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The effect of myoglobin concentration on aerobic dive limit in a Weddell seal

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Summary

One physiological adaptation for prolonged dive duration in marine mammals is an elevated myoglobin (Mb) concentration in skeletal muscle. To determine the influence of Mb concentration on the aerobic dive limit (ADL), we modified a previously published model that simulated aerobic dives in a Weddell seal (*Leptonychotes weddellii*) and ran it for four Mb concentrations: 5, 27, 54 and 108 g Mb kg^{-1} muscle representing 7%, 50%, 100% and 200%, respectively, of the normal Mb concentration in Weddell seal skeletal muscle. The model was run at increasing levels of muscular exertion and under postabsorptive and postprandial conditions to determine their effect on ADL. For each set of conditions, the model was also run at different levels of cardiac output (i.e. the dive response was varied) to determine the level of convective oxygen transport that optimized the ADL. In a postabsorptive state at a routine level of muscular exertion for a diving Weddell seal, a decrease in Mb concentration to 7% of normal caused a 39% decrease in the ADL (18 min to 11 min), while doubling the Mb concentration increased the ADL by 30% (18 min to 24 min). Under postprandial conditions at a routine level of muscular exertion, doubling the Mb concentration did not increase the ADL (12 min). The convective oxygen transport needed to meet the metabolic demands (Heat Increment of Feeding, HIF) of the splanchnic organs during digestion and assimilation required a cardiac output that was not optimal for the efficient use of muscle oxygen stores. This resulted in an over perfusion of the muscles and incomplete use of myoglobin-bound oxygen. As a result, the postprandial ADL was limited by the amount of oxygen stored in the blood, and increasing the Mb concentration had no effect on the ADL. We hypothesize that myoglobin concentration is optimized for the type and duration of dives routinely made by Weddell seals, and that a further increase may not increase the ADL for most free-ranging dives.

Key words: Weddell seal, *Leptonychotes weddellii*, myoglobin, diving, ADL, postabsorptive, postprandial.

Introduction

Weddell seals and other marine mammals exhibit physiological adaptations and behavioral strategies that increase dive duration. These include an elevation in total body oxygen stores through increases in blood volume, hematocrit (Hct; the percentage of blood volume occupied by red blood cells) and muscle myoglobin (Mb) concentration. In addition, marine mammals use efficient modes of locomotion (e.g. gliding during descent, stroke-and-glide swimming) that keep oxygen consumption low during diving (Williams et al., 2000; Williams, 2001). For Weddell seals, these physiological adaptations and behavioral strategies result in an aerobic dive limit (ADL) of about 20 min (Davis and Kanatous, 1999; Ponganis et al., 1993a; Kooyman et al., 1980).

If body oxygen stores are the primary physiological limit to dive duration, why are they not larger? During the evolution of marine mammals, what physiological factors may have set the upper limit to blood volume, Hct and muscle Mb concentration? Weddell seals have a blood volume as high as 21% of their body mass (Ponganis et al., 1993), almost three times larger than predicted for a terrestrial mammal of the same size (Stahl, 1967). The upper limit to blood volume may be a compromise between increasing oxygen stores and the resultant increase in body mass or abdominal volume.

The Hct of Weddell seals (ca. 60%), which is 1.5-times higher than in most terrestrial mammals, increases blood oxygen stores and maintains convective oxygen transport to organs and tissues as the partial pressure of oxygen in the blood decreases during diving. However, the increased Hct also increases blood viscosity, circulatory resistance and heart work (Elsner and Meiselman, 1995). As a result, the large spleen of Weddell seals sequesters red blood cells, lowers the hematocrit, and decreases blood viscosity when they are at the surface. Only when they begin diving does the spleen contract and release the red blood cells into the circulation, which increases the hematocrit while heart rate is reduced due to the dive response (Hurford et al., 1996). A hematocrit greater than 60% would further increase blood viscosity, increase heart

work, and could decrease rather than increase convective oxygen transport. (Hedrick and Duffield, 1986). Consequently, the elevated Hct of Weddell seals and other marine mammals may be at its physiological maximum for optimizing blood oxygen storage and convective oxygen transport.

The concentration of Mb in the skeletal muscles of Weddell seals is about 10-times greater than in most terrestrial mammals (Snyder, 1983). Oxygen bound to Mb represents one-third of the total oxygen store in Weddell seals, so it is a major factor in setting the ADL (Davis and Kanatous, 1999; Kooyman and Ponganis, 1998). However, it is not clear what physical or physiological factors may have set the maximum concentration of muscle Mb. The objective of this study was to model the effects of different muscle Mb concentrations on the ADL of Weddell seals. Specifically, we wanted to know how increasing or decreasing Mb concentration beyond normal levels would affect the ADL. Although lowering the Mb concentration would obviously decrease the ADL, would increasing the concentration automatically increase it? To answer this question, we used a previously published model of convective oxygen transport and tissue oxygen consumption (Davis and Kanatous, 1999). We ran the model at different myoglobin concentrations for various levels of muscular exertion under postabsorptive and postprandial conditions to determine their effect on ADL.

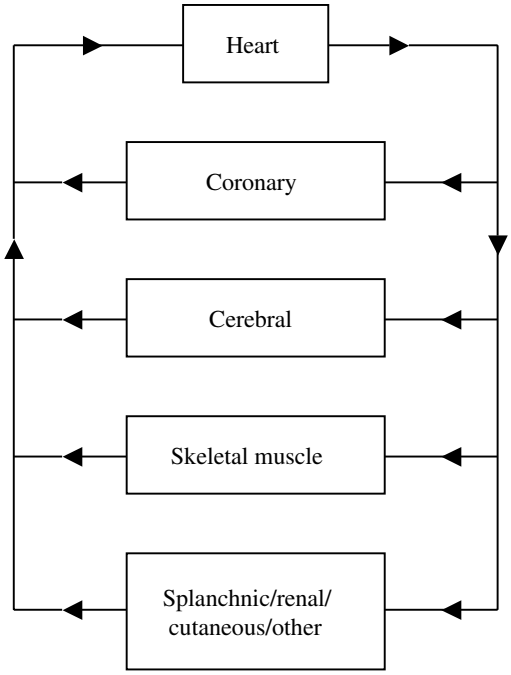

Fig. 1. Simplified circulatory system used in the model. The cardiovascular system was divided into four regional circulations: coronary, cerebral, skeletal muscle and a combined category that included the splanchnic, renal, cutaneous and other circulatory beds.

