transport and metabolic limitations on VO2 and iPO2. Furthermore, model predictions provide an efficient basis on which to design critical, new experiments to investigate the distinctive effects of O2 convection and diffusion in limiting O2 utilization during muscle contraction.

**APPENDIX A**

**Dynamic Mass Balance**

The model is spatially distributed in blood and tissue compartments. All metabolites are present in the tissue compartment. The blood compartment only includes O2, lactate, and pyruvate.

### Blood compartment.

\[
\frac{\partial C_{O_2,b}}{\partial t} = -Q \frac{\partial C_{O_2,b}}{\partial v} + D_{O_2,b} \frac{\partial^2 C_{O_2,b}}{\partial v^2} - \frac{f_{O_2}}{\gamma_{O_2,b}} \quad (A1)
\]

\[
\frac{\partial C_{lac,b}}{\partial t} = -Q \frac{\partial C_{lac,b}}{\partial v} - \frac{f_{lac}}{\gamma_{lac,b}} \quad (A2)
\]

\[
\frac{\partial C_{pyr,b}}{\partial t} = -Q \frac{\partial C_{pyr,b}}{\partial v} - \frac{f_{pyr}}{\gamma_{pyr,b}} \quad (A3)
\]

### Tissue compartment.

\[
\frac{\partial C_{O_2,t}}{\partial t} = \frac{f_{O_2}}{\gamma_{O_2,t}} + D_{O_2,t} \frac{\partial^2 C_{O_2,t}}{\partial v^2} - \frac{f_C \phi_{Oxp}}{\gamma_{O_2,t}} \quad (A4)
\]

\[
\frac{\partial C_{lac}}{\partial t} = \frac{f_{lac}}{f_C} + \phi_{LDH} \quad (A5)
\]

\[
\frac{\partial C_{pyr}}{\partial t} = \frac{f_{pyr}}{f_C} + \phi_{PK} - \phi_{LDH} \quad (A6)
\]

\[
\frac{\partial C_{GAP}}{\partial t} = \Psi_{GP} - \phi_{PGLM} \quad (A7)
\]

\[
\frac{\partial C_{PGM}}{\partial t} = \phi_{PGLM} - \phi_{PGI} \quad (A8)
\]

\[
\frac{\partial C_{PPK}}{\partial t} = \phi_{PGI} - \phi_{PFK} \quad (A9)
\]

\[
\frac{\partial C_{ADP}}{\partial t} = \phi_{ADP} - 2\phi_{ADK} + \phi_{GAPDH} \quad (A10)
\]

\[
\frac{\partial C_{AMP}}{\partial t} = -\phi_{ADK} \quad (A11)
\]

\[
\frac{\partial C_{PCr}}{\partial t} = \phi_{CK} \quad (A12)
\]

\[
\frac{\partial C_{NADH}}{\partial t} = \phi_{GAPDH} - \phi_{LDH} \quad (A13)
\]
O\textsubscript{2} Equilibrium

\(O_2\) can be free or bound to Hb or Mb. The sum of free and bound \(O_2\) is total \(O_2\) concentration

\[
C_{O_2}^{T} = C_{O_2}^{B} + C_{O_2}^{c}
\]

Free and bound \(O_2\) concentrations are related by local chemical equilibria. In blood, the relationship is

\[
C_{O_2}^{B} = 4Hct \frac{K_{Hb}(C_{O_2}^{f})^{nH}}{1 + K_{Hb}(C_{O_2}^{f})^{nH}}
\] (B2)

and in tissue the relationship is

\[
C_{O_2}^{B,c} = \frac{K_{Mb}C_{O_2}^{f}}{1 + K_{Mb}C_{O_2}^{f}}
\] (B3)

Transport Fluxes

The axial fluxes for the substrates (\(O_2\), lactate, and pyruvate) present in the blood and tissue compartments are as follows

\[
J_{O_2}^{b,c} = PS(C_{O_2}^{f} - C_{O_2}^{c})
\] (B4)

\[
J_{lac}^{b,c} = T_{lac} \left( \frac{C_{lac,b}^{c}}{M_{lac} + C_{lac,b}^{c}} - \frac{C_{lac,c}}{M_{lac} + C_{lac,c}} \right)
\] (B5)

\[
J_{pyr}^{b,c} = T_{pyr} \left( \frac{C_{pyr,b}^{c}}{M_{pyr} + C_{pyr,b}^{c}} - \frac{C_{pyr,c}}{M_{pyr} + C_{pyr,c}} \right)
\] (B6)

Metabolic Fluxes

Most of these fluxes are calculated using ordered bi-bi Michaelis-Menten kinetics. The exceptions are the creatine kinase flux, the oxidative phosphorylation flux, and the ATPase flux.

