Effect of temperature on the myoglobin-facilitated transport of oxygen in skeletal muscle

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ABSTRACT An analysis of thermal effects on the facilitative transport of oxygen in skeletal muscle fibers is presented. Steady-state mass and energy transport balances are written and solved analytically or numerically using a finite-difference procedure. It is shown that no significant spatial thermal gradients exist due to internal reactions or bulk conduction effects across a muscle fiber. At typical muscle conditions, it is predicted that increased global temperature reduces the fraction of oxygenated myoglobin, increases local oxygen concentrations, and increases the percentage of oxygen flux attributed to oxy-myoglobin. The maximum supportable oxygen consumption rate, $\dot{m}_{O_2}^{max}$, is defined as the highest consumption rate sustainable without developing anoxic regions at the center of the fiber. By considering only temperature sensitive effects within fibers, $\dot{m}_{O_2}^{max}$ is found to increase slightly with temperature at low temperatures. This increase is due to thermal effects on the diffusion coefficients as opposed to effects associated with the kinetics of the myoglobin-oxygen reaction. If the simulations include the temperature effect associated with oxygen solubility in blood plasma, $\dot{m}_{O_2}^{max}$ decreases with temperature. A sensitivity analysis was performed by varying the values of relevant parameters. The maximum consumption rate was least affected by parameters associated with the kinetic and equilibrium constants and most affected by the diffusion coefficients and the concentration of myoglobin.

1. INTRODUCTION

Myoglobin is a protein which reversibly binds oxygen, and the role of myoglobin in the facilitated transport of oxygen has drawn much interest. In addition to its function as an oxygen store in diving mammals and birds, where very large concentrations of myoglobin have been found, it also plays a significant role in oxygen transport. Two modeling approaches have been presented in the literature. The first considers the diffusion of oxygen and myoglobin through a layer of tissue without oxygen consumption. The boundary concentrations of oxygen are hypothesized as being different on the two sides of the tissue providing the driving force for transport. Consumption enters this problem only indirectly as the mechanism needed to maintain the low oxygen concentration boundary. This approach was used by Kutchai et al. (1970) to study hemoglobin facilitation of oxygen, and later by Gonzalez-Fernandez and Atta (1986) to study myoglobin facilitation. The latter group has also modeled facilitative transport in tissue with myoglobin impermeable membrane layers (Gonzalez-Fernandez and Atta, 1982; Gonzalez-Fernandez, 1989, 1990). All of these models show increased oxygen fluxes in the presence of myoglobin.

The second approach is to consider oxygen consumption within the tissue. Physiochemically, the two processes of transport and consumption are coupled. Murray (1974) was the first to take this approach, assuming oxygen consumption to be spatially constant. Van Ouwerkerk (1977) then modeled separately the oxygen-rich and anoxic regions that occur at relatively large consumption rates. More recently, Covell and Jacquez (1987) considered oxygen consumption to occur not homogeneously but in bands corresponding to anatomical layers of mitochondria, and Loiselle (1987) considered oxygen consumption to be dependent on the free oxygen concentration.

In addition, there have been a few published models that tie together the problem of oxygen transport within fibers with external transport mechanisms. Fletcher (1980) and Salathé and Kolkka (1986) have presented models that include transport from capillary blood, and Stroeve (1982) has considered myoglobin-facilitated transport from the perspective of fibers being imbedded within a heterogeneous matrix of tissue. All of these models include terms for oxygen consumption within individual fibers.

None of the previous modeling work has directly studied the effect of temperature on the facilitation process. Stevens and Carey (1981) and Stevens (1982) argue that one of the adaptive advantages of being warm

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0006-3495/91/07/160/12 $2.00

blooded is an enhancement of myoglobin-facilitated oxygen transport. They also postulate that a similar benefit exists for animals when muscle temperatures rise during exercise. This temperature-enhanced transport is believed to be caused by effects associated with the presence of myoglobin and not by effects associated with the diffusion of free oxygen. Temperature does have an influence on contraction properties, for example, increasing temperature has been found to increase the maximum contraction speed and the isometric tension in muscle fibers (Lännergren et al., 1982) and in whole muscle (Ranatunga, 1982). Temperature has also been found to affect exercise response, with maximal work duration and oxygen uptake correlating positively with temperature increases (Bergh and Ekblom, 1979).

In this work we investigate two questions. First, should we expect to find temperature gradients within actively contracting muscle? In principle, increased temperature at the fiber core would enhance the dissociation of oxygen from myoglobin. This could produce larger oxy-myoglobin concentration gradients resulting in a greater total oxygen flux. Second, regardless of whether there are spatial gradients in temperature, what is the effect of a global increase in temperature on the facilitation process? A range of temperatures is studied to cover the possible adaptive benefits for homeotherms (20–37°C) and the possible benefits related to temperature increases that occur during exercise (33–42°C).

GLOSSARY

A	Arrhenius activation energy
c', c_1–c_4	dimensionless constants
D_{O_2}, D_{Mb}, D_{MbO_2}	diffusion coefficients for oxygen, myoglobin, and oxy-myoglobin
J_{total}, J_{fac}	molar flux of total oxygen, facilitated oxygen flux
k, k'	association and dissociation myoglobin-oxygen rate constants
K_c, K_p	myoglobin-oxygen equilibrium constants
k_{eff}	thermal conductivity
L	diffusional distance (half the tissue thickness)
Mb	myoglobin
MbO_2	oxygenated myoglobin or oxy-myoglobin
$\dot{m}_{O_2}$	oxygen consumption rate
$\dot{m}_{O_2}^{max}$	maximum oxygen consumption rate
$[O_2]_b$	oxygen concentration at boundary ($\chi = 0$)
P	total myoglobin concentration
P_{O_2}	oxygen partial pressure
Q	calorific oxygen equivalent
R	gas constant
r	particle radius
$-r_{O_2}$	net rate of myoglobin-oxygen reaction
T	temperature
T_b	boundary temperature
x	distance into fiber
Y	dimensionless oxy-myoglobin concentration
α	Henry's law constant or oxygen solubility
ΔH	heat of myoglobin-oxygen reaction
Θ	dimensionless temperature
K	Boltzmann's constant
κ	Arrhenius frequency factor
μ	viscosity
ω	dimensionless oxygen concentration
χ	dimensionless distance

2. MODEL DEVELOPMENT

Model equations

(All symbols used in the development presented below are defined in the glossary.)