Materials and methods

Theoretical basis for the model: Fick's Principle

A numerical integration technique was used to model the relationship between regional convective oxygen transport ($\dot{Q}_{O_2}$) and the rate of oxygen consumption ($\dot{V}_{O_2}$) in a hypothetical Weddell seal during aerobic dives at different levels of muscle oxygen consumption ($\dot{V}_{MO_2}$) (see List of symbols and abbreviations). A detailed description of the model and an explanation of the assumptions and equations under postabsorptive conditions has been published (Davis and Kanatous, 1999). This study differed from that of Davis and Kanatous in that we ran the model with four different muscle Mb concentrations during aerobic dives under both postabsorptive and postprandial conditions. The numerical process iteratively determined arterial blood oxygen concentration (Ca_{O_2}) and venous blood oxygen concentration (Cv_{O_2}) for various tissues and organs based on the circulatory diagram shown in Fig. 1 and Eqn 1 (Fick's principle):

$$\dot{V}_{O_2} = \dot{Q}(Ca_{O_2} - Cv_{O_2}) , \quad (1)$$

where $\dot{Q}$ is blood flow rate ($l\,min^{-1}$). Cerebral, coronary and skeletal muscle regional circulations were incorporated into the model individually, while splanchnic, renal and cutaneous circulations were grouped together with all other organs and tissues (e.g. bone and fat). The average temporal resolution (i.e. the period between consecutive computations) was 0.22 min.

This model considers only dives that are within the seal's ADL (Kooyman et al., 1980; Ponganis et al., 1993a). The term ADL was used in this model to describe the maximum duration of an aerobic dive under specific conditions. The basal contribution of anaerobic metabolism in harbor seals has been shown to constitute approximately 2% of ATP production in a resting state and 1% during active swimming (Davis et al., 1991). For this model, this small basal contribution of anaerobic metabolism is ignored, and tissues are considered aerobic as long as there is no increased reliance on anaerobic metabolism resulting in an increase in blood lactate over resting levels. While terms such as diving lactate threshold (DLT) and calculated aerobic dive limit (cADL) are useful for certain applications (Butler and Jones, 1997), they were not applicable to all conditions used to terminate a dive in this model. DLT was not used because increased blood lactate resulting from anaerobic metabolism was not necessary to terminate a dive in this model. The term cADL is historically used to denote a calculation of aerobic dive limit based on total useable oxygen stores divided by whole body metabolism. While this model does calculate an ADL, it does so through modeling of blood flow and metabolism in individual tissues, which can produce vastly different results than whole body calculations in some metabolic states. The rate of oxygen consumption in the tissues is maintained until convective oxygen delivery falls below a critical level and endogenous oxygen stores (skeletal muscle only) are depleted, resulting from a

combination of ischemic and hypoxic hypoxia. When any organ (e.g. splanchnic organs) or tissue (e.g. skeletal muscle) no longer has sufficient oxygen to support aerobic metabolism (i.e. the point at which anaerobic energy metabolism commences), then the ADL has been reached and the dive is terminated.

Assumptions and equations

Organ and tissue masses were based on published values for a 450 kg adult Weddell seal (Fujise et al., 1985; Zapol et al., 1979) as described by Davis and Kanatous in their table 1 (Davis and Kanatous, 1999). The resting $\dot{V}_{O_2}$ values for Weddell seal organs and tissues were estimated from the metabolic mass-adjusted $\dot{V}_{O_2}$ for the equivalent organs of a human or rat (Diem and Lentner, 1970; Field et al., 1939; Kety, 1957). The basal, whole body $\dot{V}_{O_2}$ (897 ml O_2 min^{-1} or 2.0 ml O_2 min^{-1} kg^{-1}) was calculated by combining individual organ and tissue metabolic rates. The calculated basal metabolic rate was similar to the minimum metabolic rates measured for adult Weddell seals during rest or sleep (Castellini et al., 1992b; Ponganis et al., 1993a; Williams et al., 2004).

Resting heart rate (f_H) (51.5 beats min^{-1}), cardiac output ($\dot{V}b$) (42.7 l min^{-1}) and stroke volume (V_S) (0.83 l) were based on measured values for Weddell seals (Zapol et al., 1979). During a simulated dive, $\dot{V}b$ was varied from 19–131% of resting levels [(see Davis and Kanatous, 1999), table 2]. For brevity, we hereafter refer to these percentages of resting, predive $\dot{V}b$ as percent $\dot{V}b$ (e.g. 19%$\dot{V}b$). When $\dot{V}b$ was below resting levels, most of the reduction resulted from a decrease in f_H (i.e. bradycardia). However, based on studies of seals during forced submergence and voluntary dives (Blix and Folkow, 1983; Kjekshus et al., 1982; Ponganis et al., 1990; Sinnet et al., 1978; Zapol et al., 1979), V_S was also reduced as f_H declined. The maximum reduction in V_S in the model was 25% of the resting value and was proportionate to the reduction in f_H. The reduction in cardiac output (i.e. the severity of the dive response) was immediate and remained constant throughout a dive. An 'anticipatory' increase in $\dot{V}b$ toward the end of a dive was not included in the model. Except for the brain, where circulation was always maintained at resting levels, we assumed that blood flow to the rest of the body decreased proportionately with $\dot{V}b$ during a dive due to reduced $\dot{V}b$ and peripheral vasoconstriction (Blix et al., 1976; Elsner et al., 1964). Peripheral vasoconstriction was assumed to occur in the large arteries (e.g. the renal artery), making it independent of tissue level metabolic dilators that affect arterioles (White et al., 1973). Because vasoconstriction was assumed to occur high in the vascular tree, blood flow was not adjusted independently to individual tissue beds.

Body oxygen stores were confined to the blood and skeletal muscle in this model, since no oxygen storage capability exists in the splanchnic organs (Dodd et al., 1987) and the heart represents less than 2% of the total muscle mass. We assumed that lung oxygen was not available during a dive due to the complete functional pulmonary shunt that occurs in Weddell seals at pressures greater than 3–5 atmospheres (2280–3800 mmHg; 1 mmHg=0.133 kPa; approximately 30–50 m deep) (Falke et al., 1985; Reed et al., 1994). Even if lung oxygen were available during a dive, it represents only 5% of the total body oxygen store in Weddell seals (Kooyman and Ponganis, 1998).

