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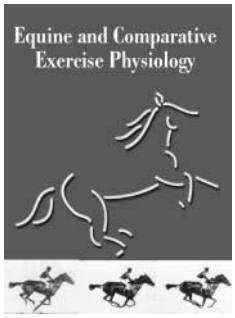

Current concepts of oxygen transport during exercise

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Abstract

This brief review examines the athletic potential of mammals in general and the horse in particular as it relates to oxygen (O_2) transport and utilization. The horse has been bred selectively for over six millennia based upon its ability to run fast. Whereas this has optimized cardiovascular and muscle function and the capacity to deliver and utilize O_2, it has resulted in lung failure during intense exercise. Horses in their athletic prime are considered and attention is focused on their maximal capacities as related to O_2 transport, irrespective of age *per se*. Following a few comments on the history of O_2, this review moves from established principles of O_2 transport at the integrative organ level to the microcirculation and the processes and principles that govern O_2 offloading, where much remains to be discovered. Four principal questions are addressed: (1) as an athlete, what are the most outstanding physiological characteristics of the horse? (2) what anatomical and physiological capacities facilitate this superlative performance and such prodigious O_2 fluxes (i.e. maximal $\dot{V}O_2$)? (3) do cardiovascular dynamics or intramuscular energetic processes limit $\dot{V}O_2$ kinetics (i.e. the speed at which $\dot{V}O_2$ increases at the onset of exercise)? $\dot{V}O_2$ kinetics determine the size of the O_2 deficit and as such represent an important determinant of muscle metabolism and fatigue; and (4) what determines the efficacy of muscle microcirculatory O_2 exchange?

Keywords: Horse; cardiovascular; muscular; oxygen exchange; haemoglobin; oxygen uptake kinetics; blood flow; oxygen diffusion

Introduction

A historical perspective on oxygen

When the solar system was created approximately 4.6 billion years ago[1], Earth's atmosphere was completely devoid of oxygen (O_2). Fossils, some 3.5 billion years old, provide evidence of algae-like micro-organisms that captured solar energy and manufactured organic molecules. These creatures were powered by anaerobic fermentation and released O_2 into their surroundings. It took 2 billion years for them to create an atmosphere in which O_2 represented a little over one out of every five molecules. The direct descendants of those early organisms are called cyanobacteria and a few colonies still exist. For example in Shark Bay, Western Australia, they cluster several billions to the square metre on stromalite formations in the coastal shallows and continue to pump out molecular O_2.

In the O_2-rich atmosphere created by the cyanobacteria, unicellular eukaryotic (i.e. with a nucleus) aerobic life appeared about 1.5 billion years ago[2]. These organisms performed those basic functions which characterize cellular activity today; they were metabolically active, excitable, and capable of locomotion and reproduction. Along with almost all subsequent forms of life on Earth, these cells possessed mitochondria which provided adenosine triphosphate (ATP) to power cellular functions[3,4].

The development of larger animals required a solution to the O_2 transport problem. In the fluid environment, O_2 diffuses well over distances of micrometres, but not millimetres or more. Around 700 million years ago, larger animals could develop only by maintaining a small individual cell size and assembling millions or billions of cells together[5]. Loss of immediate contact with the external milieu necessitated the development of elegant O_2 transport mechanisms over distances from several millimetres to metres. Diverse strategies have evolved to facilitate efficient gas exchange. For example, insects have networks of tubular airways (tracheae) to bring air to their cells, and fish possess gills that project outwards into the aquatic environment. Mammals have evolved lungs

that are turned inwards and which warm and moisten the inspired air before it reaches the alveolar gas exchange structures. The cardiovascular system then transports O_2 to the muscles, the activity of which provides by far the greatest stress to the mammalian O_2 transport system. Indeed, skeletal muscle has an astonishing capacity to increase its metabolic rate 50- to 600-fold above resting levels.

Today, we live in an atmosphere that contains 20.94% O_2. Despite the fact that *Homo sapiens sapiens* have been around for at least 35 000 years, man's knowledge of O_2 is fairly recent. Oxygen itself was discovered only around 1772 through the independent experiments of Joseph Priestley (1733–1804) and Carl Wilhelm Scheele (1742–1786). Both scientists communicated their discovery to the brilliant French chemist Antoine Lavoisier (1743–1794) in Paris, who named this 'eminently respirable' air 'oxygine' for its acid-forming properties[6,7].

The following sections explore the co-ordinated function required for O_2 transport among the pulmonary, cardiovascular and muscular systems during exercise that is necessary to supply the mitochondria with O_2. It is this co-ordination which facilitates effective matching of O_2 delivery ($\dot{Q}O_2$) to O_2 requirements and limits the reliance on glycolysis and other finite non-O_2 energy stores (which constitute the so-called 'O_2 deficit'). The horse, which man has bred selectively for over six millennia based upon its ability to run fast (Fig. 1), is presented as a model of superb aerobic performance. As we shall see below, the capacity of the horse to take up, transport and utilize O_2 is absolutely extraordinary.

Great athletes in the animal kingdom

The designation of any particular mammal as the greatest athlete is very much dependent upon the criterion utilized. For example, in terms of absolute speed, the cheetah reigns supreme, topping well in excess of

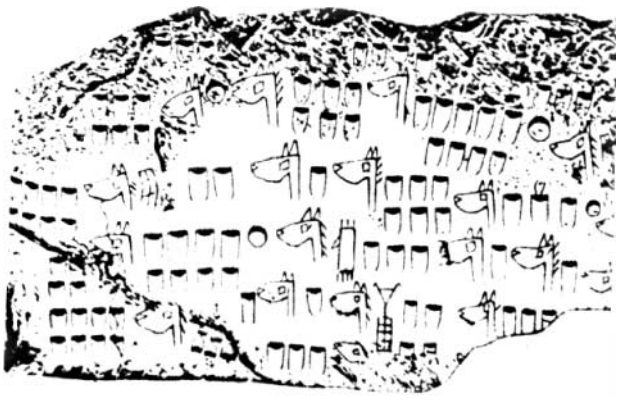

FIG. 1 Chaldean pedigree chart (*circa* 4000 BC) demonstrating that selective breeding of horses was practised at least 6000 years ago. Reproduced from Lyons and Petrucelli[8] and the World Health Organization, Geneva, with permission

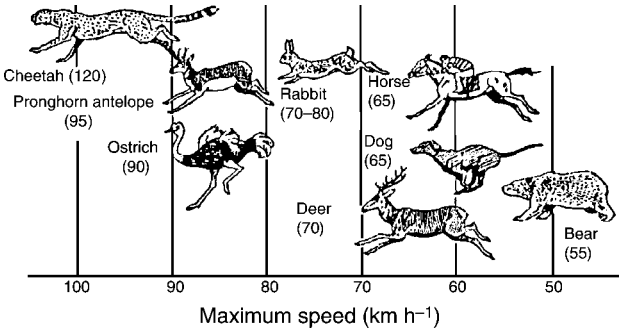

FIG. 2 Approximate maximum speeds for the Thoroughbred horse and a variety of terrestrial mammalian species including the ostrich. Please note that the Quarter horse[9] has been clocked close to 90 km h^{-1} (55 miles h^{-1}) whereas maximal speeds for the Thoroughbred are somewhat lower, as indicated in this figure. In addition, without the encumbrance of a rider and saddle, the horse would be considerably faster. Despite this consideration, the horse is clearly the fastest large land animal, i.e. body mass over 300 kg. For converting to other commonly used units: 10 km h^{-1} = 6.2 miles h^{-1} or 2.8 m s^{-1}. Revised from Kubo[10]

70 miles h^{-1} (120 km h^{-1}). Indeed as seen in Fig. 2, the Thoroughbred racehorse, which peaks close to 40 miles h^{-1} (65 km h^{-1}), compares rather poorly with the antelope (and even the gnu). In all fairness though, without the encumbrance of the rider (10–15% of its own body mass) and saddle, the racehorse is likely to be substantially faster. The fastest human clocks in at a rather pedestrian 27 miles h^{-1} (43 km h^{-1}). Over very short distances, the fastest horses ever timed are Quarter horses[9], which may reach speeds of up to 50–55 miles h^{-1} (88 km h^{-1}). In many respects, it seems rather unfair to compare the speeds of small and large animals, in part because muscle length (and thus absolute shortening velocity) scales to body length. A more equitable basis for trans-species comparison might be running speed as a function of body length, rather than absolute speed[11]. By this criterion, the Merriam kangaroo rat can achieve an astounding 110 body lengths per second! To place this in perspective, the cheetah at top speed moves at 32, and the horse at ~10 body lengths per second.