Three geometric arrangement of muscle fibers relative to the capillary bed have been considered in the literature: (*a*) a tissue of large radius perfused by a centrally located capillary in which diffusion occurs into a widening area (the Krogh model); (*b*) a cylinder of tissue completely surrounded by an annular blood pool in which components diffuse into a contracting area; (*c*) a flat sheet of material surrounded on both sides by blood with the same concentration of dissolved oxygen.

Covell and Jacquez (1987) describe the anatomical features of skeletal muscle as it pertains to this problem. They show that the physical arrangement of fibers and capillaries is such that there are four to six evenly spaced capillaries embedded around individual fibers. None of the geometries listed above ideally describe this anatomy, the appropriate configuration being something between the two radial extremes. Gonzalez-Fernandez and Atta (1986) note that the one-dimensional flat sheet geometry is an intermediate approximation of the two cylindrical extremes in that diffusion does not occur into an expanding or contracting region of tissue. While this geometry does not represent the physical structure, it is a representation that attempts to account for the reduced radial dispersion that occurs between two capillaries due to the surrounding capillaries. Hence, this geometry has been used in this work.

Myoglobin has a single oxygen binding site and is present in the muscle cytosol. The protein binds to oxygen in a reversible reaction of the form

$$Mb + O_2 \Leftrightarrow MbO_2. \quad (1)$$

The net rate of oxygen binding to myoglobin can be expressed as

$$-r_{O_2} = k[O_2][Mb] - k'[MbO_2] \quad (2)$$

which can be rewritten

$$-r_{O_2} = k[O_2][Mb] - \frac{k}{K_c}[MbO_2] \quad (3)$$

by incorporating the reaction equilibrium constant, K_c. This formulation enables one to represent the temperature effects of the kinetic constants in terms of the association rate constant and the equilibrium constant. This is preferable because more information is available on the temperature dependence of the equilibrium constant.

The steady-state diffusion of oxygenated myoglobin and free oxygen can be expressed as

$$D_{MbO_2}\frac{d^2[MbO_2]}{dx^2} = r_{O_2} \quad (4)$$

$$D_{O_2}\frac{d^2[O_2]}{dx^2} = -r_{O_2} + \dot{m}_{O_2}, \quad (5)$$

as has been derived by others (see, for example, Murray, 1974, or Loiselle, 1987). Each of these equations states that the change in concentration due to diffusion is just counterbalanced by the change due to chemical reaction. For the oxygen equation two reactions are considered: (*a*) the reversible reaction with myoglobin and (*b*) the irreversible metabolic consumption.

Writing a steady-state energy balance on the muscle tissue gives

$$k_{eff}\frac{d^2T}{dx^2} = \Delta H(-r_{O_2}) - Q\dot{m}_{O_2}, \quad (6)$$

where ΔH is the heat of reaction and Q is the calorific oxygen equivalent. Physically, this equation can be interpreted as stating that the net rate of thermal conduction (LHS) is equal to the rate of heat generated by the myoglobin-oxygen reaction (first term, RHS) plus the heat generated by other metabolic processes in the tissue (second term, RHS).

For simplicity, the rate of oxygen consumption will be taken as a homogeneous constant throughout the muscle tissue. This is a simpler formulation than that presented by Covell and Jacquez (1987), who used a sinusoidal function to account for a nonhomogeneous distribution of mitochondria. They found that, except at very low boundary oxygen concentrations (boundary $P_{O_2} < 5$ torr), the oxygen profiles did not show large spatial variations corresponding to the regions of high and low oxygen consumption. Apparently, diffusion is rapid enough relative to the oxidation rate to smooth the profiles. At $P_{O_2} \geq 10$ torr the concentration profiles did not differ significantly from those obtained by considering homogeneous consumption.

Energy balance analysis

Defining the following dimensionless variables:

$$\chi = \frac{x}{L} \text{ and } \Theta = \frac{T}{T_b}$$

and substituting into Eq. 6 gives

$$\frac{d^2\Theta}{d\chi^2} = \frac{L^2}{k_{eff}T_b}\{\Delta H(-r_{O_2}) - Q\dot{m}_{O_2}\}. \quad (7)$$

Comparing the magnitude of the two terms within the brackets, it can be shown that the second term is much larger than the first, even when considering the maximum possible heat produced by the myoglobin reaction (by setting $[MbO_2] = 0$ in Eq. 3). Neglecting the first term this becomes

$$\frac{d^2\Theta}{d\chi^2} \cong -\frac{L^2}{k_{eff}T_b}Q\dot{m}_{O_2} = -c' \quad (8)$$

which, in general, describes the steady-state temperature profile that results from internal heat production in any oxygen-consuming homogeneous material.