The metabolic flux expression for each reaction is reported as follows.

Glycogen phosphorylase

\[
\Phi_{GPB} = \frac{V_{max,f,GPB}C_{gly}C_{p}}{K_{gly}K_{gly}^{f}} - \frac{V_{max,r,GPB}C_{gly}C_{gbp}}{K_{gly}K_{gly}^{r}}
\] (B7)

Phosphoglucomutase

\[
\Phi_{PGM} = \frac{V_{max,f,PGM}C_{gbp}}{K_{gbp}K_{gbp}^{f}} - \frac{V_{max,r,PGM}C_{gbp}}{K_{gbp}K_{gbp}^{r}}
\] (B10)

Phosphoglucoseisomerase

\[
\Phi_{PGI} = \frac{V_{max,f,PGI}C_{gbp}}{K_{gbp}K_{gbp}^{f}} - \frac{V_{max,r,PGI}C_{gbp}}{K_{gbp}K_{gbp}^{r}}
\] (B11)

Phosphofructokinase

\[
\Phi_{PFK} = \frac{V_{max,f,PFK}C_{ATP}C_{gbp}}{K_{ATP}K_{gbp}} - \frac{V_{max,r,PFK}C_{ADP}C_{gbp}}{K_{ADP}K_{gbp}}
\] (B12)

\[
\Delta = \left( 1 + \frac{C_{gbp}^{f}}{K_{gbp}} \right) \left( 1 + \frac{C_{ATP}}{K_{ATP}} \right) \frac{C_{ADP}}{K_{ADP}} \frac{C_{gbp}^{r}}{K_{gbp}} \left( 1 + \frac{C_{ADP}}{K_{ADP}} \right)
\] (B13)

\[
\Delta = \left( 1 + \frac{C_{gbp}^{f}}{K_{gbp}} \right) \left( 1 + \frac{C_{ATP}}{K_{ATP}} \right) \frac{C_{ADP}}{K_{ADP}} \frac{C_{gbp}^{r}}{K_{gbp}} \left( 1 + \frac{C_{ADP}}{K_{ADP}} \right)
\] (B14)

Aldolase

\[
\Phi_{ALD} = \frac{V_{max,f,ALD}C_{gbp}}{K_{gbp}K_{gbp}^{f}} - \frac{V_{max,r,ALD}C_{gbp}}{K_{gbp}K_{gbp}^{r}}
\] (B16)

Triose phosphate isomerase

\[
\Phi_{TPH} = \frac{V_{max,f,TPH}C_{gbp}}{K_{gbp}K_{gbp}^{f}} - \frac{V_{max,r,TPH}C_{gbp}}{K_{gbp}K_{gbp}^{r}}
\] (B17)

Glyceraldehyde-3-phosphate dehydrogenase

\[
\Phi_{GAPDH} = \frac{V_{max,f,GAPDH}C_{ebp}C_{NAD}C_{p}}{K_{gbp}K_{NAD}K_{p}^{f}} - \frac{V_{max,r,GAPDH}C_{13bp}C_{NADH}}{K_{13bp}K_{NADH}^{r}}
\] (B18)

where \(D\) is calculated as
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.S., G.M.S., and N.L. are responsible for conception and design of the research; J.S., L.B.G., B.G., G.M.S., and N.L. analyzed the data; J.S., L.B.G., B.G., G.M.S., and N.L. interpreted the results of the experiments; J.S. prepared the figures; J.S., G.M.S., and N.L. drafted the manuscript; J.S., L.B.G., B.G., G.M.S., and N.L. edited and revised the manuscript; J.S., L.B.G., B.G., G.M.S., and N.L. approved the final version of the manuscript; L.B.G. and B.G. performed the experiments.

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