For exercise physiologists who study humans, the aerobic capacity or maximal oxygen uptake ($\dot{V}O_{2max}$) has become the gold standard for assessing the capacity to take up, transport and utilize O_2. $\dot{V}O_{2max}$ may be expressed in absolute terms (l O_2 min^{-1}), or perhaps more equitably across species in relative terms as a function of body mass (typically per kilogram per minute, i.e. ml O_2 kg^{-1} min^{-1}). Thus, absolute $\dot{V}O_{2max}$ for terrestrial mammals varies over five orders of magnitude as body mass increases, from the world's smallest mammal – the diminutive 2 g Etruscan shrew (~0.004 l O_2 min^{-1}) – to values of 80–100 l O_2 min^{-1} in the elite ~500 kg Thoroughbred racehorse. Relative $\dot{V}O_{2max}$ tells a somewhat different story. The Etruscan shrew[11,12] can consume a prodigious ~200–400 ml

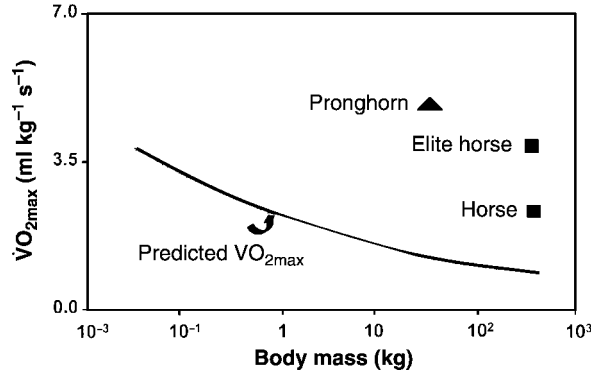

FIG. 3 Body-mass-specific maximal O_2 uptake ($\dot{V}O_{2max}$) plotted as a logarithmic function of body mass among a wide variety of mammals (solid line). Notice the extraordinary values plotted for the pronghorn antelope and the horse. Note units are in ml kg^{-1} s^{-1}. Redrawn from Lindstedt *et al.*[13]

O_2 kg^{-1} min^{-1} and, as seen in Fig. 3, with increased body mass across different mammalian species, relative $\dot{V}O_{2max}$ declines predictably. Two exceptions to this general pattern are the pronghorn antelope[13] ($\dot{V}O_{2max}$ ~300 ml O_2 kg^{-1} min^{-1}) and the Thoroughbred horse[11,14] ($\dot{V}O_{2max}$ > 200 ml O_2 kg^{-1} min^{-1}). Each can deliver ($\dot{Q}O_2$) and consume ($\dot{V}O_2$) more O_2 *in toto* and per unit body mass than any other mammal of their respective size. Moreover, as the horse is so much larger than the pronghorn antelope, the absolute $\dot{V}O_{2max}$ of the horse is several-fold greater (Fig. 4).

Determinants of O_2 delivery ($\dot{Q}O_2$) and $\dot{V}O_{2max}$

Role of the heart and cardiovascular system

In performance horses, skeletal muscle comprises over half of the body mass[15] and the mitochondrial oxidative capacity of that muscle exceeds the ability of the respiratory and cardiovascular systems to deliver O_2.

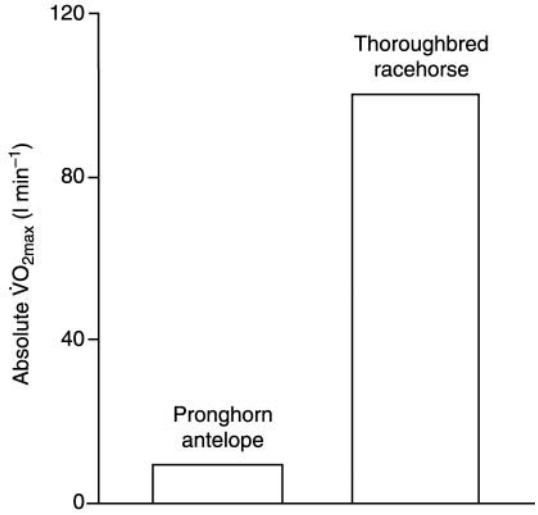

FIG. 4 Absolute maximal O_2 uptake ($\dot{V}O_{2max}$) plotted for the pronghorn antelope and the Thoroughbred racehorse

This has been termed O_2 supply limitation, and has been convincingly demonstrated in a range of species where interventions that increase muscle O_2 delivery increase $\dot{V}O_{2max}$. For example, increasing stroke volume during exercise by running horses on an incline[16], or removing the pericardium from the dog[17] and pig[18], increases $\dot{V}O_{2max}$. Raising the inspired O_2 above 21% elevates arterial O_2 content and $\dot{V}O_{2max}$ in horses[19] and humans[20]. One particularly dramatic unveiling of this O_2 supply limitation is seen in humans when the exercising muscle mass is restricted to 2–3 kg (knee extensors) instead of the 15–20 kg recruited during running. Specifically, once the cardiac output ceiling has been avoided, blood flow to the knee extensors reaches ~4 l kg^{-1} min^{-1} enabling attainment of a $\dot{V}O_{2max}$ in excess of 600 ml kg^{-1} min^{-1} for the knee-extensor muscles themselves[21]! Compare this value with the ~150 ml kg^{-1} min^{-1} for the same muscles during conventional cycle ergometry at $\dot{V}O_{2max}$[22]. In human athletes, blood doping has been popular for decades. This involves re-infusion of autologous red cells, which increases circulating haemoglobin concentrations and thus $\dot{V}O_{2max}$[23]. The doubling of systemic haematocrit by the horse's spleen at the onset of high-intensity exercise constitutes a physiological form of blood doping, removal of which reduces $\dot{V}O_{2max}$ by 20–30%[24] (see below).

In most species including the human, dog and horse, the strongest determinant of $\dot{V}O_{2max}$ is the capacity of the cardiovascular system to transport O_2 to the exercising muscles. However, there exists a series of diffusive (blood–gas barrier in the lungs, capillary–myocyte interface in muscle) and conductive (O_2 movement into the lungs, blood O_2 transport, muscle blood flow) steps from the atmosphere to the site of O_2 utilization by the muscle mitochondria that may limit $\dot{V}O_{2max}$[25–27]. During maximal exercise in the horse for example, the disproportionality in the capacities of the pulmonary and cardiovascular systems impairs O_2 loading in the lung and arterial hypoxaemia results. Moreover, the finite O_2-diffusing capacity of skeletal muscle means that there must always be some residual O_2 within the venous-blood-draining muscles. Figure 5 integrates the conductive and diffusive determinants of O_2 transport to show how the impressive $\dot{V}O_{2max}$ of the horse is attained.

From the above, it is clear that the horse reaches its prodigious $\dot{V}O_{2max}$ by achieving superlative rates of O_2 delivery ($\dot{Q}O_2$). Hence, it is useful to consider the components of $\dot{Q}O_2$:

$$\dot{Q}O_2 = \dot{Q}_{TOT} \times C_aO_2 \quad = HR \times SV \times [Hb] \times \%Sat$$

where $\dot{Q}_{TOT}$ is cardiac output, C_aO_2 is arterial O_2 content; HR is heart rate, SV is stroke rate, [Hb] is

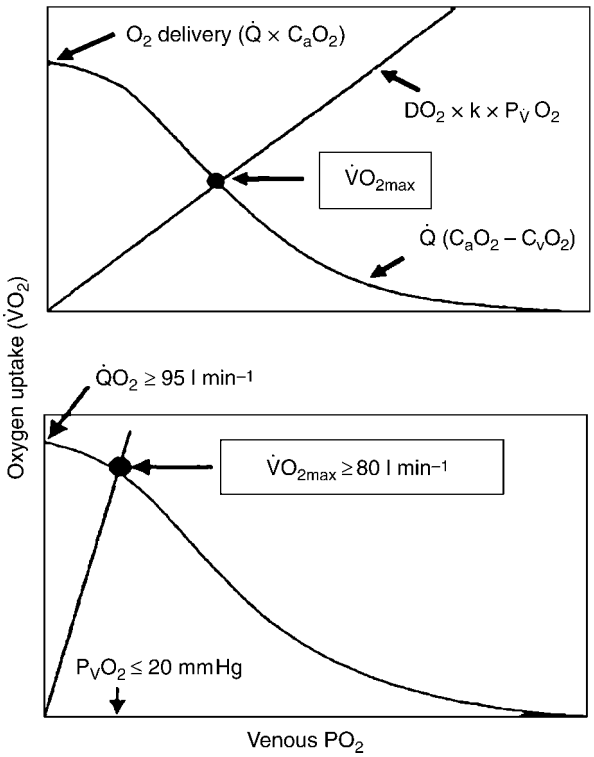

FIG. 5 Determination of maximal O_2 uptake ($\dot{V}O_{2max}$) by conductive ($\dot{Q}O_2$) and diffusive (DO_2) movement of O_2 by the cardiovascular and muscle microcirculatory systems ('Wagner' diagram, Wagner *et al.*[19,25,26]). Curved line denotes mass balance according to Fick's principle, and the straight line from the origin represents Fick's law of diffusion. DO_2 is effective diffusing capacity and '*k*' is a constant that relates measured venous partial pressure of O_2 (P_vO_2) to calculated mean capillary PO_2 ($P_{cap}O_2$). Thus $\dot{V}O_2 = DO_2(P_{cap}O_2 - P_{mito}O_2)$, where $P_{mito}O_2$ is the partial pressure of O_2 in the mitochondrion. C_aO_2 and $C_{\bar{v}}O_2$ are the concentrations of O_2 in arterial and mixed venous blood, respectively. $\dot{V}O_{2max}$ occurs at the intersection of the two lines. Top: general schematic redrawn from Wagner *et al.*[26]. Bottom: values for fit Thoroughbred racehorses at maximal running speeds. The principal determinants of $\dot{Q}O_2$ and DO_2 during maximal exercise are analysed subsequently in this review

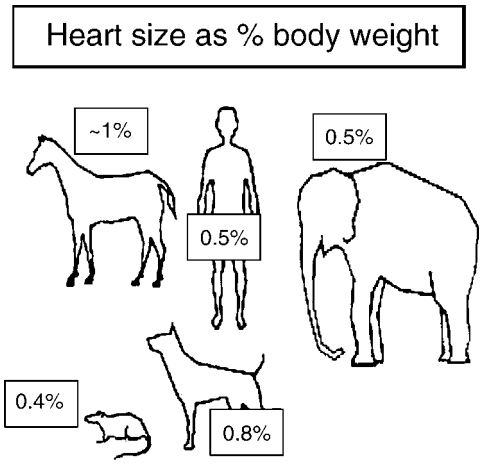

FIG. 6 Relative heart size (expressed as a percentage of body mass) for various mammalian species differing in body mass from the 0.25 kg (0.55 lb) rat to the 4000–12 000 kg (8800–26 400 lb) elephant. See text for more details

haemoglobin concentration and %Sat is % saturation of Hb O_2 binding sites.