Table 1 summarizes the values of the constants used in Eq. 8 and the equations to follow. A value for the thermal conductivity of biological tissue has been reported by Jain et al. (1979) to be 0.764×10^{-3} cal/(cm s °C). This value is a little less than that typically used for whole muscle calculations (see Seagrave, 1971), the difference being that the standard value for whole tissue incorporates some element of convective transport from blood flow in addition to the conductive transport. Because no convective transport is present within individual muscle fibers, the value reported by Jain et al. (1979) is appropriate for this work. Values for the calorific oxygen equivalent are discussed by Seagrave (1971). A value of 4.8 cal/(ml O_2) is applicable for the heat generated from a typical diet. For the diffusional distance or the half-tissue thickness we have used a reference value of 20 μm. This is approximately half the diameter of red muscle fibers (e0–35 μm) and is within the range used by other modelers: 10–20 μm (Gonzalez-Fernandez and Atta, 1986), 15–30 μm (Loiselle, 1987), and 25 μm (Murray, 1974; Covell and Jacquez, 1987; Gonzalez-Fernandez, 1989, 1990). Oxygen consumption rates for skeletal muscle have been reported by Folkow and Halicka (1968) for both resting and active tissue. A value of 6.0×10^{-8} mol/(cm^3 s) or 8.0×10^{-2} ml O_2/(ml min) for active muscle is used here, which corresponds their value for red soleus muscle. Similar values have been used by Jacquez (1984) and Covell and Jacquez (1987). Murray used a slightly smaller value of 5.0×10^{-8} mol/(cm^3 s).

Integrating the heat equation (ignoring the small

TABLE 1 **Parameters values used in the thermal analysis of myoglobin-facilitative oxygen transport in muscle tissue**

Parameter	Value	Units	Reference
k_{eff}	0.764×10^{-3}	*cal/(cm s °C)*	Jain et al. (1979)
Q	4.8	*cal/ml* O_2	Seagrave (1971)
	(1.08×10^5)	*(cal/mol)*	
L	20.0	*μm*	see text
	(2.0×10^{-3})	*(cm)*	
D_{Mb} and D_{MbO_2} (37°C)	1.0×10^{-6}	cm^2/s	Jacquez (1984)
D_{O_2} (37°C)	1.6×10^{-5}	cm^2/s	Jacquez (1984)
$\dot{m}_{O_2}$	8.0×10^{-2}	*ml* $O_2/(cm^3\ s)$	Folkow and Halicka (1968)
	(6.0×10^{-8})	$(mol/(cm^3\ s))$	
P	8.0	*mg/ml*	Jacquez (1984)
	(4.44×10^{-7})	$(mol/(cm^3))$	
$[O_2]_b$	2.52×10^{-8}	mol/cm^3	equilibrium value with P_{O_2} = 20 torr at 37°C (solubility from Siggaard-Andersen (1974))
ΔH	-1.3×10^4	*cal/mol*	Rossi-Fanelli and Antonini (1958)
A	5.5×10^3	*cal/mol*	see Antonini and Brunori (1971)
k (20°C)	1.4×10^{-7}	$1/(M\ s)$	see Antonini and Brunori (1971)
K_c (20°C)	8.11×10^5	$1/M$	see text

contribution of the myoglobin-oxygen reaction) with the boundary conditions

$$\Theta = 1 \text{ at } \chi = 0 \quad \frac{d\Theta}{d\chi} = 0 \text{ at } \chi = 1$$

gives

$$\Theta - 1 = \frac{(T - T_b)}{T_b} = c'\chi\left[1 - \frac{\chi}{2}\right]. \tag{9}$$

We find that the largest temperature differences occur at the center of the tissue ($\chi = 1$) and that the maximum variation will be $c'/2$. For typical fiber conditions this gives a temperature rise of 1.7×10^{-5} °C at the tissue core. This estimate was confirmed numerically by solving the coupled problem with the terms for the myoglobin-oxygen reaction and was found to be $\sim 1.1 \times 10^{-5}$ °C (Murali, 1989). It is clear from Eqs. 8 and 9 that the temperature difference increases with the square of the fiber thickness. To generate a difference of 0.1% or 0.3°C, the diffusional distance needs to be on the order of 1,500 μm. Because active skeletal muscle fibers have much smaller radii, significant temperature gradients apparently do not occur because of thermal effects associated with internal reactions.

It is possible that a thermal gradient could exist because of external effects; e.g., the muscle is embedded within tissue that lies along the conductive path between a high body core temperature and ambient conditions. Temperature differences have been reported in human quadriceps muscle by Saltin et al. (1968). At an ambient temperature of 20°C and for muscle in a resting state, temperature differences on the order of one degree were found with 20 mm of tissue depth. Scaling this difference across an individual fiber, a negligible gradient of 0.001 °C per half fiber is calculated. Hence, we conclude that there are no significant temperature gradients within individual muscle fibers due to either internal reactions or imposed external conditions. The temperature of the muscle tissue then can be taken as constant and equal to that of the locally perfusing blood.

Although there apparently are no significant thermal gradients, muscle temperatures do change globally with prolonged periods of activity. Brooks et al. (1971) found temperature increases of 8°C in rats after exhaustive treadmill running. In human studies researchers have shown that the temperature change can be several degrees celsius with submaximal levels of activity (Saltin and Hermansen, 1966; Saltin et al., 1968). The change found at any one location could be 5–6°C, and temperatures ranged from between 33°C (at rest) and 41°C (after an hour of activity). Stevens and Carey (1981) suggest that there are metabolic advantages in operating at higher temperatures. They believe that this advantage is related to thermal effects on the myoglobin-facilitated transport of oxygen within muscle fibers. Stevens (1982) has conducted experiments using a liquid-membrane system with hemoglobin that suggests there is a temperature effect on the rate of facilitated transport, although

it is not clear how the time constant he measured is related to physical parameters, or how the conditions of the experiment are related to in vivo muscle conditions. Therefore, we now investigate the effect of a global temperature change on the facilitation process. Because muscle fibers are spatially isothermal, the steady-state heat and mass balance equations are uncoupled, and one need focus only on how the temperature affects the coefficients in the mass balances.