To calculate total oxygen stores in the blood, we assumed that the blood volume for a 450 kg Weddell seal was 96 liters (Ponganis et al., 1993a) and that 33% of this volume was arterial blood and 67% was venous blood (i.e. venules, small and large veins, hepatic sinus and spleen) (Hurford et al., 1996; Rowell, 1986). The blood hemoglobin (Hb) concentration (assuming complete splenic contraction) was 260 g l^{-1}, and the oxygen binding capacity of Hb was 1.34 ml O_2 g^{-1} Hb (Kooyman et al., 1980; Ponganis et al., 1993a; Qvist et al., 1986). This gave a capacitance coefficient of oxygen in blood (β_{BO_2}) of 348 ml O_2 l^{-1} (260 g Hb l^{-1} blood × 1.34 ml O_2 g^{-1} Hb). At the beginning of a dive, we assumed that the arterial blood was 100% saturated with oxygen as a result of predive hyperventilation (Kooyman et al., 1980; Qvist et al., 1986; Ponganis et al., 1993a). Mixed venous blood was calculated from Eqn 2 to be 86% saturated at the beginning of a dive assuming an oxygen content that was 5% by volume less (Ponganis et al., 1993a) than an initial Ca_{O_2} of 348 ml O_2 l^{-1} blood.

$$S\bar{v}_{O_2} = [(348–50)/348] \times 100 = 86\% , \quad (2)$$

where $S\bar{v}_{O_2}$ is the oxygen saturation of mixed venous blood. Arterial and venous blood oxygen stores were calculated as:

$$\text{arterial blood oxygen (ml)} = 96\times0.33\times348 = 11\,025 , \quad (3)$$

$$\text{venous blood oxygen (ml)} = 96\times0.67\times348\times0.86 = 19\,250 . \quad (4)$$

We assumed that 35% of the seal's body mass was skeletal muscle. For this study, we ran the model with four Mb concentrations: 5, 27, 54 and 108 g Mb kg^{-1} muscle, representing 7%, 50%, 100% and 200%, respectively, of the normal Mb concentration in Weddell seal skeletal muscle (Ponganis et al., 1993a). The Mb concentration of 5 g kg^{-1} muscle is typical of terrestrial mammals such as a dog, human or rat (Snyder, 1983). We assumed an oxygen binding capacity of 1.34 ml O_2 g^{-1} Mb, and complete saturation at the beginning of a dive (Gayeski et al., 1987; Schenkman et al., 1997).

Muscle oxygen stores were calculated as:

$$\text{skeletal muscle oxygen (ml)} = 450\times0.35\times1.34\times[\text{Mb}] . \quad (5)$$

The total oxygen store (sum of arterial, venous and muscle oxygen) was therefore 31 330 ml O_2 (69.6 ml O_2 kg^{-1}), 35 973 ml O_2 (79.9 ml O_2 kg^{-1}), 41 672 ml O_2 (92.6 ml O_2 kg^{-1}) or 53 068 ml O_2 (117.9 ml O_2 kg^{-1}), based on the four Mb concentrations, respectively. However, not all of this oxygen is available for metabolism during a dive (Davis and Kanatous, 1999).

As blood circulates through the four vascular beds (Fig. 1), the organs and tissues extract oxygen from the blood to meet

their respective $\dot{V}_{O_2}$ requirements. Cv_{O_2} was calculated for each circulatory bed according to Fick's Principle:

$$C_{BV_{O_2}} = Ca_{O_2} - (\dot{V}_{BO_2} / \dot{Q}_B) , \quad (6)$$

$$C_{HV_{O_2}} = Ca_{O_2} - (\dot{V}_{HO_2} / \dot{Q}_H) , \quad (7)$$

$$C_{MV_{O_2}} = Ca_{O_2} - (\dot{V}_{MO_2} / \dot{Q}_M) , \quad (8)$$

$$C_{SRCV_{O_2}} = Ca_{O_2} - (\dot{V}_{SCRO_2} / \dot{Q}_{SRC}) , \quad (9)$$

where $\dot{Q}$ is blood flow rate, $\dot{V}$ is the rate of oxygen consumption, the letters B, H, M indicate brain, heart and skeletal muscle respectively, and SRC indicates splanchnic, renal and cutaneous organs and other peripheral tissues. However, the extraction coefficient of oxygen from the blood (E_{BO_2}), where $E_{BO_2}=(Ca_{O_2}-Cv_{O_2})/Ca_{O_2}$, could never exceed 0.8 (i.e. maximum E_{BO_2} at critical oxygen delivery) during a single pass of the blood through an organ or tissue (Samsel and Schumacker, 1994; Torrance and Wittnich, 1994; Nelson et al., 1988). The mixed venous blood oxygen concentration ($C\bar{v}_{O_2}$) was calculated for the four vascular beds as the difference between the Ca_{O_2} and the total oxygen extracted per ml of blood:

$$C\bar{v}_{O_2} = Ca_{O_2} - [(\dot{V}_{BO_2}+\dot{V}_{HO_2}+\dot{V}_{MO_2}+\dot{V}_{SRCO_2}) / (\dot{Q}_B+\dot{Q}_H+\dot{Q}_M+\dot{Q}_{SRC})] . \quad (10)$$

The arterial blood oxygen saturation (Sa_{O_2}) and venous blood oxygen saturation (Sv_{O_2}) were calculated for the blood of each vascular bed as the quotient of their respective oxygen concentrations (Eqn 6–9) and a β_{BO_2} of 348 ml O_2 l^{-1} blood. The arterial (Pa_{O_2}) and venous (Pv_{O_2}) blood oxygen partial pressures were calculated from their respective Sa_{O_2} and Sv_{O_2} using two polynomial equations fitted to the oxy-hemoglobin dissociation curve (P_{50}=26.9 mmHg=0.133 kPa) for adult Weddell seals (Qvist et al., 1981).

Evidence obtained during the forced submergence of harbor seals and Weddell seals indicates that $\dot{Q}_B$ is generally maintained and $\dot{V}_{BO_2}$ does not decline (Blix and Folkow, 1983; Kerem and Elsner, 1973; Zapol et al., 1979). In this model, we assumed that $\dot{Q}_B$ and $\dot{V}_{BO_2}$ remained at resting levels during a dive and were independent of $\dot{V}b$. We also assumed that the minimum Pa_{O_2} and $P\bar{v}_{O_2}$ for normal cerebral metabolism and function were 22 mmHg (Sa_{O_2}=38%) and 18 mmHg ($S\bar{v}_{O_2}$=27%), respectively. This is comparable to the average Pa_{O_2} (24.5±2.86 mmHg; mean ± s.d., N=7) in Weddell seals 2 min before surfacing and to the end tidal P_{O_2} (24 mmHg) of the first exhalation (assuming that this approximates arterial P_{O_2}) after 17 min aerobic dives (Ponganis et al., 1993a; Qvist et al., 1986). As a result, the model terminated a dive if Pa_{O_2} decreased below 22 mmHg in the model. However, the Pa_{O_2} of blood perfusing the brain was generally not a consideration in determining ADL.