During maximal exercise in the horse, SV is set primarily by cardiac size (which in turn is influenced by blood volume) and HR can exceed 4 Hz (i.e. 240 beats min^{-1}). As a consequence of splenic contraction, arterial haemoglobin concentration ([Hb]) increases almost 100% from rest. Athletic species such as the horse and dog have larger hearts and spleens relative to their body mass than their pedestrian counterparts (e.g. elephant or man, Fig. 6)[28,29]. Thus, despite the heart of a 4400 kg elephant weighing in at an impressive 22.5 kg (49.5 lb), it constitutes only ~0.5% (or less) of its body mass (Fig. 6). It should be noted that the dog illustrated in Fig. 6 is the average. The greyhound may have a heart mass averaging up to 1.3% of body mass (individual dogs may approach 1.9%)[29].

It has been recognized for several decades that the structural dimensions of the heart represent a key determinant of maximum SV, $\dot{Q}_{TOT}$ and hence $\dot{V}O_{2max}$ and exercise performance. Post-mortem examination of superlative human distance runners, such as Olympic champion Paavo Nurmi and seven-time Boston marathon winner Clarence DeMar, has revealed heart sizes substantially larger than predicted on the basis of their body mass[30]. Heart mass in horses ranges from 0.9% of body mass in untrained horses up to 1.1% of body mass in trained horses[31]. The largest horse breeds, for example draught horses, may have an immense body mass (Fig. 7) but a smaller relative heart mass than their fleeter brethren. Specifically, racing horses have a proportionally greater heart size, and some of the largest healthy hearts ever recorded have come from famous horses such as Sham, Mill Reef, Key to the Mint, Phar Lap and Eclipse. Figure 8 compares Key to the Mint's 7.2 kg (15.8 lb) heart to

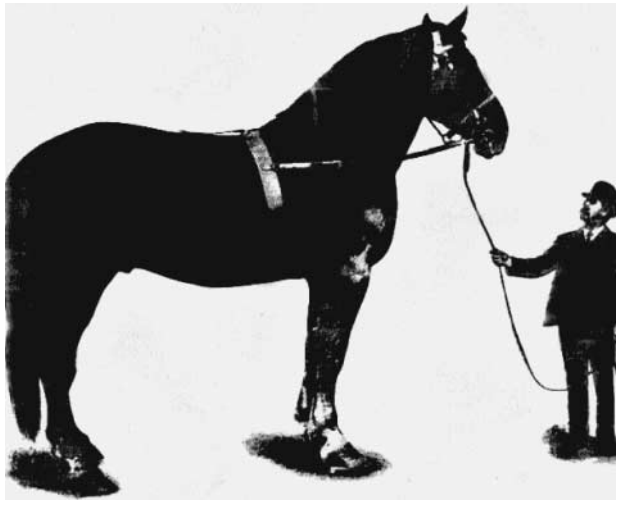

FIG. 7 One of the world's largest horses, Dr. Le Gear weighed in at 3940 lb in 1903. Photograph courtesy of Mr Weldon Dudley

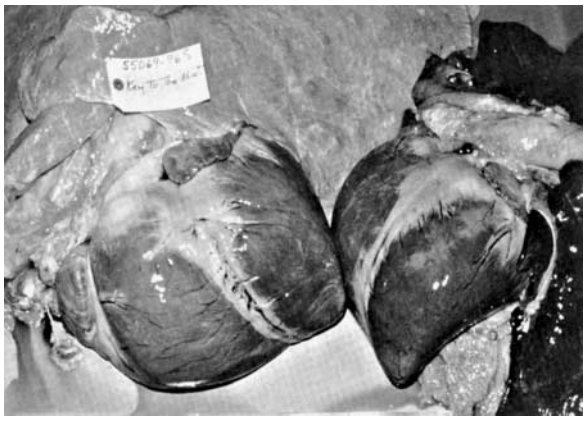

FIG. 8 Comparison of Key to the Mint's heart on the left (7.2 kg; 15.8 lb) with that of an unremarkable stallion on the right (5.5 kg; 12 lb). Photograph courtesy of Dr Thomas Swerczek, with permission

that of an unexceptional stallion. Sham, who was consistently runner-up to the great Secretariat, had a healthy heart that weighed in at 8.2 kg (18 lb). Secretariat himself was a Triple Crown winner and performed some of the greatest feats in horseracing history. Unfortunately, his heart was never weighed at necropsy, but the pathologist Dr Thomas Swerczek, who weighed Sham's heart, estimated Secretariat's heart to be about 10 kg (22 lb, ~2% of body weight) and perfectly healthy[30]. Assuming that weight to be accurate, Fig. 9 predicts that Secretariat may have been capable of reaching cardiac outputs in excess of 500 l min^{-1} (Fig. 9a) and $\dot{V}O_{2max}$ over 120 l min^{-1} (>240 ml kg^{-1} min^{-1}) (Fig. 9b). Given the decisive advantage of possessing a large healthy heart, there has been substantial interest in identifying the gene, located on the X chromosome, that codes for heart mass[30].

Arterial blood O_2 content (C_aO_2): role of spleen and lungs

Spleen

As seen above, maximal O_2 transport ($\dot{Q}O_2$) is determined by the product of cardiac output ($\dot{Q}_{TOT}$) and arterial O_2 content (C_aO_2). The equine spleen is of paramount importance for setting the high exercising blood [Hb] and thus C_aO_2 of the horse. The spleen is under sympathetic control and, when it contracts, 121 or more of red blood cells (RBCs) are dumped into the circulation. This effectively doubles the volume of circulating RBCs[24,33,34]. The capacity of the spleen correlates with splenic mass and total blood volume, and is substantially greater in racing than in non-racing breeds, reaching 0.4–0.5% of body weight[31,33,35]. C_aO_2 is determined principally by [Hb] and the % saturation of O_2 binding sites. In the resting horse, arterial [Hb] is 12–14 g 100 ml^{-1} (haematocrit ~35–40%) whereas at maximal exercise the spleen has expelled sufficient RBCs to increase this to 21–24 g 100 ml^{-1} (haematocrit ~60–70%) (see Fig. 10b for the effect of this haemoconcentration on O_2 content). This haemoconcentration also contributes to the high pulmonary artery pressures found during exercise[36]. The O_2-carrying capacity of the blood at maximal exercise (if all O_2 binding sites on Hb were filled) may be as high as 31 ml O_2 100 ml^{-1}. However, as we shall see below, due to a mismatching of the pulmonary and cardiovascular capacities that results in part from the physical limitation to increasing lung (but not heart) volume imposed by the thoracic dimensions, the actual C_aO_2 during exercise is considerably less than this value.

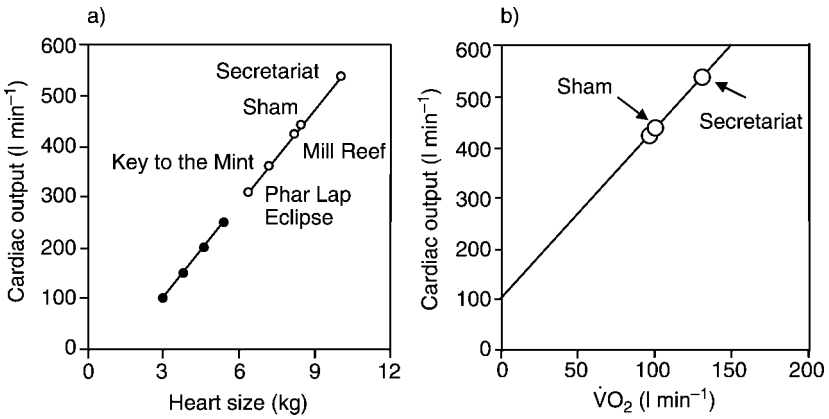

FIG. 9 Relationship between cardiac output ($\dot{Q}$) and heart size (a), and cardiac output and O_2 uptake ($\dot{V}O_2$) at maximum exercise (b). In (a), solid symbols are determined from the data of Evans and Rose[32]; the hollow symbols are determined from that relationship and the measured or estimated (Secretariat) heart weights published for each named horse[30]. In (b), an arterial–venous O_2 difference of 22.8 ml 100 ml^{-1} of blood is assumed to estimate maximal O_2 uptake ($\dot{V}O_{2max}$) values for Secretariat and Sham (the unlabelled point is Mill Reef). Note Secretariat's extraordinary cardiac output (~540 l min^{-1}) and $\dot{V}O_{2max}$ (>120 l min^{-1}, which would be 240 ml O_2 kg^{-1} min^{-1} at 500 kg body weight)