Mass balance analysis

Dimensionless myoglobin and oxygen concentrations can be defined as

$$Y = \frac{[MbO_2]}{P} \omega = \frac{[O_2]}{[O_2]_b},$$

where P represents the total myoglobin concentration. With these definitions Eqs. 5 and 6 can be written:

$$\frac{d^2Y}{d\chi^2} = -c_2\left\{(1 - Y)\omega - \frac{Y}{c_1}\right\} \tag{10}$$

$$\frac{d^2\omega}{d\chi^2} = c_3\left\{(1 - Y)\omega - \frac{Y}{c_1}\right\} + c_4 \tag{11}$$

with the boundary conditions:

$$\omega = 1 \quad \text{at} \quad \chi = 0 \qquad \frac{d\omega}{d\chi} = 0 \quad \text{at} \quad \chi = 1$$

$$\frac{dY}{d\chi} = 0 \quad \text{at} \quad \chi = 0, 1.$$

The assumption is made that any resistance to oxygen transport through the fiber membrane at the boundary can be ignored. Thus the boundary oxygen concentration is equal to the plasma concentration. Because myoglobin cannot diffuse through the fiber membrane, its gradient is set as zero at the boundary. At the center of the fiber both species gradients must be zero because of symmetry.

The four dimensionless constants that appear in Eqs. 10 and 11 are defined as

$$c_1 = K_c[O_2]_b \quad c_2 = \frac{L^2}{P}\frac{k_1[O_2]_b}{D_{MbO_2}}$$

$$c_3 = \frac{L^2Pk_1}{D_{O_2}} \quad c_4 = \frac{L^2\dot{m}_{O_2}}{D_{O_2}[O_2]_b}.$$

The numerical value of each of these constants is influenced by temperature through the temperature dependence of the diffusion coefficients and/or the kinetic and equilibrium constants.

For diffusion of spherical particles in liquids the Stokes-Einstein equation states that the diffusion coefficient is proportional to the temperature and inversely proportional to the viscosity:

$$D = \frac{KT}{6\pi\mu r}. \tag{12}$$

At 37°C the diffusion coefficients for myoglobin and oxygen are taken as 1.0×10^{-6} and 1.6×10^{-5} cm^2/s, respectively, and the coefficient for the oxygen-myoglobin complex is assumed the same as for the unbound myoglobin. These values are derived from diffusivities measured in protein solutions at 20°C by Riveros-Moreno and Wittenberg (1972) and corrected for temperature by Jacquez (1984). The viscosity (μ) in the Stokes' equation also depends on temperature, and Jacquez has accounted for this by using the known temperature effects on the viscosity of water. Potentially, changes in the cytosol viscosity will affect the intracellular diffusion coefficients. But it is not known if temperature influences the viscosity of concentrated protein solutions within cells, and until this phenomena is demonstrated it is not appropriate to include this additional complexity. Therefore no viscosity corrections have been included in the temperature dependence of the diffusion coefficients. This dependence can then be expressed as

$$D(T) = D(37°C)\frac{T(°K)}{310.2}. \tag{13}$$

Hence, values of the diffusion coefficients will change in proportion to the absolute temperature; i.e., a change in muscle absolute temperature of 3% (10°C) will produce a 3% change in the diffusion coefficients.

Studies of the kinetics and equilibrium of the myoglobin-oxygen reaction have been reviewed by Antonini and Brunori (1971). The dependence of the forward rate constant, k, on temperature can be expressed in an Arrhenius form:

$$k = \kappa \exp[-A/RT], \tag{14}$$

where κ is the Arrhenius frequency factor and A is the activation energy. At 20°C, the association rate constant and activation energy for horse myoglobin have reported values of 1.4×10^7 (Ms)$^{-1}$ and 5,500 cal/mol, respectively. These values have been used to calculate the Arrhenius frequency factor. The temperature dependence of the equilibrium constant is given by the van't Hoff expression:

$$K_c(T) = K_c(20°C) \exp\left\{\frac{-\Delta H(20°C)}{R}[1/T - 1/(293.2°K)]\right\}. \tag{15}$$

The heat of reaction for human myoglobin has been studied by Rossi-Fanelli and Antonini (1958), who report a value of -1.3×10^4 cal/mol. This value did not vary significantly over the temperature range studied (10 to 40°C). The same authors also report equilibrium constant data that is expressed relative to the partial pressure of oxygen. This equilibrium constant is related to K_c and the solubility of oxygen, α, by

$$K_c = \frac{[MbO_2]}{[Mb][O_2]} = \frac{[MbO_2]}{[Mb](P_{O_2}\alpha)} = \frac{K_p}{\alpha}. \quad (16)$$

A solubility expression derived by Siggaard-Andersen (1974) was used to derive the equilibrium constant needed in Eq. 15. At 20°C, K_c is equal to 8.11×10^5 M^{-1}.

Two of the dimensionless constants appearing in Eqs. 10 and 11 depend on the concentration of myoglobin. A wide range of myoglobin concentrations have been used in past modeling, ($2.8–8.0 \times 10^{-7}$ mol/cm^3) and we have chosen an intermediate value of 4.77×10^{-7} mol/cm^3 (8 mg/ml). This number is in agreement with the values determined experimentally by Wittenberg (1970) for red skeletal muscle, although Jacquez (1984) argues that this value may be low because the volumes of fibrillar proteins and mitochondria have not been excluded in the calculations.