We assumed that $\dot{Q}_H$ and $\dot{V}_{HO_2}$ changed proportionately with $\dot{V}b$ (Blix and Folkow, 1983; Blix et al., 1976; Kjekshus et al., 1982). When convective oxygen transport to the myocardium changed during a dive, it was proportional to the change in heart work, and the myocardium always received sufficient blood oxygen to maintain aerobic metabolism.

$\dot{Q}_M$ was also assumed to change proportionately with $\dot{V}b$. Oxygen transported to the muscles in the blood was always used (up to a maximum E_{BO_2} of 0.8) before oxygen bound to Mb because of the lower affinity of Hb for oxygen (Schenkman et al., 1997). Oxygen not provided by the blood was obtained from oxymyoglobin stores to meet $\dot{V}_{MO_2}$ requirements. $\dot{V}_{MO_2}$ was assumed to be independent of $\dot{Q}_M$ as long as the combination of convective oxygen transport and oxymyoglobin stores was sufficient to meet metabolic demand. If at any time the combination of these two were no longer sufficient to maintain aerobic muscle metabolism, the dive was terminated.

Postabsorptive $\dot{V}_{O_2}$ (3.73±0.88 ml O_2 min^{-1} kg^{-1}) and postprandial $\dot{V}_{O_2}$ (5.24±0.88 ml O_2 min^{-1} kg^{-1}) during aerobic dives were based on indirect calorimetry measurements by Williams et al. for foraging and non-foraging Weddell seals (Williams et al., 2004). We assumed that the average difference in $\dot{V}_{O_2}$ (1.51 ml O_2 min^{-1} kg^{-1} or 680 ml O_2 min^{-1} for a 450 kg seal) between postabsorptive and postprandial dives of 7–23 min in duration resulted from the metabolic cost of prey warming, digestion, absorption and assimilation, which we refer to as the Heat Increment of Feeding (HIF). This increase in $\dot{V}_{O_2}$ was added to the postabsorptive $\dot{V}_{SRCO_2}$ to give a postprandial $\dot{V}_{SRCO_2}$ of 1234 ml O_2 min^{-1} (a 2.2-fold increase). We assumed that the $\dot{V}_{SRCO_2}$ was maintained as long as: (1) convective oxygen transport was sufficient to support oxygen demand, (2) E_{BO_2} did not exceed 0.8 and (3) Pa_{O_2} was greater than 22 mmHg (Kvietys and Granger, 1982; Schlichtig et al., 1992).

Computations

The model was run on a standard spreadsheet program (Quattro Pro for Windows Version 6.0, Novell Applications Group, Orem, UT, USA) for eight levels of $\dot{V}b$, sixteen levels of $\dot{V}_{MO_2}$ up to a maximum whole-body $\dot{V}_{O_2}$ of 10.7 ml O_2 min^{-1} kg^{-1} and four different Mb concentrations under postabsorptive conditions, which produced 512 combinations. These were then compared to postprandial conditions for the normal and elevated Mb concentration adding an additional 256 combinations. The general procedure was to select a Mb concentration, set the $\dot{V}b$ at a particular level (e.g. 37% of the resting level) and then vary the $\dot{V}_{MO_2}$ from 1 to 16 times the resting level. This process was then repeated for each Mb concentration and $\dot{V}b$. $\dot{V}_{O_2}$ for the four vascular beds and the entire body were calculated for each combination. The ADL was reached and the dive terminated when: (1) any non-muscle organ or tissue did not receive sufficient oxygen through convective oxygen transport to maintain aerobic metabolism, (2) convective oxygen transport and oxymyoglobin stores were no longer sufficient to maintain aerobic muscle metabolism, or (3) when the Pa_{O_2} fell below 22 mmHg.

Results

The role of $\dot{V}b$ in optimizing the ADL at different levels of muscle metabolism

The role of $\dot{V}b$ in optimizing the ADL at different levels of muscle metabolism has been described (Davis and Kanatous,

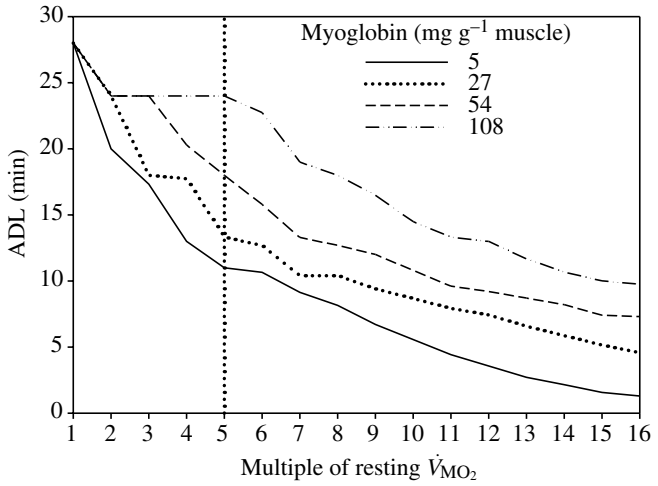

Fig. 2. Calculated postabsorptive aerobic dive limit (ADL) for four myoglobin concentrations (mg g^{-1} muscle) as a function of skeletal muscle oxygen consumption ($\dot{V}_{MO_2}$). Vertical dotted line marks the estimated routine level of diving $\dot{V}_{MO_2}$ for a Weddell seal.

1999). Briefly, the ADL decreases in a non-linear fashion with increasing $\dot{V}_{MO_2}$ for different levels of $\dot{V}$b (range=19–131% of resting levels) (Davis and Kanatous, 1999; Davis et al., 2004) (Figs 2, 3). For each level of $\dot{V}_{MO_2}$, there is an optimal $\dot{V}$b that gives a maximum ADL, and this optimal $\dot{V}$b increases (i.e. the dive response is less pronounced) as $\dot{V}_{MO_2}$ increases [(see Davis and Kanatous, 1999), fig. 4 and table 4]. Since the ADL is inversely proportional to $\dot{V}_{MO_2}$ (assuming a constant level of blood and muscle oxygen depletion), the optimal $\dot{V}$b decreases as the ADL increases [(see Davis and Kanatous, 1999), fig. 5].