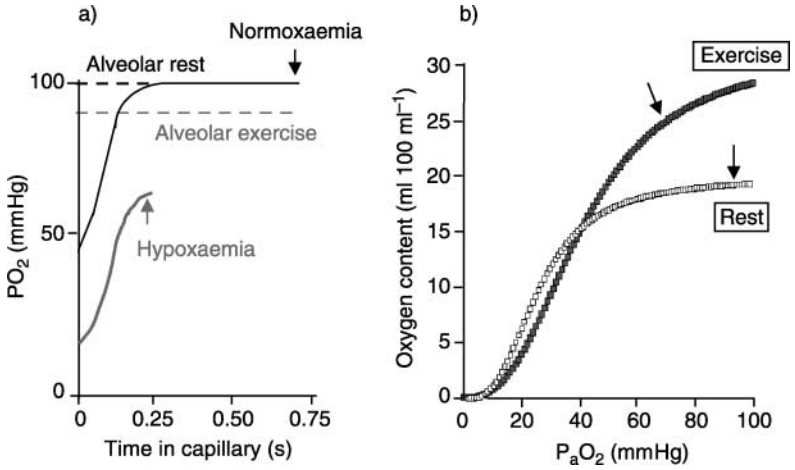

FIG. 10 Oxygen loading in the lungs. (a) Partial pressure of O_2 (PO_2) in the red blood cell as a function of pulmonary capillary transit time at rest and during maximal exercise. Note the reduction in alveolar PO_2 during exercise (due in part to alveolar hypoventilation) and the profound decrease in red cell PO_2 as it enters and leaves the capillary during maximal exercise compared with rest. (b) O_2 dissociation curves in the Thoroughbred racehorse at rest and during maximal exercise (where P_aO_2 is arterial partial pressure of O_2). The arrows denote the arterial points under each condition

Lungs

Maximal exercise reduces arterial O_2 saturation[24,37–39] from ~95% at rest to <85% and this lowers C_aO_2 to below 26 ml 100 ml^{-1} (Fig. 10). Of those mechanisms responsible for the exercise-induced hypoxaemia prevalent in the horse, the most important quantitatively are alveolar hypoventilation, diffusion limitation and the rightward shift of the O_2 dissociation curve. Man experiences a carotid body-mediated hyperventilation during high-intensity exercise; conversely, the horse exhibits a profound hypoventilation, and the pressure of arterial carbon dioxide (P_aCO_2) may increase from 40 mmHg at rest to values in excess of 65 mmHg[24,37–39]. This effectively reduces the alveolar partial pressure of O_2 (PO_2) from ~100 mmHg at rest to ~90 mmHg at maximal exercise and impairs the rate of O_2 diffusion across the blood–gas barrier (Fig. 10).

Alveolar–capillary O_2 diffusion limitation may account for roughly two-thirds of the alveolar-to-capillary O_2 pressure differential present during maximal exercise in the horse. In the presence of a lowered alveolar PO_2, a significant alveolar-to-end capillary O_2 pressure gradient is developed[37] as a result of several factors. First, in fit horses, the extraordinary rest–exercise increase in cardiac output ($\dot{Q}_{TOT}$, ~13-fold) is several-fold greater than the elevations in capillary blood volume that result from recruitment and distension in the pulmonary vasculature. This reduces mean capillary RBC transit time from ~0.75 s at rest to below 0.2–0.3 s, such that there is insufficient time for O_2 equilibration across the blood–gas barrier. Whereas morphometric calculations place mean exercising capillary RBC transit time at 0.3–0.5 s[40,41], this value is not likely to be representative of that in a fit racehorse with a $\dot{Q}_{TOT}$ in excess of 400 l min^{-1}. Moreover, because there will be a considerable spread in the individual capillary RBC transit times, even a mean value of 0.4 s is probably indicative of a significant portion of the RBCs that have a much shorter capillary transit time. The sigmoidal shape of the O_2 dissociation curve impairs the ability of those cells with longer transit times to load sufficient O_2 to compensate for those that do not equilibrate with the alveolar gas. In addition, as detailed above, the mean alveolar PO_2 falls below 100 mmHg due to hypoventilation, which will set an upper limit for O_2 loading even in those capillaries with longer RBC transit times. Second, the very high pulmonary artery pressures will increase fluid extravasation into the alveolar space, which will serve effectively to thicken the blood–gas barrier and lower pulmonary diffusing capacity[42,43]. Third, a ventilation-to-perfusion ($\dot{V}$:$\dot{Q}$) mismatch (≤1% shunt) does develop during exercise, but this may be too small in healthy horses to constitute a quantitatively important contribution to the arterial hypoxaemia[36,44]. Finally, because of their great muscle mass and capacity to generate heat, coupled with a relatively modest ratio of surface area to body mass, horses experience substantial increases in blood temperature[38,45] (>43°C). These high temperatures, combined with arterial hypercapnia (increased P_aCO_2) and a lactic acidosis, act to reduce the haemoglobin–O_2 affinity and further exacerbate alveolar–capillary PO_2 disequilibrium.

Muscle O_2 extraction

$\dot{V}O_{2max}$ is the product of $\dot{Q}_{TOTmax}$ and the extraction of O_2 (primarily by the muscles) as described by the Fick

equation:

$$\dot{V}O_{2max} = \dot{Q}_{TOTmax} \times (C_aO_2 - C_vO_2),$$

where C_aO_2 and C_vO_2 denote arterial and venous O_2 contents, respectively.

Figure 5 demonstrates that the effective muscle O_2-diffusing capacity (D_mO_2, estimated by the slope of the line projecting from the origin) determines the level to which C_vO_2 (and venous O_2 pressure, P_vO_2) will fall at maximal exercise (i.e. O_2 extraction) and also the $\dot{V}O_{2max}$. The determinants of muscle O_2-diffusing capacity are considered below under Muscle microcirculation and microvascular O_2 exchange.

$\dot{V}O_2$ kinetics at exercise onset

How fast can $\dot{V}O_2$ increase at the onset of exercise?

At the onset of exercise or across the transition to a higher work rate or running speed, the energy demand (ATP requirement) increases instantaneusly. In contrast, the O_2 utilization ($\dot{V}O_2$) increases with a finite speed or kinetics that has been defined in several species. The speed of the $\dot{V}O_2$ kinetic response is of crucial importance because that ATP not generated aerobically must be taken either from very limited stores of phosphocreatine (PCr) or anaerobic glycolysis, leading to lactic acid accumulation. Thus, when $\dot{V}O_2$ kinetics is slow (large time constant or τ of the response, where τ is the time to 63% of the final response), the so-called O_2 deficit will be large (Fig. 11) and this mandates a greater degree of intracellular perturbation that includes lowered PCr concentration and elevated concentrations of free adenosine diphosphate ($[ADP]_{free}$), creatine ([Cr]), inorganic phosphate ([Pi]) and hydrogen ions ($[H^+]$). The larger the change in these metabolites from rest, the greater will be the stimulation of glycolysis and thus the rate of utilization of very modest intramuscular glycogen stores. When these intramuscular glycogen stores are reduced or depleted, the muscle fatigues[5].

The profile of $\dot{V}O_2$ increase at the transition to a higher running speed is seen in Fig. 12 for moderate (Fig. 12a, 7 m s^{-1}) and heavy (Fig. 12b, 10 m s^{-1}) exercise intensity domains in the Thoroughbred racehorse. There are three phases of the $\dot{V}O_2$ increase that correspond to discrete physiological events[46-48]. Specifically, Phase I corresponds to the immediate increase in $\dot{V}O_2$ that is initiated within the first breath at the onset of exercise. This increase is driven by the elevated $\dot{Q}_{TOT}$ and venous return that push more deoxygenated blood through the lungs. Phase II is initiated as venous blood from the exercising muscles, with its lowered O_2 content, arrives at the lungs. The continued increase in $\dot{V}O_2$ throughout Phase II reflects any continued increase in $\dot{Q}_{TOT}$ coupled with the greater deoxygenation of the venous blood. Phase III reflects

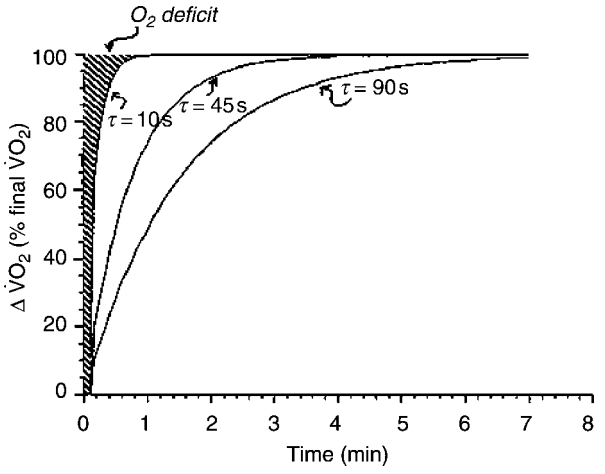

FIG. 11 Effect of different O_2 uptake ($\dot{V}O_2$) kinetics on the size of the O_2 deficit (shaded area) as a function of relative $\dot{V}O_2$. The time constant, τ, indicates the time to 63% of the final response ($\dot{V}O_2$ steady-state). τ values given −10, 45 and 90 s – correspond to the Thoroughbred horse, healthy human and heart failure patient, respectively. Notice that for the longer τ values, $\dot{V}O_2$ kinetics are slower and the size of the O_2 deficit will therefore be greater