In modeling the effect of temperature on oxygen transport, the plasma oxygen concentration boundary condition can be specified in two alternative ways. Either the oxygen partial pressure or the dissolved oxygen concentration at the plasma-fiber interface can be fixed. By using a constant partial pressure the modeling will account for the solubility of oxygen in the blood plasma, but it ties the problem to temperature-sensitive processes that are external to the fibers and can obscure the cause-effect analysis of internal events. By fixing the oxygen concentration, the modeling will elucidate only the temperature-sensitive events directly related to the facilitation process. Except where noted, all of the results are derived from this latter choice. For the boundary oxygen concentration, we use a value of 2.5×10^{-8} mol/cm^3, which corresponds to an equilibrium partial oxygen pressure of 20 torr at 37°C.

3. RESULTS AND DISCUSSION

Numerical simulations were carried out using a finite-difference method to solve the coupled two-point boundary-value equations. A NAG (Numerical Algorithms Group, Oxford, UK) library algorithm D02RAF was used as the core of the program (see also Pereyra, 1979). This program enables the user to specify error tolerances on each dependent variable and gradient. A specified mesh of solution points is not required but was found useful. Additional mesh points are added to the specified mesh automatically until the error tolerances can be satisfied. Unlike other reported numerical procedures, this technique did not require an extremely accurate initial estimate of the solution. The same method was used by Kemp and Noble (1983) to study the effect of temperature gradients on carrier-mediated transport processes in liquid membranes. Our initial results were found to be identical to those generated with the boundary-value problem solver COLNEW based on a collocation procedure (Ascher et al., 1981; Bader and Ascher, 1987).

Solution for a specified oxygen consumption

The results of simulation over a temperature range of 20–42°C are given in Figs. 1–3. The same oxygen consumption rate that was used in the energy-balance analysis is used here. Fig. 1 shows the dimensionless oxy-myoglobin concentration as a function of dimensionless distance. For the conditions studied, myoglobin is predominantly oxygenated (85–95%), which agrees with the results of Covell and Jacquez (1987). The effect of increased temperature was to decrease the concentrations of oxygenated myoglobin consistent with the exothermic nature of the reaction which favors dissociation at higher temperatures. The drop over the range of 20°C was ~12%. Oxy-myoglobin gradients are also noticeably larger at the higher temperatures. We also note that the inflection point in these curves corresponds to the only point within the tissue where equilibrium exists between myoglobin and oxygen ($d^2Y/d\chi^2 = 0$). Between this point and the plasma boundary, the oxy-myoglobin concentration is less than the equilibrium concentration, whereas at distances further from the boundary, the oxy-

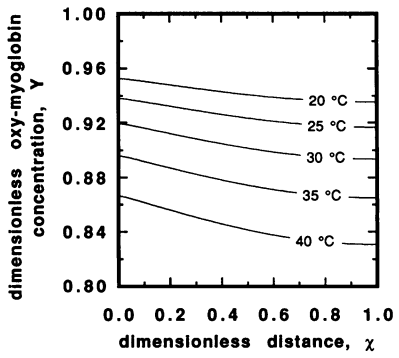

FIGURE 1 Dimensionless oxy-myoglobin concentration plotted versus dimensionless distance for skeletal muscle fibers at temperatures between 20° and 40°C.

myoglobin concentration is greater than its equilibrium value. Increasing the temperature affected the location of this point by moving it closer to the plasma interface.

The corresponding oxygen concentration curves are shown in Fig. 2. Centerline concentrations decreased to ~70–75% of the boundary value. Increasing the temperature resulted in slightly larger local oxygen concentrations. Also plotted in this figure are the corresponding curves for the oxygen profiles for fibers without myoglobin. With this condition the model can be simplified by setting the oxy-myoglobin concentration to zero. The resulting equation can be solved analytically to give

$$\omega = 1 - c_4\chi\left(1 - \frac{\chi}{2}\right). \tag{17}$$

At any given temperature, local oxygen concentrations are higher when myoglobin is present as Murray (1974) and Covell and Jacquez (1987) have reported. Temperature also increases the local oxygen concentrations when myoglobin is not present. This increase must occur because of larger oxygen diffusivities and the same mechanism is at least partly responsible for the increases when myoglobin is present.

A flux ratio can be defined as the local flux of oxy-myoglobin divided by the local total oxygen flux and is shown plotted against dimensionless distance in Fig. 3. For this problem the total oxygen flux is a linear function of position as is found by integrating the sum of the two mass balance equations. Because of the myoglobin gradient boundary condition, the flux ratio must be zero at the $\chi = 0$ boundary. This ratio increases sharply near the plasma-fiber boundary and establishes a value that gradually increases with depth into the tissue. The combined effect of an increasing oxy-myoglobin gradient with a linearly decreasing total oxygen gradient is

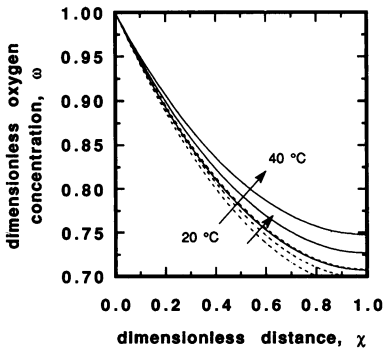

FIGURE 2 Dimensionless oxygen concentration profiles in skeletal muscle fibers (with and without myoglobin) at 20°, 30°, and 40°C. Dashed lines indicate no myoglobin. The arrows point in the direction of higher temperature.