The effect of Mb concentration on the postabsorptive ADL

In the postabsorptive state, the resting ADL (28 min) was independent of Mb concentration (Fig. 2). At a resting level of

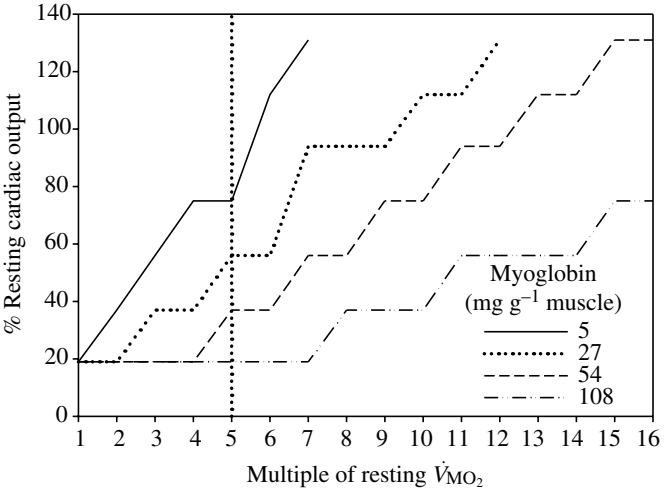

Fig. 3. Optimal cardiac output as a function of skeletal muscle metabolism ($\dot{V}_{MO_2}$) for four myoglobin concentrations (mg g^{-1} muscle). Vertical dotted line marks the estimated routine level of diving $\dot{V}_{MO_2}$ for a Weddell seal.

Table 1. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at a reduced [Mb] of 5 mg g^{-1} in a postabsorptive state*

			Myoglobin O_2 consumed by muscle during dive	
$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min kg^{-1})	ADL (min)	ml	%
1	1.8	28.0	221	21
2	2.3	20.0	353	33
3	2.8	17.3	644	61
4	3.4	13.0	407	39
5	3.9	11.0	1026	97
6	4.4	10.7	687	65
7	4.9	9.1	575	54
8	5.4	8.1	1008	95
9	5.9	6.7	1030	98
10	6.4	5.6	1013	96
11	6.9	4.4	960	91
12	7.3	3.6	1001	95
13	7.8	2.7	974	92
14	8.3	2.1	984	93
15	8.8	1.6	964	91
16	9.3	1.3	1020	97

For explanations of symbols and abbreviations, see List.

$\dot{V}_{MO_2}$, the lowest level of convective oxygen transport ($\dot{V}$b=19%) was still sufficient to supply 97% of the oxygen needed by the skeletal muscle. As a result, very little Mb oxygen (ranging from 21% to 1% of endogenous oxymyoglobin for concentrations from 5 to 108 mg g^{-1}, respectively) was used while resting submerged, and it was not a factor that limited the ADL (Tables 1–4). The only way to increase the use of Mb oxygen at rest was to decrease convective oxygen transport even further (i.e. $\dot{V}$b<19%). However, when we ran the model at a $\dot{V}$b of 9%, the ADL decreased because convective oxygen transport to the splanchnic organs and kidneys was insufficient. Hence, at rest there was no optimal $\dot{V}$b that provided sufficient oxygen delivery for the kidneys and splanchnic organs while utilizing more Mb-bound oxygen, regardless of the Mb concentration. As a result, there was no difference in ADL for Mb concentrations of 54 and 108 mg g^{-1} until $\dot{V}_{MO_2}$ exceeded 3-times resting.

At muscle Mb concentrations of 5 and 27 mg g^{-1}, the postabsorptive ADL decreased in a curvilinear fashion with increasing $\dot{V}_{MO_2}$ and whole body $\dot{V}_{O_2}$. At normal and elevated Mb concentrations, the ADL decreased in a curvilinear fashion with the exception of a common plateau at 24 min for $\dot{V}_{MO_2}$ of 2- to 3-times resting and 2- to 5-times resting for these two Mb concentrations, respectively (Fig. 2). At these low levels of $\dot{V}_{MO_2}$, the ADL was limited by blood oxygen stores, and Mb oxygen was not a limiting factor (Tables 3 and 4). These two curves diverge at higher levels of exertion as muscle oxygen stores are consumed and contribute significantly to setting the ADL. Only when $\dot{V}_{MO_2}$ exceeded 3-times resting did an

Table 2. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at a reduced [Mb] of 27 mg g^{-1} in a postabsorptive state*

$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min^{-1} kg^{-1})	ADL (min)	Myoglobin O_2 consumed by muscle during dive	
			ml	%
1	1.8	28.0	221	4
2	2.3	24.0	3390	59
3	2.8	18.0	2232	39
4	3.3	17.8	5607	98
5	3.8	13.3	3372	59
6	4.3	12.7	5609	98
7	4.9	10.4	2775	49
8	5.4	10.4	4609	81
9	5.8	9.4	5577	98
10	6.3	8.7	4903	86
11	6.8	7.9	5671	100
12	7.3	7.4	5300	93
13	7.8	6.6	5488	96
14	8.3	5.9	5609	98
15	8.8	5.1	5617	99
16	9.3	4.6	5662	99

For explanations of symbols and abbreviations, see List.

Table 4. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at an increased [Mb] of 108 mg g^{-1} in a postabsorptive state*

$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min^{-1} kg^{-1})	ADL (min)	Myoglobin O_2 consumed by muscle during dive	
			ml	%
1	1.8	28.0	221	1
2	2.3	24.0	3390	15
3	2.8	24.0	8574	38
4	3.3	24.0	13758	60
5	3.7	24.0	18942	83
6	4.2	22.8	22759	100
7	4.7	19.0	18660	82
8	5.2	18.0	21291	93
9	5.7	16.5	22767	100
10	6.2	14.5	22684	100
11	6.7	13.3	20523	90
12	7.2	13.0	22714	100
13	7.6	11.7	22502	99
14	8.1	10.7	22593	99
15	8.7	10.0	21030	92
16	9.2	9.8	22517	99

For explanations of symbols and abbreviations, see List.

increase in the Mb concentration above 54 mg g^{-1} increase the ADL.