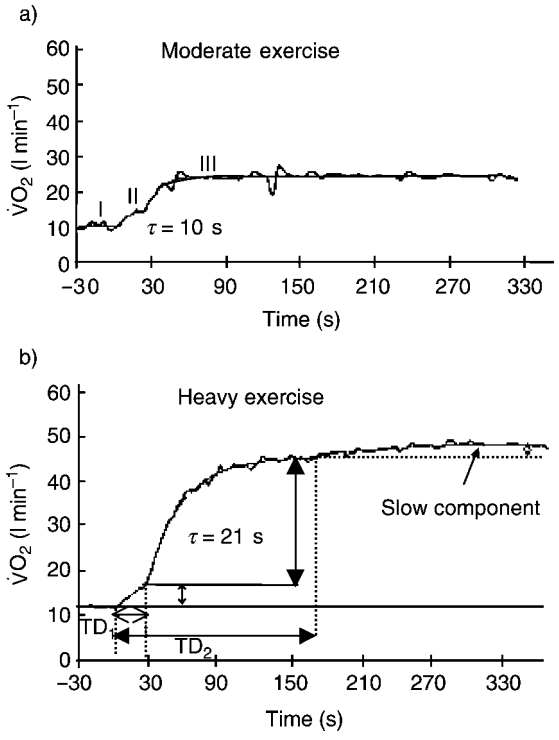

FIG. 12 O_2 uptake ($\dot{V}O_2$) response at the onset of moderate (7 m s^{-1}, a) and heavy (10 m s^{-1}, b) exercise in the Thoroughbred horse (redrawn from Langsetmo *et al.*[55]). I, II and III correspond to the respective Phases of the $\dot{V}O_2$ response. TD_1 and TD_2 denote time delays before onset of Phase II (primary fast exponential response) and slow component $\dot{V}O_2$ responses, respectively. Note slowing of the fast exponential response for heavy compared with moderate exercise. Please see text for additional details

attainment of the steady state. Typically, for moderate-intensity exercise, these responses are best modelled as a time delay (corresponding approximately to the duration of Phase I) followed by a mono-exponential increase (Phase II, characterized by τ) in $\dot{V}O_2$ to the steady state (Phase III)[49,50]. For heavy-intensity exercise, a secondary slow component increase of $\dot{V}O_2$ is superimposed on the primary fast exponential increase associated with exercise onset[46,51]. In humans and horses, this slow component increase is initiated about 80–120 s following the exercise transition, and elevates $\dot{V}O_2$ progressively above that predicted for a given speed or work rate from the exponential response[52–54]. In addition, for heavy exercise in the horse, the rapid exponential response is markedly slowed (longer τ) from that seen for moderate exercise[55] (Fig. 12).

Within humans and across a broad range of species, the speed of the $\dot{V}O_2$ kinetics is correlated with the individual's $\dot{V}O_{2max}$[56,57]. This is evident, for example, when examining a group of people with a broad range of $\dot{V}O_{2max}$ values, or alternatively following the same individuals before and after a period of exercise training[56,58]. Moreover, cardiac patients who suffer from a severely dysfunctional capacity for cardiovascular O_2 transport and muscle O_2 exchange/utilization have pathologically slowed $\dot{V}O_2$ kinetics[59,60] (Fig. 13). In stark contrast to these patients, the horse demonstrates exceedingly fast kinetics which is in keeping with its prodigious $\dot{V}O_{2max}$. Thus, for a sizeable increase in $\dot{V}O_2$, the horse will generate a relatively modest O_2 deficit[55]. This effect is demonstrated across a range of τ values from the very slow kinetics evidenced in a heart-transplant patient – or seen in the ghost crab ($\tau = 90$ s[61]) – to the healthy human ($\tau = 45$ s[46,53,54,62]) and the Thoroughbred horse ($\tau = 10$ s[55]) (Fig. 11).

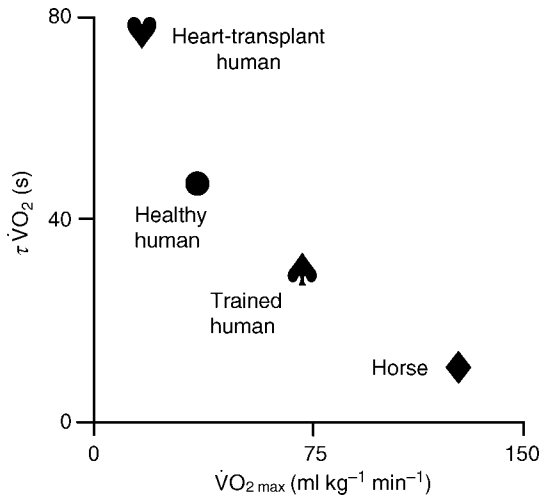

Fig. 13 Time constant (τ) of the O_2 uptake ($\dot{V}O_2$) kinetics plotted as a function of maximal O_2 uptake ($\dot{V}O_{2max}$) for human heart-transplant patients and their healthy controls[59], trained individuals[56] and the Thoroughbred horse[55]

Does O_2 delivery or oxidative enzyme inertia limit $\dot{V}O_2$ kinetics?

Given that there are finite energy stores (e.g. PCr, glycogen) within skeletal muscle, coupled with the pernicious effects of H^+, Pi and possibly lactate[63] on the contractile apparatus and cellular function, it is obviously beneficial to limit the size of the O_2 deficit as much as possible. As mentioned above, exercise training speeds $\dot{V}O_2$ kinetics – most likely due to the elevated mitochondrial volume found after training. In addition, there may also be improved O_2 delivery and a more sensitive vascular control[64]. Despite these observations, the precise mechanisms that limit the speed of the $\dot{V}O_2$ kinetics, particularly for heavy exercise (above the lactate threshold), remain the subject of great scientific contention[65–68]. Specifically, for moderate exercise (below the lactate threshold) in healthy individuals, there is no evidence of a muscle O_2 shortage across the rest–exercise transition in muscle from rats[69], dogs[70] or humans[62,71]. Moreover, elevated arterial O_2 or a faster/greater delivery of O_2 does not speed the dynamics[66,70]. Consequently, the compelling weight of evidence suggests that it is the inertia of the mitochondrial enzyme system that sets the maximum speed of the $\dot{V}O_2$ kinetics. Experiments in the horse have demonstrated clearly that reduction of nitric oxide (NO), via L-NAME (N^G-L-nitro-arginine methyl ester) blockade of NO synthase, speeds the $\dot{V}O_2$ kinetics for moderate[72] (Fig. 14) and heavy[73] exercise. The most likely mechanism for this effect is relief of the NO inhibition of cytochrome oxidase within the respiratory chain. This constitutes extremely powerful evidence that muscle O_2 delivery does not limit $\dot{V}O_2$ dynamics in the horse, because NO synthase blockade

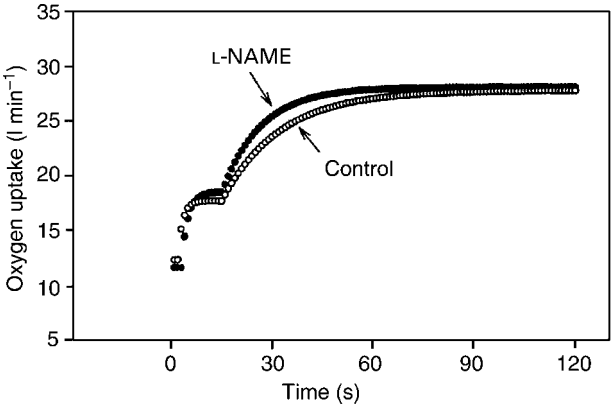

Fig. 14 Inhibition of nitric oxide production by L-NAME (N^G-L-nitro-arginine methyl ester; an inhibitor of nitric oxide synthase) significantly speeds the O_2 uptake kinetics at the onset of moderate speed running (7 μm s^{-1}) in the Thoroughbred horse. Redrawn from Kindig *et al.*[72]

reduces cardiac output[38] ($\dot{Q}_{TOT}$) and probably muscle blood flow[74]($\dot{Q}_m$) and O_2 delivery whilst simultaneously speeding $\dot{V}O_2$ kinetics[72,73].

It is apparent that much remains to be determined regarding the control of $\dot{V}O_2$ kinetics. Pathological conditions that greatly impair muscle O_2 delivery clearly slow $\dot{V}O_2$ kinetics[59,60] (Fig. 13), as does a lowering of the inspired O_2 fraction (i.e. hypoxia)[75] and supine exercise in humans[65]. Thus, it is likely that conditions such as airway inflammation, exercise-induced pulmonary haemorrhage and cardiac dysrhythmias that act to reduce O_2 delivery will also slow $\dot{V}O_2$ kinetics in the horse. Other than exercise training[57], blockade of NO production[72,73] and breathing hyperoxic gas mixtures during heavy-intensity exercise[76], no other methods of speeding $\dot{V}O_2$ kinetics have been identified. However, the beneficial effects of fast $\dot{V}O_2$ kinetics are unequivocal and, all else being equal, the individual who generates the smallest O_2 deficit for a given change in metabolic rate will preserve muscular function and exercise capacity better than their counterpart who is constrained by slower $\dot{V}O_2$ kinetics and hence a larger O_2 deficit.

Muscle microcirculation and microvascular O_2 exchange

Muscle blood flow ($\dot{Q}_m$) during exercise

From rest to maximal exercise, arterial and arteriolar smooth muscle in the gut and splanchnic regions constricts, and that within active skeletal muscle dilates. This facilitates a massive redistribution of $\dot{Q}_{TOT}$ such that muscle $\dot{Q}$ ($\dot{Q}_m$) increases from 10–20% of $\dot{Q}_{TOT}$ at rest to as much as 90% during exercise. Multiple sources/mechanisms are responsible for this vasoactive control and $\dot{Q}_{TOT}$ redistribution including: muscle metabolites, prostacyclins, NO and conducted vasodilation (dilation) or sympathetic stimulation, angiotensin and endothelin (constriction)[77–80]. Also important in facilitating the >50-fold increase in $\dot{Q}_m$ and muscle O_2 delivery ($\dot{Q}_mO_2$) during exercise in the horse is the increased arterial driving pressure, mean arterial pressure (MAP; ~200 mmHg, which is substantially higher than in most other mammals) and the action of the muscle pump[38,79,81–86].