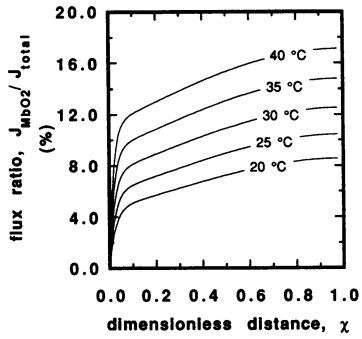

FIGURE 3 The local flux ratio as a function of dimensionless distance for muscle fiber temperatures between 20° and 40°C.

evident near the boundary, while further into the tissue the increases illustrate that it is the consumption of free oxygen that drives the diffusion process. The tissue depth where the transition occurs corresponds to the point where oxygen and myoglobin exist in equilibrium. At the conditions considered, the centerline ratio varies between 8 and 17%, somewhat smaller than the values found by Loiselle (1987), who tied oxygen consumption to the local oxygen concentration. The ratio increases ~4% with a 10°C temperature rise. Therefore, at higher temperatures oxygenated myoglobin accounts for a larger proportion of the total oxygen flux. Because the diffusion coefficients are both linear in temperature, this results from increased local gradients in the oxygenated myoglobin and decreased local gradients of oxygen with temperature, which are apparent in Figs. 1 and 2.

Solution for the maximum supportable oxygen consumption

Increasing the level of metabolic activity in the muscle increases the demand for oxygen. Because oxygen delivery is limited by transport considerations, increasing oxygen consumption results in reduced oxygen and oxy-myoglobin concentrations within the tissue. Eventually, as activity is increased, a point is reached when the two concentrations will drop to zero. In this work this condition is called the maximum rate of oxygen consumption, or $\dot{m}_{O_2}^{max}$, and it corresponds to the tissue aerobic capacity. Further increases in activity must be supported by anaerobic means and result in local regions of oxygen debt. Because oxygen consumed at steady-state must be equal to oxygen transported, an increase in $\dot{m}_{O_2}^{max}$ is taken as an indication of an increased ability to transport oxygen. We now investigate the effect temperature has on this maximum rate of oxygen consumption.

For tissue without myoglobin, an analytical solution for the maximum oxygen consumption can be obtained

by integrating the oxygen diffusion equation with [Mb] = 0. Solving Eq. 11 with the boundary conditions

$$\omega = 0 \quad \text{and} \quad \frac{d\omega}{d\chi} = 0 \quad \text{at } \chi = 1$$

gives

$$c_4 \,[\text{with } \dot{m}_{O_2} = \dot{m}_{O_2}^{max} \text{ (no Mb)}] = 2 \tag{18}$$

or in dimensional form

$$\dot{m}_{O_2}^{max} \text{ (no Mb)} = \frac{2D_{O_2}(T)[O_2]_b}{L^2} \tag{19}$$

as was first derived by Warburg. Fig. 4 shows the effect temperature has on the maximum oxygen consumption rate in the absence of myoglobin. If the temperature dependence of D_{O_2} and $[O_2]_b$ is neglected, the maximum oxygen consumption rate is a constant. With the parameter values given in Table 1, this rate is 0.2714 ml O_2/(ml min) and has been used as a normalizing constant for all the data plotted in Figs. 4–6. It is interesting to note that this rate consumption, without myoglobin, is three times greater than the largest oxygen consumption rates measured by Folkow and Halicka (1968). If the diffusional thermal effects are included, $\dot{m}_{O_2}^{max}$ increases linearly with temperature. The magnitude of this change will correspond to the magnitude of the change in the diffusion coefficients ($\sim 3\%$ per 10°C temperature rise).

The above analysis considers only physical events occurring within muscle fibers. Effects due to transport limitations within the blood plasma are not described as we are considering the oxygen concentration as constant at the fiber-plasma interface. When the temperature dependence of the plasma oxygen solubility is included (by maintaining the partial pressure of oxygen constant at the interface instead of the oxygen concentration), a sharp decrease in the maximum oxygen consumption is found with temperature. This curve is also shown in Fig. 4. At temperatures below 37°C, $\dot{m}_{O_2}^{max}$ is greater when the partial pressure is held constant. Because the solubility of oxygen in plasma decreases with temperature, this is due to the larger oxygen concentrations at the boundary. At temperatures above 37°C the opposite is true. The large decrease in $\dot{m}_{O_2}^{max}$ with temperature indicates that increases in the maximum rate of oxygen consumption can be reversed by temperature-sensitive events external to the fibers. All three lines plotted intersect at 37°C because the baseline or standard values of the diffusion coefficients and boundary oxygen concentration are referenced to this temperature.

For tissues that contain myoglobin no similar analytical solution is available, and numerical methods must be invoked. In principle the problem can be solved by adjusting the value of $\dot{m}_{O_2}$ in Eqs. 10 and 11 until one achieves a zero concentration of oxygen at the tissue center. A simpler computational approach is to back-calculate the boundary oxygen concentration with the specification that the oxygen concentration and the oxygen gradient be zero at the center of the tissue. The new boundary conditions become

$$\omega = 0 \quad \text{at} \quad \chi = 1 \qquad \frac{d\omega}{d\chi} = 0 \quad \text{at} \quad \chi = 1$$

$$\frac{dY}{d\chi} = 0 \quad \text{at} \quad \chi = 0, 1.$$

The diffusion equations are then solved by systematically varying the value of $\dot{m}_{O_2}$ until the original oxygen concentration boundary condition ($\omega = 1$ at $\chi = 0$) is matched.

Fig. 5 shows the results of calculating $\dot{m}_{O_2}^{max}$ at tempera-

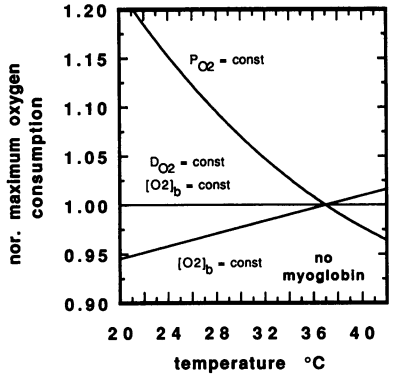

FIGURE 4 Maximum rate of oxygen consumption or aerobic capacity as a function of muscle temperature (without myoglobin). All values are normalized to the value at 37°C.