Based on the findings of Williams et al. (Williams et al., 2004), we assumed an average postabsorptive diving $\dot{V}_{O_2}$ of 3.8 ml O_2 min^{-1} kg^{-1}, which was equivalent to a $\dot{V}_{MO_2}$ of 5-times resting in our model. At this routine level of diving metabolism, a reduction of Mb concentration from 54 mg g^{-1} to 27 mg g^{-1} and 5 mg g^{-1} reduced the ADL from 18 min to 12.7 min (29% reduction) and 11.0 min (39% reduction), respectively. Doubling the normal Mb concentration increased the ADL 33% from 18 to 24 min (Fig. 2).

Table 3. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at a normal [Mb] of 54 mg g^{-1} in a postabsorptive state*

$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min^{-1} kg^{-1})	ADL (min)	Myoglobin O_2 consumed by muscle during dive	
			ml	%
1	1.8	28.0	221	2
2	2.3	24.0	3390	30
3	2.8	24.0	8574	75
4	3.3	20.3	11279	99
5	3.8	18.0	9627	84
6	4.3	15.8	11355	100
7	4.8	13.3	9003	79
8	5.2	12.7	11081	97
9	5.8	12.0	10729	94
10	6.3	10.8	11389	100
11	6.8	9.6	9894	87
12	7.3	9.2	11262	99
13	7.8	8.7	10404	91
14	8.3	8.2	11258	99
15	8.8	7.4	10030	88
16	9.3	7.3	11298	99

For explanations of symbols and abbreviations, see List.

For all four Mb concentrations, the optimal $\dot{V}$b (i.e. the $\dot{V}$b that gave the maximum ADL) increased as muscular exertion increased (Fig. 3). The optimal $\dot{V}$b increased more quickly with increasing levels of exertion (i.e. the slope of the trend line was greater) for low muscle Mb concentrations compared to normal and elevated Mb concentrations. As Mb increased, the optimum $\dot{V}$b for each level of $\dot{V}_{MO_2}$ decreased. For example, at the average diving $\dot{V}_{MO_2}$ of 5-times resting, the optimal $\dot{V}$b at Mb concentrations of 5, 27, 54 and 108 g Mb kg^{-1} were 75%, 56%, 37% and 19% of resting levels, respectively. As Mb increases, $\dot{V}$b and muscle blood flow must decrease (i.e. more pronounced dive response) for the muscle to fully use this Mb bound oxygen.

At a $\dot{V}_{MO_2}$ of 5-times resting for normal and elevated Mb concentrations, convective oxygen transport at the optimal $\dot{V}$b was insufficient to support the aerobic metabolic needs of the muscle. As a result, muscle Mb oxygen stores were used from the beginning and throughout the dive (Fig. 4). In contrast, the optimal $\dot{V}$b for reduced Mb concentrations was greater (i.e. less pronounced dive response), resulting in increased convective oxygen transport to the muscles and a delay in the use of Mb oxygen until well into the dive (7 min and 1.33 min for Mb concentrations of 5 and 27 mg g^{-1}, respectively). With optimal matching of $\dot{V}$b to $\dot{V}_{MO_2}$, almost all myoglobin oxygen was

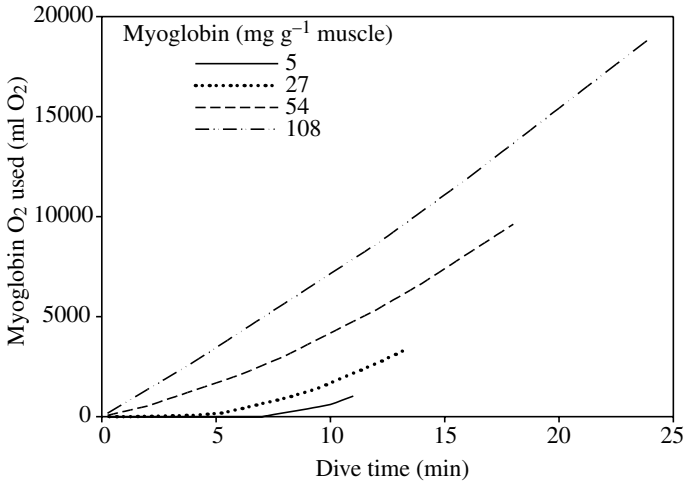

Fig. 4. Myoglobin oxygen used during diving at a muscular exertion of 5-times resting $\dot{V}_{MO_2}$ for four Mb concentrations.

consumed at this routine level of exertion regardless of myoglobin concentration.

The effect of Mb concentration on the postprandial ADL

Under postprandial conditions, the ADL decreased at all levels of exertion because of the increased oxygen consumption of the splanchnic organs associated with prey warming, digestion and assimilation. At a routine $\dot{V}_{MO_2}$ of 5-times resting and normal Mb concentration, the postprandial ADL (12 min) was 33% less than under postabsorptive conditions (Fig. 5). The convective oxygen transport needed by the splanchnic organs required a $\dot{V}$b that was not optimal for the complete use of muscle oxygen at a routine diving $\dot{V}_{MO_2}$ of 5-times resting. Not until $\dot{V}_{MO_2}$ exceeded 7-times resting did this level of perfusion allow for complete utilization of muscle

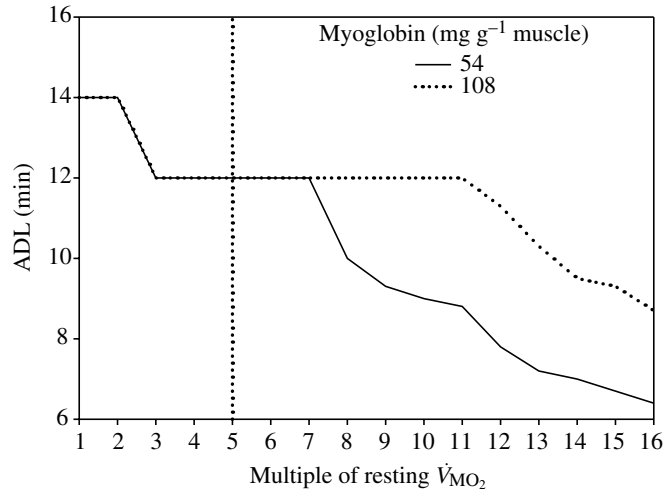

Fig. 5. Aerobic dive limit (ADL) as a function of muscle oxygen consumption ($\dot{V}_{MO_2}$) for a postprandial Weddell seal with normal and elevated Mb concentrations. Vertical dotted line marks the estimated routine level of diving $\dot{V}_{MO_2}$ for a Weddell seal.