During exercise, $\dot{Q}_m$ is distributed heterogeneously between and within muscles depending on their recruitment, oxidative capacity, fibre type and $\dot{V}O_2$ demands[79] ($\dot{V}_mO_2$). Thus, in the exercising horse, $\dot{Q}_m$ in heavily recruited, highly oxidative red muscles in the limbs and respiratory system may achieve peak values[87–89] of 1–3 l min^{-1} kg^{-1}. Specifically, Armstrong *et al.*[89] measured $\dot{Q}_m$ of the *vastus intermedius* at 1.5 l min^{-1} kg^{-1} in Standardbred horses running at $\dot{V}O_{2max}$ (134 ml kg^{-1} min^{-1}; $\dot{Q}_{TOT}$ 288 l min^{-1}). Within the thigh muscles sampled, there was a strong correlation ($r = 0.947$) between $\dot{Q}_m$ at $\dot{V}O_{2max}$ and citrate synthase activity (used as a marker of oxidative enzyme activity). During exercise, the matching between $\dot{Q}_mO_2$ and $\dot{V}_mO_2$ is so well balanced that fractional O_2 extraction approaches 90% (Fig. 5) and this has inspired the Panglossian suggestion that there exists an O_2 sensor located within the muscle or its vascular bed[78]. However, if present, such a sensor remains to be identified.

$\dot{Q}_m$ traverses multiple orders of muscular arterioles that decrease in luminal diameter progressively between the feed artery and the capillary bed that ramifies from the small terminal arterioles. Whereas these arterioles may participate in O_2 exchange[90], the capillary bed is the predominant site for blood–myocyte O_2 transfer, certainly under exercising conditions. The capillary wall does not contain smooth muscle and therefore can be extremely thin ($\ll 1\,\mu m$), in order to facilitate gas exchange (Fig. 15b). The density, volume and surface area of the skeletal muscle capillary bed are correlated closely with oxidative capacity[92,93]. Equine locomotory skeletal muscles are highly oxidative and the capillary bed is correspondingly rich, with between 400 and 800 capillaries mm^{-2}, each with a mean diameter of 4–6 μm[89,94]. These capillaries contain over three-quarters of the intramuscular blood volume. As discussed earlier with regard to the pulmonary capillaries, the skeletal muscle capillary volume is crucial for setting RBC transit time in the capillary and facilitating O_2 offloading during exercise (Fig. 15a).

As mentioned above, for more prolonged exercise (i.e. >60–90 s duration) where approximately steady-state conditions can be achieved beyond the initial transient at exercise onset, $\dot{Q}_mO_2$ and $\dot{V}_mO_2$ are matched closely, which suggests that $\dot{Q}_mO_2$ is ultimately controlled by muscle metabolism[20–22,77,84,95]. At the immediate onset of exercise, however, $\dot{Q}_mO_2$ increases within the first one or two contractions (Fig. 16), which is faster than that of $\dot{V}_mO_2$[86,96] (Fig. 12) and thus cannot be explained by either metabolic feedback or other vasodilatory mechanisms currently described[57,97]. Indeed, $\dot{Q}_mO_2$ rises so rapidly that fractional O_2 extraction falls and effluent venous O_2 content rises[62]. By a process of elimination, the muscle pump is thought to be responsible for increasing the pressure differential, and therefore flow, across the muscle vascular bed and augmenting flow within the first contraction cycle[85,86,96] (Fig. 16). Another mechanism that may operate rapidly to increase $\dot{Q}_m$ is conducted vasodilation that is initiated within the capillaries adjacent to active muscle fibres, and which induces vasodilation upstream within the

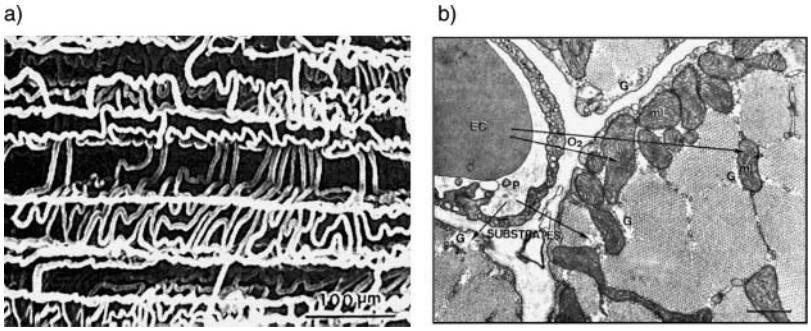

FIG. 15 The skeletal muscle capillary bed possesses a complex three-dimensional geometry with extensive branching and capillaries that become extremely tortuous at short muscle sarcomere lengths. (a) Corrosion cast (muscle fibres have been corroded away) of mouse *soleus* muscle showing three-dimensional geometry of the muscle capillary bed (from Ishikawa *et al.*[91], with permission). (b) Electron micrograph depicting the passage of O_2 from the red blood cell (RBC), or erythrocyte (EC), to the mitochondria (mi). P – plasma; G – glycogen granules. Bar = 0.5 µm. Note the extremely short physical space between the RBC and the subsarcolemmal cytoplasm. From Weibel[12], with permission

arteriolar bed[80,98]. The aforementioned demonstration by Kindig *et al.*[72,73] that NO inhibition speeds $\dot{V}O_2$ kinetics at exercise onset, despite any concomitant $\dot{Q}_m$ reduction, suggests that NO itself is probably not essential to the immediate $\dot{Q}_m$ increase at exercise onset[38,99] (Fig. 14).

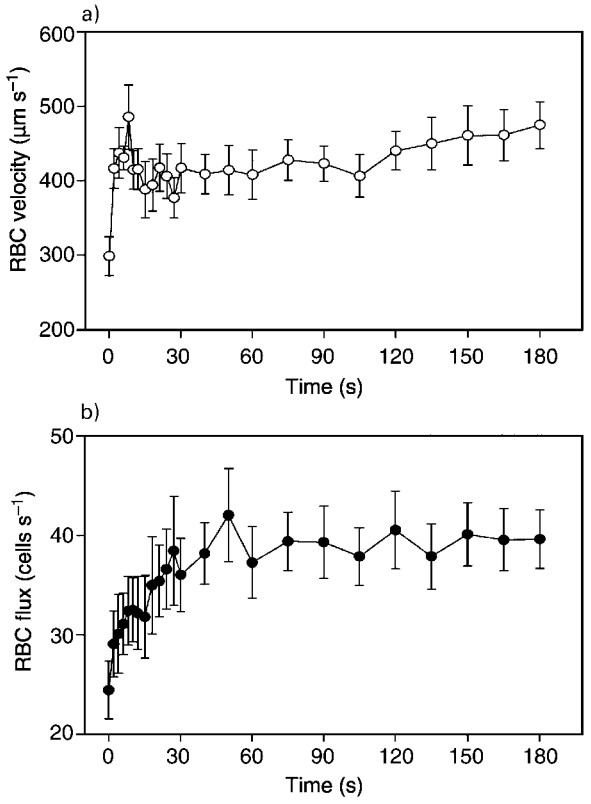

FIG. 16 Capillary red blood cell (RBC) velocity (a) and flux (b) increase within the first contraction cycle at the start of muscle contractions (electrical stimulation at 1 Hz). From Kindig *et al.*[86], with permission

Determinants of O_2 exchange within skeletal muscle: the microcirculation

Despite their tenacious presence in medical/physiology textbooks and the classroom, there are certain commonly held misconceptions regarding the processes of muscle microcirculatory blood–myocyte O_2 exchange that do not cohere with recent experimental evidence. Paramount among these misconceptions are the following:

- capillaries in skeletal muscle are straight, unbranched structures aligned with the muscle fibres;
- the haematocrit in the capillary is the same as in the systemic circulation;
- during exercise, muscle O_2-diffusing capacity (D_mO_2) must increase by recruitment of more capillaries;
- because of very high $\dot{Q}_{TOT}$, blood flow to individual muscles is so great that capillary RBC transit time becomes limiting; and
- large diffusion distances in muscle lead to anoxic regions and limit mitochondrial O_2 delivery and function.

This section addresses these issues within the context of blood–myocyte–mitochondrial O_2 delivery during exercise. Please note that, owing to technical infeasibility, the majority of functional microcirculatory measurements presented have been made in animals other than the horse.

The size (volume, surface area) and structural geometry (luminal diameter, tortuosity, branching) of the muscle capillary network in combination with the RBC flux and distribution within that network determine the potential for O_2 exchange[94,100,101].