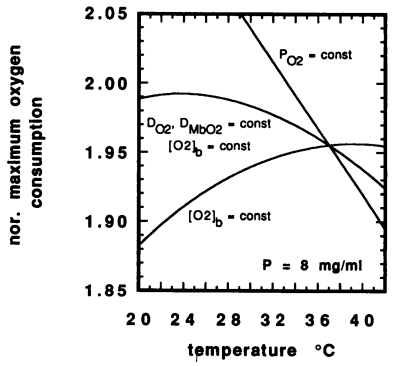

FIGURE 5 Maximum rate of oxygen consumption or aerobic capacity as a function of muscle temperature (with myoglobin). Values are normalized as in Fig. 4.

tures between 20° and 42°C for the same three cases described above but with myoglobin added. The data in this figure has been normalized by the same value used in Fig. 4 (0.2714 ml O_2/[ml min]). Thus, the plotted values indicate an elevated ability to transport oxygen in the presence of myoglobin. With the oxygen concentration held constant at the boundary, $\dot{m}_{O_2}^{max}$ increases with temperature at low temperatures. The magnitude of this effect is ~4.0% over a temperature range from 20°C to the maximum at 39.2°C. Also plotted is the curve generated by holding the diffusion coefficients constant at the 37°C values. In this instance the curve peaks at a much lower temperature (23.6°C) and exhibits a decreasing trend throughout most of the temperature range of interest. This suggests that the benefit in aerobic capacity is the result of temperature effects associated with the diffusion coefficients and not with the kinetic constants. If the partial pressure of oxygen is fixed at the plasma-fiber interface, a large decrease in the maximum oxygen consumption rate is found with increasing temperature. This can again be attributed to the decrease in oxygen solubility with increased temperature.

From the above it is clear that if there exists a benefit in elevated temperature for the myoglobin-facilitation process, its magnitude is small and it may be off-set by factors external to the fibers. The magnitude of the effect is much smaller than reported increases in the oxygen uptake rates. Bergh and Ekblom (1979) report a 15% increase in oxygen consumption with a change in muscle temperature from 35° to 39.3°C. In comparison, the percentage benefit found from the modeling is <1% over the same temperature range. Hence it appears that the increased oxygen uptake is not being driven by myoglobin-facilitated transport of oxygen. Of course, the transport of oxygen from the lungs to the mitochondria involve several processes in series, each providing some resistance to transport. At steady state each of these processes must be transporting oxygen at the same rate, so muscle fibers must be obtaining the increases found by Bergh and Ekblom. Decreases in the resistance to transport at other points along the pathway may yield greater oxygen partial pressures at the fiber boundaries. These partial pressures could overcome the decreased solubility with temperature and generate greater fluxes because of larger gradients. In addition, there may be other benefits of elevated temperature for muscle. These include temperature effects on the mechanical properties which affect contraction, effects on the neural activation process, and effects on other chemical reactions involved with contraction. Both Lännergren et al. (1982) and Ranatunga (1982) have reported positive correlations between temperature and contraction properties. In addition, Brooks et al. (1971) have found evidence that indicates that the rates of enzymatic reactions within mitochondria are sensitive to temperature.

Parameter sensitivity effects

The numerical results given above depend on the parameters describing the kinetics and equilibrium of the oxygen-myoglobin reaction and the diffusion coefficients. To investigate the sensitivity of the results relative to these parameters, simulations were run varying the value of each parameter separately. Parameters which varied included the equilibrium constant at 20°C, the heat of reaction, and the frequency factor and activation energy of the association rate constant. Also studied were deviations in the diffusion coefficients and the concentration of myoglobin.

The factors affecting the reaction kinetics or equilibrium were varied consistently with the experimental uncertainty of those factors. No statistics were given with the equilibrium constant data by Rossi-Fanelli and Antonini (1958), although it is clear from the published figure that the scatter in this data is quite small. Deviations of ±10% were considered for this parameter. For the heat of reaction the range of values reported vary between −13.1 (±0.6) kcal/mol for human myoglobin and −16.4 kcal/mol for rat myoglobin (see Antonini and Brunori, 1971; Rossi-Fanelli and Antonini, 1958). The former value was used as the baseline for this work. Values studied in this sensitivity analysis varied from −12 to −16 kcal/mol covering both the estimated standard deviation for the human value and the range of literature values. Reported data for the association rate constant at 20°C vary from 1.0×10^{-7} $(Ms)^{-1}$ to 1.9×10^{-7} $(Ms)^{-1}$. The value used in this work was 1.4×10^{-7} $(Ms)^{-1}$ as given by Antonini and Brunori (1971) for crystalline horse myoglobin. No indication of the accuracy of this number is available, so the parameter was varied over the entire range of reported experimental values. Antonini and Brunori also tabulate two literature values for the association constant activation energy. Both reports were for horse myoglobin and both were given as 5.5 kcal/mol. No accuracy estimates were given in the reports, but the constancy of the values suggests that deviations of the order of 20% (1.0 kcal) should be sufficient to cover the experimental uncertainty.

As mentioned above and by Jacquez (1984) there are difficulties in determining appropriate values for the diffusion coefficients and the concentration of myoglobin within muscle fibers. Therefore, relatively large deviations in the baseline values of these parameters were considered.