Table 5. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at a normal [Mb] of 54 mg g^{-1} in a postprandial state*

$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min kg^{-1})	ADL (min)	Myoglobin O_2 consumed by muscle during dive	
			ml	%
1	3.4	14.0	0	0
2	3.8	14.0	258	2
3	4.3	12.0	1206	11
4	4.8	12.0	3356	29
5	5.3	12.0	5948	52
6	5.8	12.0	8540	75
7	6.2	12.0	11132	98
8	6.7	10.0	11069	97
9	7.2	9.3	9930	87
10	7.8	9.0	9682	85
11	8.3	8.8	11194	98
12	8.7	7.8	11143	98
13	9.3	7.2	10120	89
14	9.7	7.0	11251	99
15	10.2	6.7	10487	92
16	10.7	6.4	11317	99

For explanations of symbols and abbreviations, see List.

oxygen stores, and Mb oxygen became limiting to the ADL (Fig. 5 and Table 5). As a result, doubling the Mb concentration did not increase the ADL until the level of muscular exertion exceeded 7-times resting. Diving at routine levels of muscular exertion in a postprandial state resulted in convective oxygen transport and not oxy-myoglobin limiting the ADL. Based on the results from our model, digesting and assimilating food while diving decreased the ADL for two reasons: (1) increased splanchnic consumption of blood oxygen and (2) the increased convective oxygen transport needed by the splanchnic organs resulted in a $\dot{V}$b that was not optimal for the complete use of muscle oxygen. As a result, the model indicated that there was no advantage in having a higher than normal myoglobin concentration during postprandial dives at routine levels of $\dot{V}_{MO_2}$.

Discussion

The role of myoglobin in diving marine mammals

Due to myoglobin's high affinity for oxygen (p50=2–3 mmHg) (Schenkman et al., 1997) compared to Hb (p50=27 mmHg) (Qvist et al., 1981), it is an endogenous oxygen store for muscle only. To use this source of oxygen, the muscle must become hypoxic by reducing convective oxygen transport. This not only decreases the muscle P_{O_2} so that the oxygen dissociates from myoglobin, but also reserves more blood oxygen for other tissues. The first publication using this model (Davis and Kanatous, 1999) showed the importance of adjusting cardiac output and convective oxygen transport to muscle according to the level of exertion, so that the total

oxygen available in the muscle and blood was used by the end of a dive. As the level of muscular exertion increased, the dive response was less pronounced and convective oxygen transport to skeletal muscle (and other peripheral organs and tissues) increased. To maximize the ADL, both oxygen stores had to be depleted simultaneously so that neither was singly responsible for limiting aerobic dive duration.

In a postabsorptive resting state, the ADL was independent of Mb concentration from 5–108 mg myoglobin g^{-1} muscle (Fig. 2). The model showed that the $\dot{V}$b needed to maintain resting metabolism in the splanchnic organs (19%) resulted in an over-perfusion of the skeletal muscle so that almost all (97%) of the oxygen used by the muscles at rest was supplied by convective oxygen transport in the blood. Greater utilization of Mb oxygen would require less convective oxygen transport to skeletal muscle. However, further reduction in $\dot{V}$b (9%) resulted in insufficient convective oxygen transport to the splanchnic organs and reduced the ADL.

At a routine diving $\dot{V}_{MO_2}$ of 5-times resting, the postabsorptive ADL increased with higher Mb concentrations (Fig. 2). In addition, Mb concentration was negatively correlated with optimal $\dot{V}$b for a dive (Fig. 3). Higher Mb concentrations (54 and 108 mg g^{-1}) required a greater reduction in cardiac output (more profound dive response). The resultant reduction in convective oxygen transport to muscles decreased the muscle P_{O_2} (i.e. made the muscle hypoxic) so that myoglobin oxygen was used throughout the dive (Fig. 4).

At a $\dot{V}_{MO_2}$ of 1 to 7-times resting in a postprandial state, the $\dot{V}$b required to maintain the elevated aerobic metabolism in the splanchnic organs resulted in an over-perfusion of the skeletal muscle, which caused the incomplete use of Mb oxygen stores (Table 5). Inefficient use of muscle oxygen stores as well as increased use of blood oxygen for digestion and assimilation resulted in blood oxygen limiting the ADL in the postprandial state until $\dot{V}_{MO_2}$ exceeded 7-times resting (Fig. 5 and Table 5). As a result, the doubling of Mb concentration did not increase the ADL under postprandial conditions until the level of $\dot{V}_{MO_2}$ exceeded 7-times resting, which is 40% higher than the routine level of exertion.

Behavioral considerations

The results of this model showed that an increase in the Mb concentration increased the ADL at a routine diving $\dot{V}_{MO_2}$ under postabsorptive conditions (Fig. 2). However, for the same $\dot{V}_{MO_2}$ under postprandial conditions, the convective oxygen transport needed for digestion and assimilation required a $\dot{V}$b which resulted in an over-perfusion of the muscle and incomplete use of muscle oxygen stores at routine levels of exertion (i.e. <7 times resting $\dot{V}_{MO_2}$) (Fig. 5 and Tables 5, 6). Castellini et al. stressed the importance of integrating physiology and behavior in considering the biology of diving (Castellini et al., 1992a). To determine what selective pressures might affect myoglobin concentration, it is important to consider the way Weddell seals routinely dive.

Davis et al. classified Weddell seal dives into four types (Davis et al., 2003). Type 1 were feeding dives with a mean duration of 15.0 min, and these accounted for 14% of all dives made and 29% of total time submerged. Given the assumptions regarding HIF, the postprandial ADL (12 min) at a routine level of exertion calculated by our model agrees well with average duration of feeding dives reported (Davis et al., 2003). Types 2 and 3 dives were relatively short in duration (mean=3.6 min and 7.9 min, respectively) and were rarely associated with feeding. Together these dives accounted for 72% of dives being made. The average duration of these dive types are well below our estimated postabsorptive ADL of 18 min and are not limited by the physiological constraints of the oxygen stores, but by behavior.

Type 4 dives were long in duration (average=24.7 min), appeared to be exploratory (non-feeding) dives, and accounted for 14% of all dives. This dive type exceeds our estimated postabsorptive ADL of 18 min and relies significantly on anaerobic metabolism. Our model indicates that an increased myoglobin concentration would prolong aerobic metabolism for this type of dive. However, these long duration dives rarely occur in free-diving Weddell seals (Kooyman, 1980).