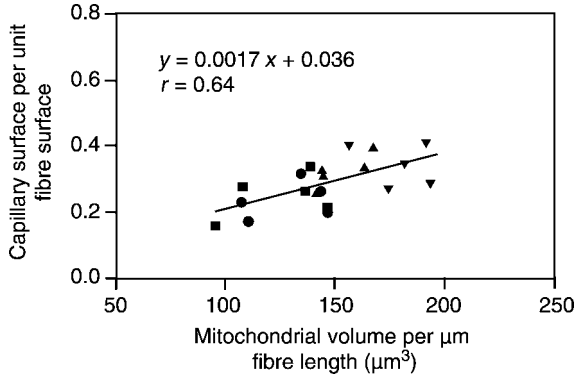

FIG. 17 In muscles of different fibre type (*soleus*: slow-twitch [triangles]; *plantaris*: fast-twitch [circles, squares]) in the untrained (control, circles, upright triangles) and exercise-trained (squares, inverted triangles) conditions, capillary surface per unit fibre surface contact correlates significantly with fibre mitochondrial volume. Redrawn from Poole and Mathieu-Costello[93]

As seen in Fig. 15a, these capillaries form a rich interconnecting network of 4–6 μm diameter vessels (for comparison, horse RBCs are 5.5 μm and a human hair may be 50–100 μm in diameter). At short muscle sarcomere lengths (<2.3–2.6 μm), capillaries are tortuous (Fig. 15a[91]), whereas at long sarcomere lengths (>2.8 μm), the capillaries become stretched and closely aligned with the muscle fibres[94,102]. As capillaries stretch, their lumen becomes narrowed and the resistance to RBC passage increases, which lowers the capillary RBC flux and velocity[103–105]. Capillary surface area within skeletal muscle appears to be regulated as a function of fibre mitochondrial volume, which is a good approximation of maximal O_2 demands[93] (Fig. 17). In the horse, there are 700–800 km of capillary length per kg (2.2 lb) of limb muscle which supply ~40–50 ml of mitochondrial volume[89,94,106], each of which is thought to be capable of utilizing ~5 ml O_2 min^{-1} ml^{-1}.

Measurement of capillary RBC flux and distribution within individual capillaries during muscle contractions is a noteworthy achievement and it is only within the last few years that this has been possible across the rest–contractions transition (Fig. 16). At rest, in the *spinotrapezius* muscle of the rat, ~80% of capillaries support RBC flow[105] at an average velocity of ~250 μm s^{-1} and a capillary tube haematocrit that averages only 25–50% of systemic values[86,107–109]. Thus, capillary surface area and therefore D_mO_2 cannot be increased substantially from rest to exercise by simple recruitment of additional capillaries. One intriguing model developed for pulmonary capillaries by Wagner[110] is that as capillary RBC velocity increases during exercise and there is a greater O_2 desaturation of venous blood in the pulmonary artery, the RBC utilizes a greater length of the pulmonary capillary for O_2 loading. In this model, additional capillary surface area is recruited longitudinally within capillaries that were already recruited at rest. Coupled with the elevated capillary haematocrit found during exercise, this longitudinal capillary recruitment may help to explain the huge increases in D_mO_2 seen in exercising muscle.

The number of RBCs adjacent to the muscle fibre is thought to determine the instantaneous muscle D_mO_2[100,101] and therefore the potential for O_2 flux will be the product of the total length of capillaries adjoining a muscle fibre and their haematocrit (i.e. RBC number per unit length of capillary). As muscle contracts and blood flow increases, capillary RBC velocity and flux are elevated rapidly (Fig. 16) and haematocrit increases towards systemic values[86]. By extrapolation from rodent to horse muscle, using equine capillary morphometric[94] and blood flow[89] data, from rest to maximal exercise, capillary haematocrit is predicted to increase from ~10% up to ~60% and mean RBC capillary transit time will decrease to no less than ~1 s. This presents an interesting scenario. Specifically, during maximal exercise, if the systemic polycythaemia is expressed at the microcirculatory level (as suggested from rodent studies), the six-fold increase in capillary haematocrit will facilitate rapid blood–myocyte O_2 exchange. Moreover, as capillary RBC transit time is not thought to become limiting for O_2 offloading until values of <0.3–0.5 s are reached, the minimal mean transit time of ≥1.0 s in horse muscle is so long that there probably exists only a small proportion of capillaries where O_2 offloading is limited. These considerations help explain how the horse achieves such excellent O_2 extractions (up to 85–90%) at very high $\dot{Q}_{TOT}$.

In skeletal muscle (as in the lung), O_2 diffuses down its pressure gradient from the capillary towards the mitochondria at a rate ($\dot{V}O_2$) that is determined by the O_2 pressure (PO_2) difference between capillary and mitochondria and the tissue diffusing capacity for O_2 (D_mO_2) according to Fick's law ($\dot{V}O_2 = D_mO_2$ ($P_{microvasc}O_2 - P_{mito}O_2$), Fig. 18). The presumption (dating back to the brilliant Nobel laureate August Krogh in 1919[111]) that there exists a large PO_2 gradient that decreases proportionally from the myocyte sarcolemma to the most distant mitochondrion, is at odds with cryomicrospectrophotometric[112] and magnetic resonance spectroscopy[113] measurements of low (<3 mmHg) intramyocyte PO_2 during exercise. Moreover, no substantial transverse or longitudinal variation in intramyocyte PO_2 has been detected[112] (Fig. 19). These observations are of crucial importance because they mean that the greatest fall in PO_2 occurs within ~1 μm or so of the RBC, with little further fall over the subsequent tens of μm to the most remote mitochondria. Historically, intramyocyte O_2 transport is thought to be facilitated by myoglobin. However, preservation of exercise capacity in mice that are genetically engineered without myoglobin[114] suggests that

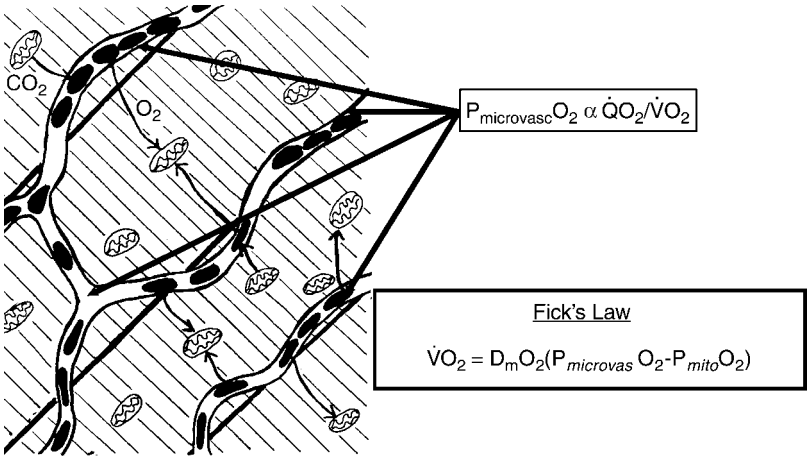

FIG. 18 Schematic illustration of Fick's law applied to blood–myocyte O_2 exchange within the microcirculation of skeletal muscle. Note: partial pressure of O_2 in mitochondria ($P_{mito}O_2$) approaches 'zero' during exercise and thus this term can be discarded in the calculation of O_2 uptake ($\dot{V}O_2$). $P_{microvasc}O_2$, O_2 partial pressure in capillary; D_mO_2, tissue diffusing capacity for O_2, $\dot{Q}O_2$, O_2 delivery. Please see text for additional details

there may be other important determinants of intramyocyte O_2 transport. For example, it is possible that the mitochondrial system, which should be thought of as a catenated network rather than a number of discrete amoeboid-shaped organelles, may serve to enhance O_2 distribution within the cell[108,115].

Structural and functional plasticity with training

The structural and functional elements of the O_2 transport pathway demonstrate a substantial plasticity or adaptability, with $\dot{V}O_{2max}$ increasing between 10 and 25% in response to training[32,57,77,92,116–122]. Lung function (diffusing capacity) and the structure and function of the cardiovascular and muscular systems undergo profound adaptations to repeated exercise bouts that result in an augmented O_2 flux and enhanced performance at maximal, as well as improved endurance at submaximal, running speeds. These adaptations have been detailed most extensively in humans, dogs and rats[57,77,92,121]. However, there also exists substantial information regarding training adaptations in the horse[32,116–120], and this section considers the broad

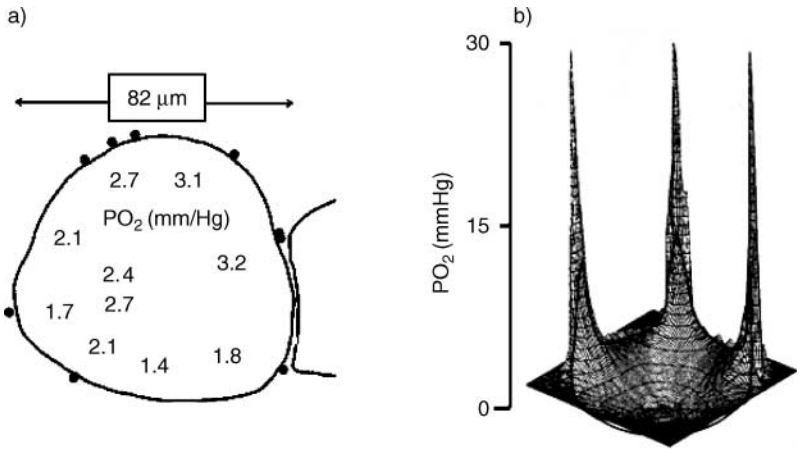

FIG. 19 Measurements of intramyocyte O_2 partial pressure (PO_2) during maximal exercise in the dog *gracilis* muscle. a) PO_2 values at discrete locations within the myocyte transverse plane. Solid circles at periphery designate capillaries with PO_2 as high as 80–90 mmHg. b) Three-dimensional reconstruction of the PO_2 profile within the muscle capillary and the contracting myocyte (redrawn from Honig *et al.*[112]). Note the principal PO_2 fall occurs in close proximity (within 1 μm) to the capillary and that the intramyocyte PO_2 profile is remarkably flat without substantial PO_2 gradients. This suggests that even relatively large intracellular O_2 diffusion distances to the mitochondria are of little consequence