Fig. 6 shows the results of the sensitivity analysis on the normalized $\dot{m}_{O_2}^{max}$ values at 37°C. Parameter deviation

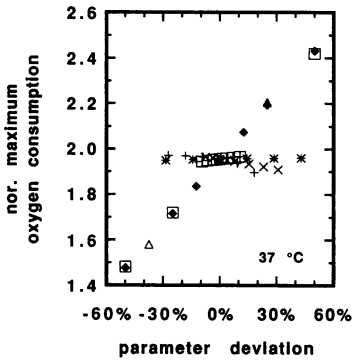

FIGURE 6 Parameter sensitivity of the normalized maximum oxygen consumption rate at 37°C. (□), D_{MbO_2}; (△), D_{O_2}; (⊞), K_p(20°C); (+), A; (*), κ; (×), ΔH; (◆), P. All values have been normalized by the same constant used in Fig. 4.

is defined as the difference between the adjusted and baseline values divided by the baseline value and expressed as a percentage. It is clear from Fig. 6 that the maximum oxygen consumption is far more sensitive to changes in the diffusion coefficient and the myoglobin concentration than to changes in the parameters affecting the kinetic constants. This explains why the maximum oxygen consumption in Fig. 5 is influenced by the relatively small temperature effects on the diffusion coefficients (Fig. 5). The positive slope of these curves indicates that increasing the value of either diffusion coefficient will increase the maximum oxygen consumption over the range of baseline deviations studied. Gonzalez-Fernandez (1990) find a similar result relative to a facilitation factor ($J_{fac} = J_{total}$ [with Mb] $- J_{total}$ [without Mb]). By varying the parameters that affect the kinetic constants, we also conclude that increasing either of the kinetic constants will increase $\dot{m}_{O_2}^{max}$. Gonzalez-Fernandez (1990) reports a biphasic response for the facilitation factor when considering a wide range of values for the kinetic constants. Restricting the analysis to near physiological values, they also find that increasing either kinetic constant increases the facilitation factor.

The parameter variations also had an effect on the temperature-induced increases in $\dot{m}_{O_2}^{max}$. The temperature maxima did shift with the parameter changes, but no substantially larger thermal benefits were found. The oxygen diffusion coefficient proved the most sensitive parameter in this respect. By increasing the value of this parameter, the temperature maximum also increased. With D_{O_2} equal to 2.0×10^{-5} cm^2/s (a 25% deviation), the temperature maximum occurred between 41° and 42°C and the benefit was ~5% between 20°C and this maximum temperature. Increasing the value to 5.0×10^{-5} cm^2/s (a 212% deviation), the maximum occurs outside the range studied and the temperature benefit was 7% between 20° and 42°C. No larger temperature effects were found.

In terms of adaptive regulation of the facilitation process, one method available to the fiber may be control of the myoglobin concentration. A series of simulations was performed at temperatures between 20° and 42°C with varying myoglobin concentrations. The results are shown in Fig. 7 where $\dot{m}_{O_2}^{max}$ is plotted vs. temperature for several myoglobin concentrations. Higher concentrations of myoglobin increase $\dot{m}_{O_2}^{max}$ substantially. The magnitude of the increase is so large as to make the temperature benefits appear negligible. The maxima, which are indicated by stars in the figure, occur at progressively lower temperatures as the myoglobin concentration is increased. It is clear from this figure that any benefit generated because of increased temperature could also be produced by a very small change in the myoglobin concentration. It is difficult then to argue that one of the adaptive advantages of being warm blooded is increased oxygen transport due to myoglobin kinetic effects when far greater benefits can be derived by slight increases in the myoglobin concentration.

4. CONCLUSIONS

There are no significant temperature gradients within individual skeletal muscle fibers because of internal metabolic reactions or externally imposed temperature gradients. Muscle fibers will be spatially isothermal and will be effectively at the same temperature as the locally perfusing blood.

Global temperature changes were found to affect the facilitation process. Elevated temperature increased

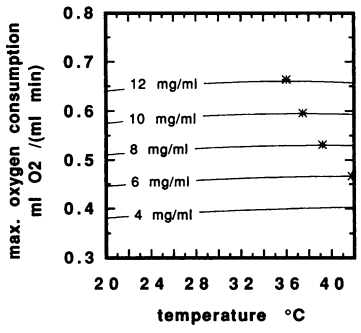

FIGURE 7 Maximum rate of oxygen consumption as a function of temperature and myoglobin concentration. Stars (*) indicate temperature maxima.

local oxygen concentrations and decreased oxy-myoglobin concentrations. An increase was also found in the fraction of the total oxygen flux carried by myoglobin. The maximum rate of oxygen consumption increased slightly with temperature at low temperatures ($< 39.2°C$). The increase was found to be attributable to thermal effects associated with the diffusion coefficients which dominated the exothermic reaction effects. This benefit could be reversed by including the temperature effect of the oxygen solubility within the plasma.

Varying the parameter values showed that oxygen transport in muscle fibers was most sensitively controlled by the diffusion coefficients and the concentration of myoglobin. Increased maximum oxygen consumption rates resulted from increasing either of the diffusion coefficients, the association or dissociation rate constant, or the myoglobin concentration.

While a small temperature effect was found on the maximum oxygen consumption, a number of points suggest that temperature is not an important factor influencing the facilitated transport of oxygen in muscle. (*a*) The temperature benefit could be reversed by including external temperature-sensitive processes. (*b*) The size of the benefit was small compared with reported experimental measurements. This suggests that the facilitation process is responding to other more temperature-sensitive processes. (*c*) Much larger benefits can be obtained by regulating other factors, e.g., the myoglobin concentration.

Received for publication 13 July 1990 and in final form 1 March 1991.

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