Table 6. *Aerobic dive limit, whole body oxygen consumption, and muscle oxygen consumption for varying multiples of muscular metabolic rate at an elevated [Mb] of 108 mg g^{-1} in a postprandial state*

$\dot{V}_{MO_2}$	Whole body $\dot{V}_{O_2}$ (ml O_2 min^{-1} kg^{-1})	ADL (min)	Myoglobin O_2 consumed by muscle during dive	
			ml	%
1	3.4	14.0	0	0
2	3.8	14.0	258	1
3	4.3	12.0	1206	5
4	4.8	12.0	3356	15
5	5.3	12.0	5948	26
6	5.8	12.0	8540	37
7	6.2	12.0	11132	49
8	6.7	12.0	13724	60
9	7.2	12.0	16316	72
10	7.7	12.0	18908	83
11	8.2	12.0	21500	94
12	8.6	11.3	22448	98
13	9.1	10.3	22471	99
14	9.6	9.5	22717	100
15	10.1	9.3	22026	97
16	10.6	8.7	22097	97

For explanations of symbols and abbreviations, see List.

Factors determining myoglobin concentration

It appears that dives of the type and duration in which an increase in myoglobin concentration would increase the ADL are rare under normal diving behavior. While an increase in myoglobin would prolong aerobic metabolism during some long duration, postabsorptive dives, it does not appear to limit the ADL in the majority of natural dives (i.e. Types 1, 2 and 3). Weddell seals make the majority of their feeding dives in bouts of many dives with short recovery periods on the surface

(Castellini et al., 1992a; Kooyman et al., 1980). As a result, many of these feeding dives probably occur in the postprandial condition. Davis et al. observed (Davis et al., 1983) that the plasma of Weddell seals became very lipemic during deep foraging dives, indicating that the digestion and intestinal absorption of fat was occurring during the 5–6 h foraging session. Increased energy expenditure for digestion during diving is added to the metabolic costs for locomotion and basal metabolism (Williams et al., 2004). This increased metabolism for digestion and assimilation is also thought to reduce the ADL of southern elephant seals during foraging bouts (McConnell et al., 1992). Digestion not only increases oxygen consumption, but also influences the optimal management of the muscle and blood oxygen stores. Our model indicated that diving with the additional metabolic cost of HIF causes blood oxygen to limit the ADL rather than myoglobin oxygen (i.e. myoglobin stores may not be completely used). We hypothesize that myoglobin concentration is optimized for the type and duration of dives routinely made by Weddell seals, and that a further increase may not increase the ADL of most free-ranging dives. Whether physiological constraints associated with the dive response and convective oxygen transport have limited the concentration of myoglobin in muscles remains uncertain, but our model does suggest a possible influence during the evolution of Weddell seals and other long duration divers. In addition, the model indicates that the calculated ADL is more complex than simply the quotient of the available oxygen stores and estimated metabolic rate.

List of symbols and abbreviations

ADL	aerobic dive limit
cADL	calculated aerobic dive limit
$C\mathrm{a}_{\mathrm{O_2}}$	arterial blood oxygen concentration (ml O_2 l^{-1} blood)
$C\mathrm{BV}_{\mathrm{O_2}}$	cerebral venous blood oxygen concentration (ml O_2 l^{-1} blood)
$C\mathrm{HV}_{\mathrm{O_2}}$	coronary venous blood oxygen concentration (ml O_2 l^{-1} blood)
$C\mathrm{MV}_{\mathrm{O_2}}$	skeletal muscle venous blood oxygen concentration (ml O_2 l^{-1} blood)
$C\mathrm{SRCV}_{\mathrm{O_2}}$	splanchnic, renal, cutaneous and other peripheral tissue venous blood oxygen concentration (ml O_2 l^{-1} blood)
$C\bar{\mathrm{v}}_{\mathrm{O_2}}$	mixed venous blood oxygen concentration (ml O_2 l^{-1} blood)
$C\mathrm{v}_{\mathrm{O_2}}$	venous blood oxygen concentration (ml O_2 l^{-1} blood)
DLT	diving lactate threshold
$E_{\mathrm{BO_2}}$	extraction coefficient of oxygen from blood $[(C\mathrm{a}_{\mathrm{O_2}}–C\mathrm{v}_{\mathrm{O_2}})/C\mathrm{a}_{\mathrm{O_2}}]$
f_{H}	heart frequency (beats min^{-1})
Hb	hemoglobin
Hct	hematocrit
HIF	heat increment of feeding
Mb	myoglobin
$P\mathrm{a}_{\mathrm{O_2}}$	arterial blood oxygen partial pressure (mmHg)
$P_{\mathrm{O_2}}$	oxygen partial pressure (mmHg)
$P\mathrm{v}_{\mathrm{O_2}}$	venous blood oxygen partial pressure (mmHg)
$\dot{Q}$	blood flow rate (l min^{-1})
$\dot{Q}_{\mathrm{B}}$	brain blood flow (l min^{-1})
$\dot{Q}_{\mathrm{H}}$	heart blood flow (l min^{-1})
$\dot{Q}_{\mathrm{M}}$	skeletal muscle blood flow (l min^{-1})
$\dot{Q}_{\mathrm{O_2}}$	convective oxygen transport in the blood (ml O_2 min^{-1})
$\dot{Q}_{\mathrm{SRC}}$	splanchnic, renal, cutaneous and other peripheral tissue blood flow (l min^{-1})
$S\mathrm{a}_{\mathrm{O_2}}$	arterial blood oxygen saturation (%)
$S\bar{\mathrm{v}}_{\mathrm{O_2}}$	mixed venous blood oxygen saturation (%)
$S\mathrm{v}_{\mathrm{O_2}}$	venous blood oxygen saturation (%)
$\dot{V}\mathrm{b}$	cardiac output (l min^{-1})
$\dot{V}_{\mathrm{BO_2}}$	brain oxygen consumption rate (ml O_2 min^{-1})
$\dot{V}_{\mathrm{CRSO_2}}$	splanchnic, renal, cutaneous and other peripheral tissue oxygen consumption rate (ml O_2 min^{-1})
$\dot{V}_{\mathrm{HO_2}}$	heart oxygen consumption rate (ml O_2 min^{-1})
$\dot{V}_{\mathrm{MO_2}}$	skeletal muscle oxygen consumption rate (ml O_2 min^{-1})
$\dot{V}_{\mathrm{O_2}}$	rate of oxygen consumption (ml O_2 min^{-1})
V_{S}	stroke volume (l)
$\beta_{\mathrm{BO_2}}$	capacitance coefficient of oxygen in blood (ml O_2 l^{-1} blood)

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