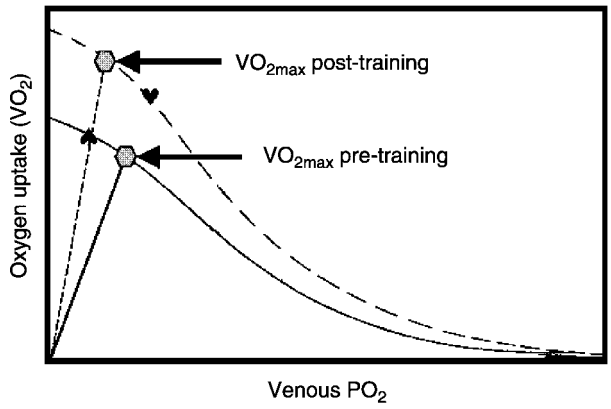

FIG. 20 Exercise training increases maximal O_2 uptake ($\dot{V}O_{2max}$) by elevating both conductive (curved line, due to increased stroke volume) and diffusive (straight line, due principally to increased muscle capillarity and events within those capillaries) O_2 transport. Note that even a modest reduction (5–10%)[116,126] in venous O_2 partial pressure (PO_2) after training requires a substantial increase (~30%) in muscle O_2-diffusing capacity (D_mO_2). Moreover, if training solely increased convective O_2 delivery and there was no augmentation of D_mO_2, venous PO_2 would rise after training (point ♥). This has not been observed. Point ♠ would theoretically be reached if D_mO_2 increased in the absence of an elevated cardiac output ($\dot{Q}_{TOT}$) (and muscle O_2 delivery, $\dot{Q}_mO_2$) at maximal exercise. For additional information see the caption to Fig. 5

mechanistic bases by which such adaptations increase $\dot{V}O_{2max}$ in this species. Specifically, the so-called 'Wagner diagram' introduced in Fig. 5 will be utilized as a graphical representation of the Fick equation and Fick's law of diffusion to explore the roles of elevated O_2 transport and muscle O_2 extraction in the increased $\dot{V}O_{2max}$ after training (Fig. 20).

The training-induced increase of $\dot{V}O_{2max}$ (Fig. 20) results from a combination of increased $\dot{Q}O_2$ and what appears to be a fairly modest improvement in O_2 extraction[116] (lowered venous PO_2 and therefore C_vO_2). At first glance, this observation appears to substantiate a multitude of reports that the elevation of $\dot{V}O_{2max}$ after training occurs in proportion to the rise in $\dot{Q}_{TOT}$ (which is driven by an augmented SV and not HR[122–124]) and that the increased O_2 extraction (and therefore change of muscle O_2-diffusing capacity, D_mO_2) is of only limited importance. Such an interpretation is superficial and misleading. Notice in Fig. 20 that with the increased O_2 delivery, if D_mO_2 (slope of line from the origin) had remained unchanged, P_vO_2 would have increased and O_2 extraction would have decreased after training (see point ♥). Thus, to maintain either the same extraction or to increase this even modestly, a sizeable elevation of D_mO_2 is required. The basis for this effect was developed initially by Piiper and Scheid[125] to explain O_2 exchange within the lung before subsequently being adapted for muscle by Wagner and colleagues[126] and is based upon the following concept:

$$\dot{V}_mO_2 = \dot{Q}_mO_2[1 - \exp(-D_mO_2/\beta\dot{Q}_m)],$$

and thus

$$\dot{V}_mO_2/\dot{Q}_mO_2 = 1 - \exp(-D_mO_2/\beta\dot{Q}_m),$$

where β is the slope of the O_2 dissociation curve in the physiologically relevant range, and the exponential term, $1 - \exp(-D_mO_2/\beta\dot{Q}_m)$, denotes O_2 extraction. Notice that O_2 extraction will decrease if increases of $\dot{Q}_m$ are not matched by those of D_mO_2, and any increase of O_2 extraction therefore requires that D_mO_2 increases to a greater extent than $\dot{Q}_m$. For example, Wagner and colleagues[126] determined that for an increased O_2 extraction of just 5–10%, D_mO_2 must increase by 30–40%. This consideration places great emphasis on understanding muscle microvascular adaptations such as increased capillarity[57,92,93], which help set D_mO_2. Because of the clear demonstration of O_2 supply limitation (i.e. oxidative enzyme capacity excess, see above), the augmented muscle mitochondrial volume density and oxidative enzyme adaptations to training[127–132] will not be considered further in the context of $\dot{V}O_{2max}$ adaptations to training. Indeed, it is entirely possible that these adaptations are more important for determining substrate utilization profiles and exercise tolerance at submaximal running speeds than $\dot{V}O_{2max}$ *per se*[92,121].

Let us consider what drives the two crucial adaptations of SV and D_mO_2 with exercise training.

Stroke volume (SV)

Training promotes expansion of RBC mass and plasma volume[133,134] which may increase central venous pressure and elevate venous return and thus ventricular preload. This in turn increases end-diastolic volume, which pre-stretches the cardiac myocytes and produces a more forceful and rapid myocardial contraction. As MAP is not altered by training, there is no change in cardiac afterload. The aforementioned observation that removal of the pericardium increases SV and $\dot{Q}_{TOTmax}$[17,18] suggests that training-induced removal of pericardial constraints to maximal SV (i.e. pericardial stretching and improved elasticity[135,136]) may be a crucial component in the increased SV seen after training. The studies of Young *et al.*[14] indicate that mitral and tricuspid valvular insufficiencies and regurgitation may accompany the training-induced elevations of cardiac mass[137,138] and serve to limit increases in $\dot{Q}_{TOTmax}$. Because $\dot{Q}_{TOTmax}$ increases after training in the absence of elevated MAP, vascular conductance (the reciprocal of peripheral resistance) must increase in proportion to elevated $\dot{Q}_{TOTmax}$. Thus, a complex interplay between the control of arteriolar resistance vessels and $\dot{Q}_{TOT}$ and $\dot{Q}_m$ must be present. Moreover, after training there is a preferential redistribution of the elevated $\dot{Q}_{TOT}$ towards[77,79,139] and within[79,108] the active

muscles, which is crucial for improving the matching of $\dot{Q}O_2$ and $\dot{V}O_2$. Whereas it is known that exercise training increases muscle vascularity and alters the sensitivity of the arteriolar endothelium and smooth muscle to vasoactive stimuli[140–142], the precise details of the training response and the role of the sympathetic nervous system in the training-induced elevation of muscle vascular conductance remain to be elucidated. What is emerging is an appreciation of the complexity of vascular control among the various orders of arterioles within skeletal muscle. For example, Laughlin and colleagues[64,79] as well as Van Teeffelen and Segal[143] have demonstrated that the relative importance of neural versus endothelial and smooth muscle control of arteriolar calibre is markedly different across the various orders of arterioles and that each may respond differently to training[64]. This is currently a very active and promising area of research.

Muscle O_2-diffusing capacity (D_mO_2)

Because exercise training does not increase arterial O_2 content (C_aO_2), an enhanced O_2 extraction must result from a fall in venous O_2 content (C_vO_2)[116]. This effect results from both a preferential redistribution of $\dot{Q}_{TOT}$ to active muscles and muscle fibres (as discussed above) and also a greater total and fractional O_2 extraction by the active muscle fibres[116,126]. As seen in Fig. 20, a greater fractional extraction, particularly in the presence of elevated $\dot{Q}_m$ at maximal exercise, demands substantial increases in D_mO_2. There are several factors that are key to determining D_mO_2. These include:

- the size of the capillary bed (i.e. capillary length and surface area per volume of muscle) and capillary-to-fibre surface contact area[86,94,144,145];
- the capillary haematocrit and the number of RBCs adjacent to the active muscle fibres at a given time[100,101];
- RBC velocity and transit time within the capillaries[108];
- the orientation of RBCs within capillaries[146]; and
- intramyocyte diffusion as determined by myoglobin and possibly other, as yet undetermined, mechanisms[114].

Of the above, the training-induced capillary proliferation[57,92,93,116,120,127,128,147,148] which occurs in proportion to elevated muscle oxidative enzyme capacity and elevated myoglobin (in mammals other than humans) remains the best characterized response[57]. As argued above, the principal site of resistance to blood–mitochondrial O_2 diffusion occurs in close proximity to the capillary endothelium and so it makes sense that capillary proliferation constitutes a key training adaptation. However, other than this providing a greater capillary surface and presumably facilitating an increased capillary RBC transit time (at any given $\dot{Q}_m$), the effects of training on events within the capillary and myocyte are largely unknown. Specifically, regarding skeletal muscle at maximal exercise, the following crucial questions remain:

- What is the haematocrit and RBC transit time (mean value and distribution) within the capillary bed?
- Is it possible to increase D_mO_2 by elevating plasma or tissue O_2 solubility[108,149,150]?
- What is the role of myoglobin in setting D_mO_2 and how is this impacted by training?
- Despite the demonstrated O_2 supply-limited property of skeletal muscle during whole-body exercise, does the elevated oxidative enzyme capacity play a role in raising $\dot{V}O_{2max}$ after training[151]?
- How does exercise training speed $\dot{V}O_2$ kinetics?

Within any intact mammalian system elucidation of these questions presents a formidable and exciting challenge that is as relevant to understanding the basis for superlative athletic performance as it is to combating the severe muscle dysfunction in diseases such as heart failure, diabetes and emphysema. Whilst it should be kept in mind that extraordinary cardiovascular and muscle O_2 transport and utilization capacities do not by themselves make a great equine athlete, truly great horses are often characterized by their superlative O_2 transport systems.

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